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Pennsylvania Soybean Board
Attn: Jennifer Reed-Harry, Executive Director
Northwood Office Center
2215 Forest Hills Drive, Suite 40
Harrisburg, PA 17112

Re: Final Report for "Prevention of Inflammation-Driven Colon Cancer by Soy Protein Concentrate"

March 28th, 2014

Dear Ms. Reed-Harry:

We appreciate the grant awarded by the Board and have conducted the proposed experiments. Please accept my apologies for the tardiness of this final report.

MAJOR FINDINGS

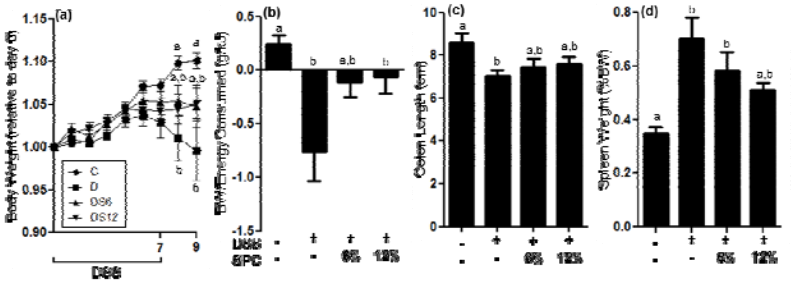

Figure 1: The effects of dietary SPC on weight gain, feeding efficiency, and gross measures of inflammation in DSS-induced CF-1 mice. (a) Body weight change relative to starting weight was determined over the course of the experiment. (b) The feeding efficiency of the mice was determined based on the ratio of body weight gain to energy intake and averaged over the last 9 d of the experiment. (c) Colon length and (d) relative spleen weight were determined at the end of the experiment. All values represent the means ± SEM and an n = 10 for each group. Different letters denote $p < 0.05$ using one-way ANOVA with a Tukey's Multiple Comparison post-test.

Soy Protein Concentrate (SPC) Dose-Dependently Reduced Colitis in DSS-Treated Mice Treatment with DSS induced body weight loss and reduced feeding efficiency (**Fig. 1**). Dietary SPC supplementation reduced the deleterious effects on body weight and feeding efficiency. Colon shortening and increased relative spleen weight are both gross markers of colitis severity. DSS treatment significantly reduced mean colon length, whereas treatment with SPC tended to increase mean colon length (**Fig. 1**). We determined the expression of several inflammatory markers in the colons of mice treated with DSS with and without dietary SPC. Analysis with ELISAs showed that protein levels of the inflammatory mediators: IL-1β, IL-6, and MCP-1 were significantly increased in colon homogenates from DSS-treated mice, and decreased by SPC (**Fig. 2**). The levels of IL-1β, IL-6, and MCP-1 were dose-dependently reduced by SPC supplementation. Mice treated with DSS had 46% higher levels of lipid peroxidation in the colon homogenate as compared to the negative control (**Fig. 2**). Treatment with SPC-supplemented diets reduced lipid peroxide levels back to control levels.

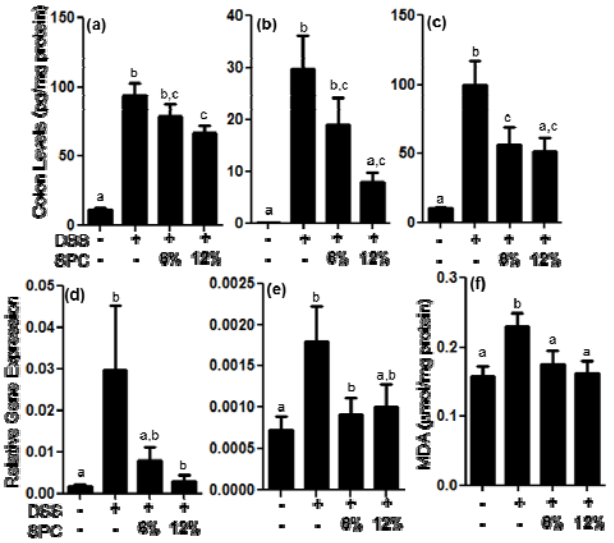

Figure 2: Biochemical markers of colonic inflammation in DSS-induced CF-1 mice. Protein levels of (a) IL-1β, (b) IL-6, (c) MCP-1 were determined in the colon homogenate using commercially-available ELISAs and normalized to the total protein of each sample. Relative fold change of mRNA expression of (d) IL-1β and (e) EMR-1 in colon homogenate was determined by quantitative reverse transcriptase PCR and normalized to the expression of GAPDH expression. (f) Lipid peroxide levels in colon homogenate were determined using the TBARs assay and normalized to total protein levels in the homogenate. All values represent the means ± SEM and an n = 10 for each group. Different letters denote $p < 0.05$ using one-way ANOVA with a Tukey's Multiple Comparison post-test.

Individuals with ulcerative colitis have compromised gut barrier function. This allows bacteria and bacterial components in colon contents to enter systemic circulation and cause inflammation. We determined the ability of SPC to modulate gut barrier function in DSS-treated mice. Decreased

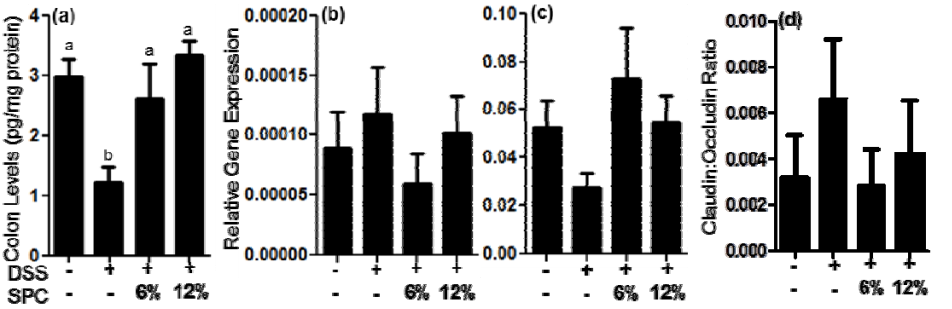

Figure 3: Biochemical markers of intestinal permeability in the colon homogenate. (**a**) GLP-2 protein levels in the colon homogenate as determined by ELISA and normalized to the total protein of each sample. Relative fold change of mRNA expression of (**b**) Claudin-1 and (**c**) Occludin in colon homogenate was determined by quantitative reverse transcriptase PCR and normalized to the expression of GAPDH expression. (**d**) The ratio of Claudin-1 to Occludin was used as a marker of gut barrier function, with a decreased ratio representing compromised barrier function. All values represent the means ± SEM and an n = 10 for each group. Different letters denote $p < 0.05$ using one-way ANOVA with a Tukey's Multiple Comparison post-test.

colonic expression of glucagon-like peptide (GLP)-1, increased expression of claudin-1, and decreased expression of occluding-1 indicate compromised gut barrier function. We found that DSS-treatment decreased expression of GLP-1 and occludin-1, and increased expression of claudin-1 (**Fig. 3**). Treatment with SPC prevented these changes indicating that it prevented loss of gut barrier function in DSS-treated mice.

Dietary SPC Reduces Tumorigenic Progression in Azoxymethane (AOM)/DSS-treated Mice Mice treated with a single injection of AOM (a colon-specific carcinogen) followed by treatment with DSS for 1 week induces colonic inflammation, hyperplasia, and dysplasia, and eventually, colon cancer. We found that dietary treatment with 24% dietary SPC reduced inflammation index (the sum of the inflammation area, ulceration, hyperplasia, and dysplasia scores) in the colons of mice treated with AOM and DSS compared to control mice (Pos) after 7 weeks of treatment (**Fig. 4a**). This reduction was due primarily to reductions in colonic inflammation area (**Fig. 4c**) and hyperplasia/dysplasia score (**Fig. 4d**).

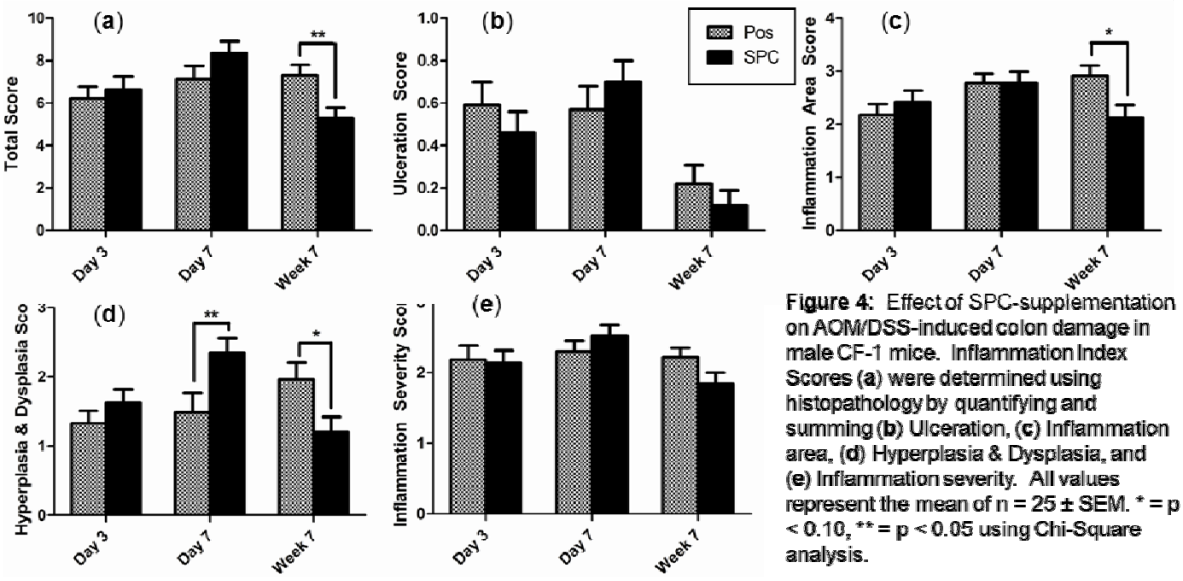

Figure 4: Effect of SPC-supplementation on AOM/DSS-induced colon damage in male CF-1 mice. Inflammation Index Scores (**a**) were determined using histopathology by quantifying and summing (**b**) Ulceration, (**c**) Inflammation area, (**d**) Hyperplasia & Dysplasia, and (**e**) Inflammation severity. All values represent the mean of n = 25 ± SEM. * = $p < 0.10$, ** = $p < 0.05$ using Chi-Square analysis.

Dietary SPC Reduces Colon Polyp Multiplicity and Tends to Reduce Colon Adenocarcinoma Incidence and Tumor Size After treatment with 24% SPC for 20 weeks, colon polyp (diameter less than 1 mm) and tumor multiplicity were determined. AOM/DSS-induced mice treated with 24% dietary SPC for 20 weeks had reduced total polyp multiplicity compared to AOM/DSS-induced controls (Pos, **Table 1**).

Table 1. Impact of Dietary SPC on Tumor/Polyp Parameters in AOM/DSS-Treated Mice

Treatment	Polyp Multiplicity (polyps/mouse)	Tumor Area (cm^2)	Non-invasive and invasive Adenocarcinoma (incidence, %mice)
Pos	5.0 ± 0.7	2.0 ± 0.5	30
SPC	3.0 ± 0.3*	0.75 ± 0.25	9

* $p < 0.01$ compared to Pos.

Total tumor area (the total area of all tumors greater than 2 mm) tended to be decreased by SPC treatment although the results are not statistically significant (**Table 1**). Similarly, the incidence of colon adenocarcinoma and invasive adenocarcinoma were elevated in AOM/DSS-treated mice, and SPC tended to reduce the incidence of these lesions, but the effect was not statistically significant (**Table 1**). Overall these results suggest that dietary SPC may have a beneficial effect against inflammation-driven colon cancer, but further studies are needed.

PUBLICATIONS

Bitzer ZT, Brownschidle AL, Tao L, Cooper TK, Vanamala J, Elias RJ, Lambert JD (2014). Dietary soy protein concentrate suppresses colonic inflammation and loss of gut barrier function *in vitro* and *in vivo*. *Carcinogenesis*. in prepartion.

SUMMARY

Our results demonstrate that dietary SPC can suppress colonic inflammation in a mouse model of ulcerative colitis, and suggest that SPC may reduce inflammation-driven colon carcinogenesis. Future studies should 1.) confirm the cancer preventive effects of SPC; 2.) identify the major bioactive peptides in SPC; 3.) explore the underlying mechanism of action; 4.) determine whether the anti-inflammatory effects of SPC translate to human subjects with ulcerative colitis.

We are in the process of preparing two grant applications to the United States Department of Agriculture and The American Institute for Cancer Research to fund future research which builds on the work reported here.

Again, thank you for your support of our research efforts. Please let me know if you require additional information. When our manuscript is published, I will forward you a final version.

With Best Regards,

Joshua D. Lambert, Ph.D.
Associate Professor