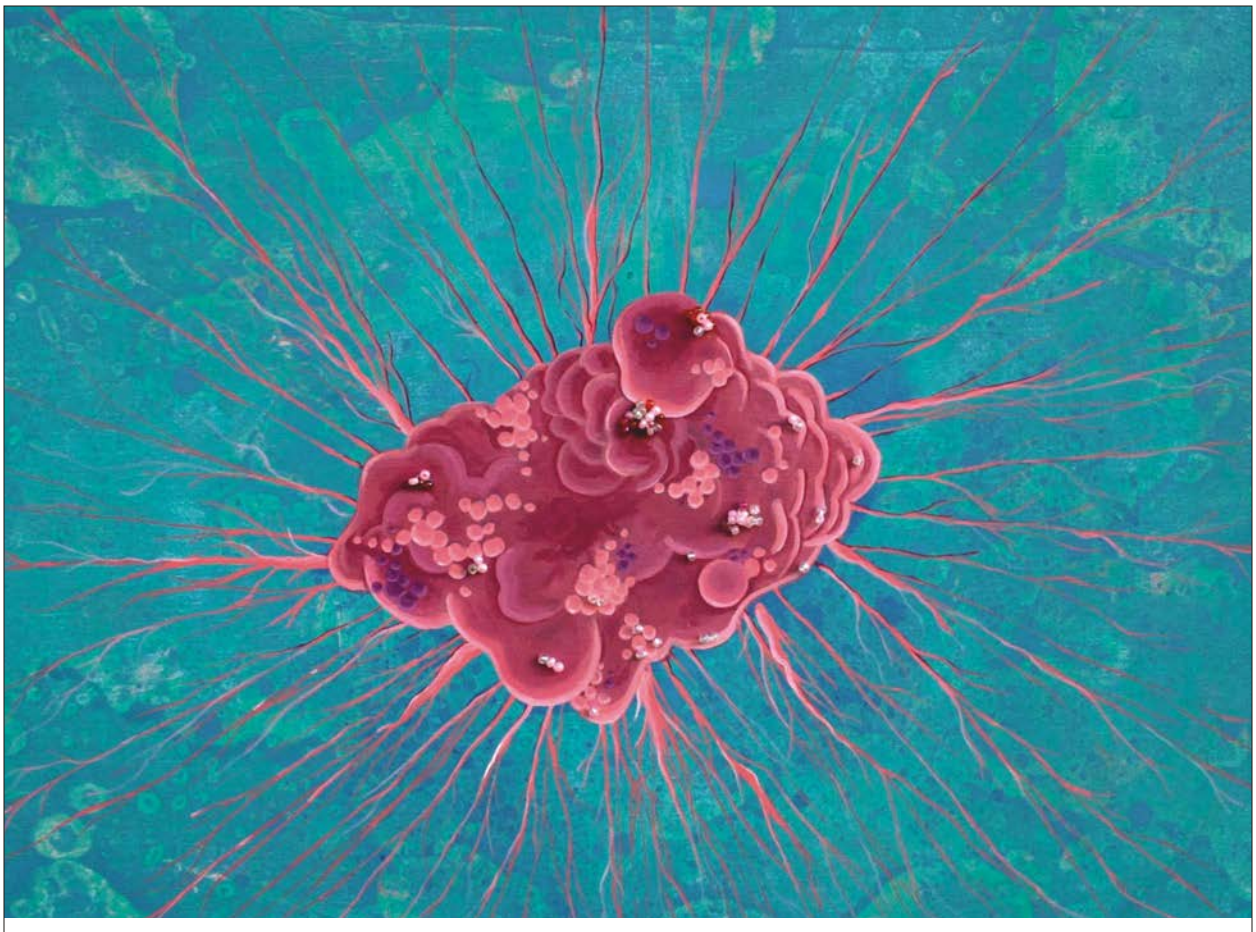

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Chemopreventive Potential of Soy Flour, Flaxseed Meal and a Probiotic in a Rat Model

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ABSTRACT

Soy flour, flaxseed meal and probiotics have been widely researched individually for their beneficial effects against various diseases, including colon cancer. Research determining possible chemopreventive synergies among the three ingredients is limited. Objective of the study was to determine the effects of feeding various combinations of Soybean Flour (SF), Flaxseed Meal (FM) and *Bifidobacterium longum* (Bl.) on azoxymethane (AOM) induced Aberrant Crypt Foci (ACF) in male Fisher 344 rats. After 1 week acclimatization period, rats were divided into Control (C) and 9 treatment groups and fed AIN-93G diets containing Bl singly, SF and FM in various combinations (5 and 10%) and combinations of Bl.+SF+FM (0.25, 5 and 10%). Rats were subcutaneously injected with AOM at 16 mg kg^{-1} body weight at 7 and 8 weeks to induce ACF. At 17 weeks of age, the rats were killed using CO_2 asphyxiation. Colons were removed and prepared for enumeration of ACF. Selected hepatic detoxification and antioxidant enzymes were also determined. Total ACF ranged from 37 in the group fed Bl.+SF+FM (0.25, 10 and 5%) to 144 in the group fed C. Enzyme activities in rats fed treatment diets were increased by 2-3 fold compared to rats fed C. Some synergies may exist among treatment ingredients. Incorporation of moderate levels of soybean flour, flaxseed meal and *Bifidobacterium longum* in diets may have implications in the incidence of colorectal cancer.

Key words: Fisher 344 male rats, *Bifidobacterium longum*, colon cancer, azoxymethane

INTRODUCTION

According to the American Cancer Society (ACS., 2011) half of all men and one-third of all women in the U.S. will develop cancer, making this disease the second leading cause of death in the US. Colon cancer specifically is the third most commonly diagnosed and cause of cancer deaths among both men and women. Colon cancer is a disease that plagues Western nations (US and European countries) at a significantly higher rate than other nations. The Western diet tends to be higher in red meat and low in fiber, both of which have been linked to increased risk of colon cancer development (Yu *et al*., 2004). Over 90% of these cancer cases are related to lifestyle patterns, specifically dietary patterns (Doll and Peto, 1981).

The bioavailability of food components is important in colon carcinogenesis research as it may imply a direct relationship between food and disease. As reported by Tammariello and Milner (2010) in a review study, there were over 1600 publications utilizing rat models for implications of colon cancer but only a little over 100 studied diets as a variable. The effects of soy flour (Yu *et al*., 2004), flaxseed meal (Williams *et al*., 2007) and probiotics (Wollowski *et al*., 2001; Verghese *et al*., 2002) have been widely researched individually with respect to their relationship to colon cancer. The effects of combination diets may prove more effective than those isolating individual components for treatment.

Soybeans have been the object of various epidemiological and animal studies with regard to its influence on various chronic diseases (Liao *et al*., 2007; Hakkak *et al*., 2001; Cavallini *et al*., 2011). Soy is high in the specific flavonoids, isoflavones which are associated with a reduced onset of certain cancers (Yuan *et al*., 2007). Certain isoflavones, genistein and daidzein are reported to act as antiproliferative agents (inhibiting tyrosine kinases) and stimulating apoptosis (Yu *et al*., 2004).

Flaxseed is made up of three beneficial components (lignans, fiber and alpha linolenic acid) that have been widely accepted. Flaxseed meal has been reported to contain 2500 mg of lignans per 100 g, more than any other plant source. Lignans are one of the phenolic compounds which serve as antioxidants and anti-inflammatory agents in the body (Bassett *et al*., 2009). Fiber is known to decrease the transit time of waste as well as increase bile acid excretion. Increased intake of fiber increases the production of the short chain fatty acids, namely butyrate. Butyrate has been shown to increase the levels of glutathione-s-transferase (an important antioxidant enzyme) in the colon (Wollowski *et al*., 2001). Dietary intake of flaxseed meal may result in a substantial decrease in colon tumor development, as well as decrease the average number of tumors and size in azoxymethane (AOM) treated rats (Williams *et al*., 2007).

In the past decade, more exploration has been delegated to confirming the beneficial attributes of probiotics. Probiotics are viable microorganisms that once consumed by the host, will act to benefit the intestinal microflora (Gibson and Wang, 1994). Probiotics include *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus* spp. bacteria. *Bifidobacterium longum*, makes up approximately 25% of the microflora population of an adult gut (Gibson and Roberfroid, 1995). Although specific mechanisms of *Bifidobacteria*'s benefits are unknown, it includes producing strong acids that lower the intestinal pH inhibiting pathogenic bacteria growth (Rasic and Kurmann, 1983), as well as acting as an immunomodulator against malignant cancer cells (Mizutani and Mitsuoka, 1980).

The dynamics of the combination of flaxseed, soy and probiotics are unknown. The objective of this study was to identify the synergistic chemopreventive potential of various combinations of soy flour, flaxseed meal and *Bifidobacterium longum* diets on azoxymethane-induced aberrant crypt foci in Fisher 344 rats.

MATERIALS AND METHODS

Experimental design, animal housing and diets: Forty Fisher 344 male weanling rats (3 weeks old) were secured from Harlan, Indiana and housed 2 rats per cage in wired stainless steel cages. Light and dark cycles were maintained for 12 h each while the relative humidity and temperature remained constant at 50% and 21°C, respectively. Rats were allowed a 1 week acclimatization period prior assignment of experimental diets. Assignment of rats to experimental groups (n = 4) was done randomly among 10 treatments. Diets consisted of AIN-93G as the Control (C) and AIN-93G using the prescribed modifications: *Bifidobacterium longum* singly

(0.25%), soy flour+flax seed meal (5%+5%), (10%+10%), (5%+10%), (10%+5%) and soy flour+flax seed meal+*Bifidobacterium longum* (5%+5%+0.25%), (10%+10%+0.25%), (5%+10%+0.25%), (10%+5%+0.25%). Diets were prepared as needed and stored at 4°C. Animals were given access to feed and water *ad libitum* over the 17 week period and maintained according to standard protocol. Daily feed intake and weekly weight gain was recorded throughout the experiment. Common dietary ingredients were obtained through MP Biomedicals (Costa Mesa, CA). Defatted soybean flour and flaxseed meal were obtained from Bob's Red Mill (Milwaukie, OR) and *Bifidobacterium longum* (BB536) from Morinaga Milk Company (Japan).

Administration of carcinogen and sample collection: To induce Aberrant Crypt Foci formation (ACF), all rats were sub-cutaneously injected with 16 mg kg^{-1} body wt., of azoxymethane (AOM) in saline at 7 and 8 weeks of age. At approximately 17 weeks of age, the rats were asphyxiated with carbon dioxide. The cecum was removed and the wall weight and pH of contents were recorded for each rat. The livers were removed, immediately frozen using liquid nitrogen, then stored at -80°C until further analysis. The colons were flushed with a potassium phosphate buffer solution (0.1 M, pH 7.2), cut lengthwise and laid flat between 2 sheets of filter paper in a 10% formalin solution overnight.

Enumeration of Aberrant Crypt Foci (ACF): Colon lengths were divided into 2 sections, proximal and distal, then cut into 2 cm subsections and stained with 2% methylene blue solution for ease of scoring. ACF enumeration was performed according to (Bird, 1995). Foci were categorized by location (proximal or distal), enumeration and crypt multiplicity (foci having 1, 2, 3, 4 or = 5 crypts).

Liver preparation and hepatic enzyme analysis: One gram of the liver samples was homogenized in 10 mL of potassium phosphate buffer (0.1 M, pH 7) using a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 10,000 g for 30 min. The supernatant was then centrifuged a second time at 10,000 g for an additional 10 min.

Hepatic Glutathione-S-Transferase (GST) was determined by measuring the conjugation of 1-chloro 2, 4-dinitrobenzene (CDNB) as described by Habig *et al.* (1974). The change in absorbance at 340 nm as a function of time was monitored (BioRad Plate Reader Model 680, MA). Total enzyme activity (U mL^{-1}) was measured at the end of 5 min of reaction using a microplate reader.

Superoxide dismutase (SOD) activity (U mL^{-1}) was determined using the protocol of Fridivich (Fridivich, 1989). The increase in absorbance at 480 nm was monitored every 30 sec for 150 sec. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of auto-oxidation.

The hepatic catalase activity was determined using a spectrophotometer at 540 nm to monitor the decomposition of H_2O_2 in accordance with the protocol of (Aebi, 1984). The catalase activity was measured against a formaldehyde standard and expressed as U mL^{-1}.

Determination of total phenolics and flavonoids: Total phenolic content was determined as described by Singleton *et al.* (1999) using the Folin-Ciocalteu colorimetric method. Extracts of soy flour and flaxseed meal were oxidized with Folin-Ciocalteu reagent then neutralized with sodium carbonate. The end product was allowed to incubate for 1.5 h and the absorbance was read at 760 nm against a blank and compared against a gallic acid standard curve.

The total flavonoid content was determined using colorimetric method (Kim *et al*., 2003). The method was modified as follows. An aliquot (25 μL) of the soybean and flaxseed extracts were mixed with 40 μL of distilled water and followed by the addition of 7.5 μL of a 5% sodium nitrite solution. After 5 min, 15 μL of 10% aluminum chloride solution was added and allowed to stand for another 5 min. Later, 50 μL of 1 M NaOH was added. The absorbance was immediately measured at 510 nm using the microplate reader (BioRad Plate Reader Model 680, MA). Catechin was used as the standard and the results will be expressed as mean (mg of catechin equivalents/1 g soy or flaxseed)±SEM for three replications.

Determination of free radical scavenging ability: To determine the free radical scavenging activity of the soy and flaxseed meal, the method developed by Brand-Williams *et al*. (1995) was used. A 1 mL aliquot of various concentrations of extract was combined with 2.9 mL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical solution. The mixture was then agitated and held at room temperature for 30 min under restricted light. Each concentration of meals was read (BioRad Plate Reader Model 680, MA) at a wavelength of 517 nm. The free radical scavenging activity was measured as the amount of extract required to decrease the initial absorbance of the DPPH by 50% compared to the control.

Determination of Ferric Reducing Antioxidant Power (FRAP): The FRAP assay was performed in accordance with methodology of Benzie and Strain (1999). A 1 mL aliquot of the diluted soy flour/flaxseed extract was combined with 3 mL of freshly prepared FRAP reagent [300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6 tri (2 pyridyl)-s-triazine (TPTZ) in 40 mL HCl and 20 mM ferric chloride ($FeCl_3H_2O$)]. The resulting mixtures were incubated for 10 min at a temperature of 37°C. Each sample was analyzed in triplicate at an absorbance of 593 nm. The change in absorbance was compared against a standard ferrous sulfate and expressed as μmol of Fe^{2+}/grams.

Statistical analysis: Statistical tests used included ANOVA to determine significant differences among treatment groups and Tukey's studentized range for separation of means where significant differences were shown at $p \leq 0.05$. The statistical analysis was conducted using a statistical analysis software, 2011 (SAS Institute Inc, Cary, NC).

RESULTS

Feed intake, weight gain, cecal weight and cecal pH of rats fed various treatments: The effects of individual treatments on feed intake, weight gain, cecal weight and pH are shown in Table 1. The treatment group fed Bl (0.25%) singly (16.77 g day^{-1}), had a significantly lower feed intake (g day^{-1}) than all other treatment groups, with the exception of the groups fed SF+FM (5%: 5%) and SF+FM+Bl (10%: 10%) at 17.98 and 17.78, respectively. Excluding the control, rats fed the treatment SF+FM (10%: 5%) had a significantly higher feed intake compared to the other treatments at 20.28 g day^{-1}. No significant difference was observed in the rats fed the combinations of SF+FM+Bl at (5%: 5%: 0.25%) (18.95), (5%: 10%: 0.25%) (18.91), (10%: 5%: 0.25%) (18.40) or SF+FM at (10%: 10%) (17.78), (5%: 10%) (18.91). When comparing the weight gain (g/17 weeks) of the rats, there was no significant differences observed between the control and the combination diets. However those fed Bl. singly had a significantly lower weight gain then treatment group fed SF+FM+Bl (5%: 5%) at 239.05 g/13 weeks. In comparing the cecal weight and the cecal

Table 1: Effect of soy flour, flaxseed meal and a probiotic on weight gain, daily feed intake, cecal pH and cecal wall weight in rats

	Weight gain			
Diets	(g/13 weeks)	Daily feed intake (g day^{-1})	Cecal wall weight (g)	Cecal pH
Control	213.50±2.31^{ab}	19.31±0.0^{ab}	1.15±0.20^{a}	8.30±0.08^{a}
Bl (0.25%)	195.00±2.33^{b}	16.77±0.00^{d}	1.00±0.09^{a}	7.98±0.5^{a}
SF+FM (5%: 5%)	212.40±7.79^{ab}	17.98±0.23^{cd}	1.25±0.12^{a}	8.31±0.4^{a}
SF+FM (10%: 10%)	216.50±6.61^{ab}	18.22±0.14^{bc}	1.23±0.11^{a}	7.94±0.11^{a}
SF+FM (5%: 10%)	221.43±10.24^{ab}	18.22±0.15^{bc}	1.05±0.21^{a}	8.31±0.12^{a}
SF+FM (10%: 5%)	224.28±11.70^{ab}	20.28±0.38^{a}	1.20±0.14^{a}	7.95±0.15^{a}
SF+FM+Bl (5%: 5%)	239.05±12.46^{a}	18.95±0.24^{bc}	1.38±0.10^{a}	8.00±0.08^{a}
SF+FM+Bl (10%: 10%)	228.43±13.71^{ab}	17.78±0.25^{cd}	1.50±0.21^{a}	7.74±0.24^{a}
SF+FM+Bl (5%: 10%)	234.35±4.79^{ab}	18.91±0.48^{bc}	1.20±0.9^{a}	7.89±0.23^{a}
SF+FM+Bl (10%: 5%)	230.63±4.55^{ab}	18.40±0.12^{bc}	1.33±0.17^{a}	8.03±0.22^{a}

SF: Soy flour, FM: Flaxseed meal, Bl: *Bifidobacterium longum*, values are expressed as Means±SEM, Mean columns with different letters are significantly different, ($p<0.05$) using Tukey's studentized range test

Table 2: Percent of crypt reduction compared to the control of rats fed soy flour, flaxseed meal and a probiotic

Diets	Distal	Proximal	Total
Control	-	-	-
Bl (0.25%)	65.82	66.30	65.82
SF+FM (5%: 5%)	65.36	45.65	59.07
SF+FM (10%: 10%)	64.29	50.00	59.72
SF+FM (5%: 10%)	67.35	58.45	64.50
SF+FM (10%: 5%)	65.01	56.21	62.20
SF+FM+Bl (5%: 5%)	71.57	76.40	73.12
SF+FM+Bl (10%: 10%)	71.87	77.64	73.71
SF+FM+Bl (5%: 10%)	78.43	75.78	77.58
SF+FM+Bl (10%: 5%)	80.76	77.08	79.58

SF: Soy flour, FM: Flaxseed meal, Bl: *Bifidobacterium longum*, values are expressed as percentages based on difference in crypt formation when compared to the control

pH of the treatment groups, they did not significantly differ from one another. The rats fed SF+FM+Bl at 10%: 10% showed an inverse relationship between cecal weight and pH, although not significant, this group had the lowest pH and highest cecal weight.

Distribution of ACF incidence and total crypts in distal and proximal colon: The ACF incidence (Fig. 1) in both the proximal and distal colon of the rats fed the control was significantly higher than rats fed any other treatments. Rats fed the any combination of SF+FM showed no significant differences in ACF incidence in either the proximal or distal sections of the colon. Similarly in the proximal colon, there was no significant difference in ACFs found in rats fed treatments consisting of Bl. singly or in any combination of SF+FM+Bl. In the distal colon, no significant difference was found between groups fed treatments consisting of SF+FM (10%: 10%), (5%: 10%) and SF+FM+Bl (5%: 5%) (10%: 10%). Rats fed SF+FM+Bl (10%: 5%) exhibited a significantly lower ACF incidence in the distal colon when compared to other treatments.

Increased crypt formation (Table 2) can be indicative of potential tumor development in colon carcinogenesis. Rats fed a diet of the control experienced overall crypt formation at minimum 2x higher than that of rats fed the treatment diets in both proximal and distal portions of the colon.

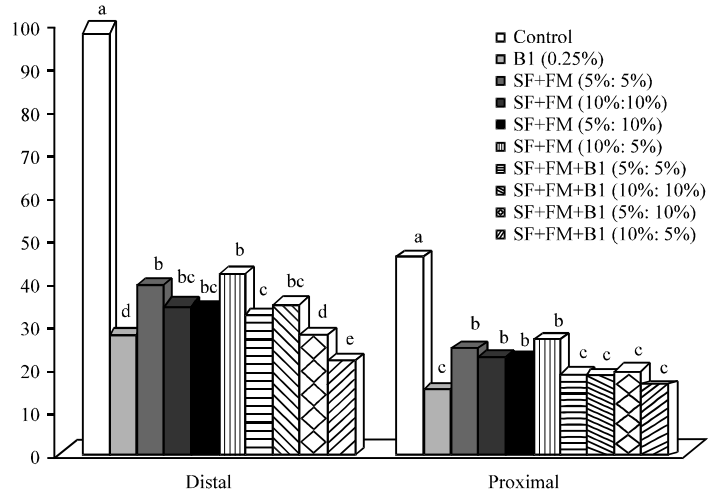

Fig. 1: Effect of soy flour, flaxseed meal and a probiotic on Aberrant Crypt Foci (ACF) incidence in colon of AOM: Induced carcinogenic rats, SF: Soy flour, FM: Flaxseed meal, Bl: *Bifidobacterium longum*. Mean columns with different letters are significantly different ($p<0.05$) using Tukey's studentized range test

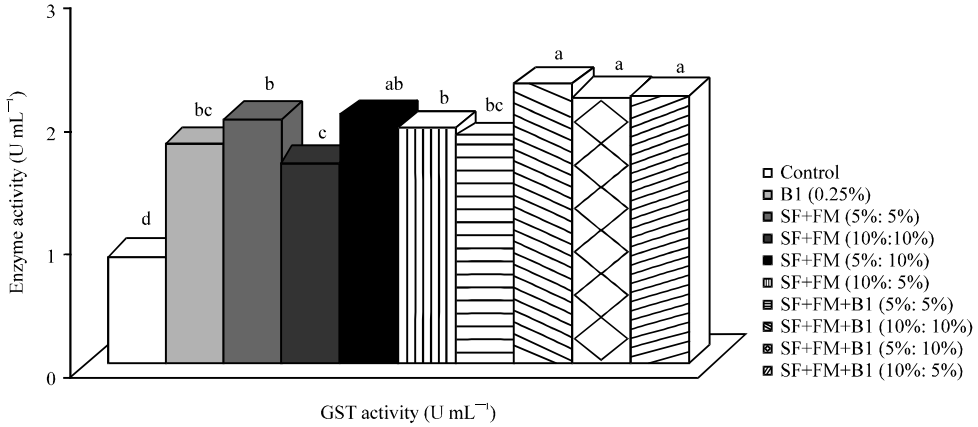

Fig. 2: Effect of soy flour, flaxseed meal and a probiotic on hepatic glutathione-s-transferase enzyme activity, SF: Soy flour, FM: Flaxseed meal, Bl: *Bifidobacterium longum*. Mean columns with different letters are significantly different ($p<0.05$) using Tukey's studentized range test

Treatment groups fed diets consisting of the various combinations of SF+FM only, exhibited a reduction rate of 59.07-64.50% when compared to the control group. Rats fed treatments consisting of the combination SF+FM+Bl. at all levels experienced the highest percentage reduction, ranging from 73.12-79.58% when compared to the control.

Hepatic detoxification and antioxidant enzyme activity: Analysis of hepatic catalase activity (U mL^{-1}) showed no significant difference between Bl. singly and the combination treatments, however the control measured significantly lower activity than all treatment groups (Fig. 2). The

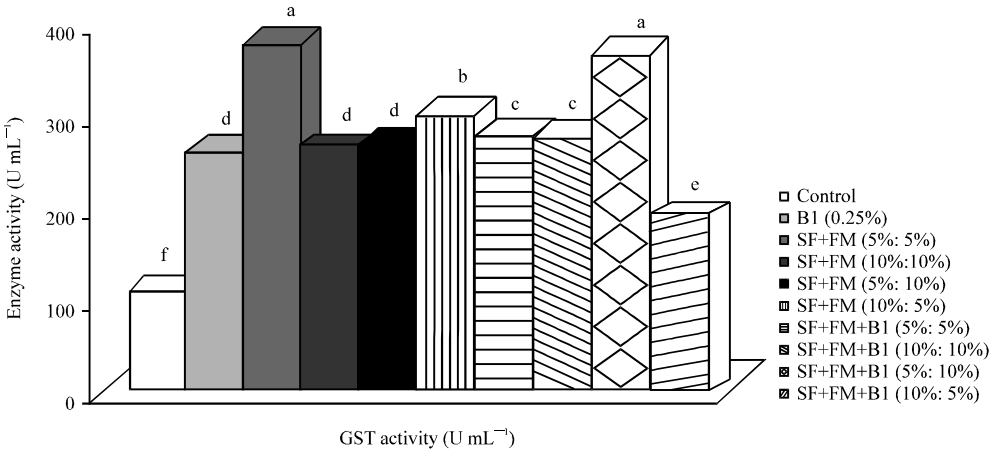

Fig. 3: Effect of soy flour, flaxseed meal and a probiotic on hepatic superoxide dismutase enzyme activity, SF: Soy flour, FM: Flaxseed meal, Bl: *Bifidobacterium longum*. Mean columns with different letters are significantly different ($p<0.05$) using Tukey's studentized range test

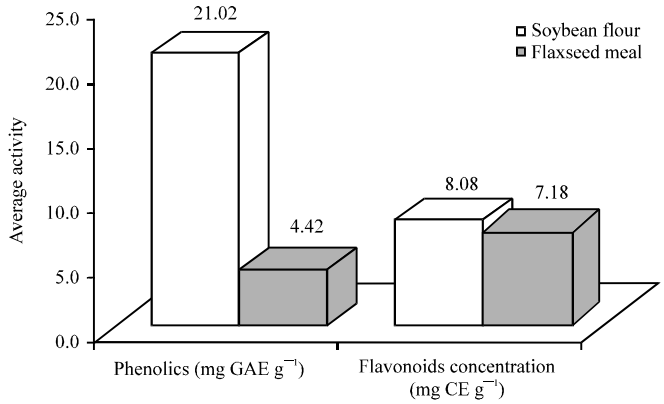

Fig. 4: Total phenolic and flavonoid content of soybean flour and flaxseed meal

Glutathione-S-Transferase (GST) level (U mL^{-1}) of the rats fed treatment diets were in all cases at least 2x that of the control with greatest activity measured in rats fed diets consisting of SF+FM+Bl. (10%: 10%) at 2.28. Similarly, superoxide dismutase (SOD) levels (U mL^{-1}) (Fig. 3) in the control were also significantly lower than the treatment groups, with all treatment diets being increased by 2-3 folds compared to the rats fed control.

Total phenolic and flavonoid content: The phenolic and flavonoid content of soy flour and flaxseed meal is given in Fig. 4. Soybean flour (21.02 mg GAE/g) had a higher phenolic content compared to the flaxseed meal (4.42 mg GAE/g). The soybean flour (8.08 mg CE/g) had a higher flavonoid content compared to the flaxseed meal (7.18 mg CE/g) (Fig. 4).

Antioxidant potential and correlation of phenolic and flavonoid content with antioxidant potential: The antioxidant power of the soybean flour and flaxseed meal was determined using the Ferric Reducing Antioxidant Potential (FRAP) and free radical oxygen

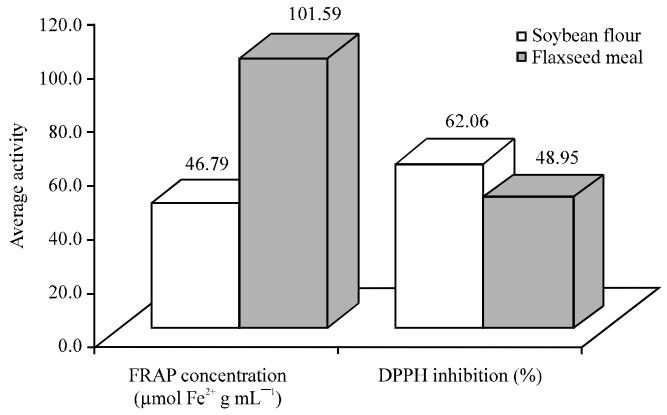

Fig. 5: Ferric Reducing Antioxidant Potential (FRAP) and free radical oxygen scavenging potential (DPPH) of soybean flour and flaxseed meal

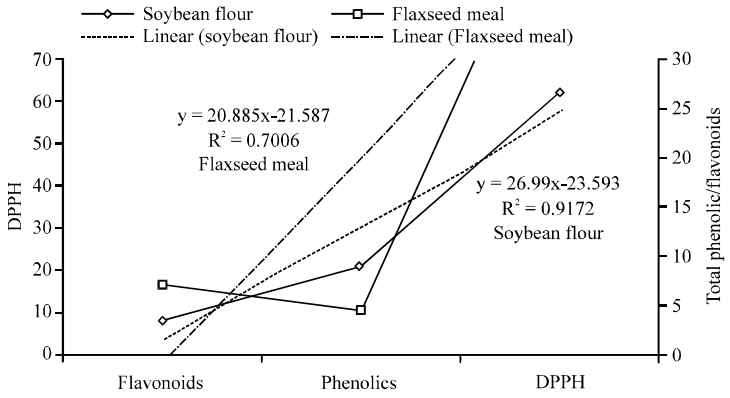

Fig. 6: Correlation of phenolics and flavonoids with antioxidant potential via DPPH in soybean flour and flaxseed meal

scavenging potential (DPPH) methods (Fig. 5). The antioxidant potential of the flaxseed meal and soybean flour may have played a role in reducing the ACF numbers and increasing the detoxification and antioxidant enzyme activity in the treatment groups. Flaxseed meal (101.59 μmol Fe^{2+} g mL^{-1}) had a higher FRAP value compared to soybean flour (46.79 μmol Fe^{2+} g mL^{-1}). Soybean flour (62.06%) has a higher scavenging ability (DPPH% inhibition) compared to flaxseed meal (48.95%). Overall, flaxseed meal had a higher antioxidant capacity compared to the soybean flour.

Figure 6 shows the correlation between the flavonoid and phenolic content and the antioxidant potential as measured by DPPH in soybean and flaxseed meal. The correlation for the phytochemicals measured was higher (R^2 = 0.9172) for soybean flour compared to flaxseed meal (R^2 = 0.7006). The phenolic and flavonoid content resulted in a higher DPPH activity for the flour tested. Figure 7 shows the correlation between the flavonoid and phenolic content and the antioxidant potential as measured by FRAP in soybean flour and flaxseed meal. The correlation

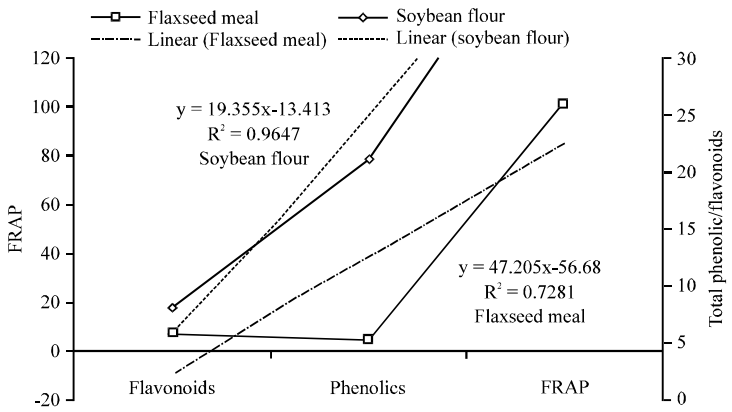

Fig. 7: Correlation of phenolics and flavonoids with antioxidant potential via FRAP in soybean flour and flaxseed meal

for the phenolic and flavonoid compounds with the FRAP measured was higher in soybean flour ($R^2 = 0.9647$) compared to flaxseed meal ($R^2 = 0.7281$). The phenolic and flavonoid content resulted in a higher FRAP activity for flour/meal tested.

DISCUSSION

Research has been widely conducted on various pure compounds such as isoflavones (Yu *et al.*, 2004) and various antioxidants in relation to their beneficial effects against the onset of certain diseases. Although, this relationship is necessary for understanding the effects of individual bioactive components, it does not gauge the ability of the food matrix to provide the same benefits. The interest of this study was to determine potential inhibitory effects of diets inclusive of soybean flour, flaxseed meal and *Bifidobacterium longum* against the formation of precancerous lesions in the colon. A secondary objective included identifying the potential synbiotic relationship associated with the inclusion of *Bifidobacterium longum* in the diets. The final objective included determining the diets' effects on important hepatic detoxification enzymes levels as a mechanism against ACF formation. Dietary supplementation of the treatment diets were administered prior and subsequently to the injections of AOM. This design suggests that the results will indicate a relationship between soy flour, flaxseed meal and *Bifidobacterium longum* and the prevention of the initiation stage of colon cancer.

The daily feed intake of the treatment groups when compared to the control were in most cases not significantly different except in the case of the Bl singly, SF+FM (5%: 5%) and SF+FM+Bl (10%: 10%). This however did not lead to significant differences in the weight gain over the 13 weeks. Likewise, no significant differences were seen in the cecal wall weight or cecal pH. Increased cecal wall weight is often attributed to hyperproliferation of the cecum cells while a decreased cecal pH is caused by an increase in production of beneficial Short Chain Fatty Acids (SCFA) from increased consumption of dietary fibers. In similar studies utilizing prebiotics and soybean meal (Gourineni *et al.*, 2011a), significant differences were found in weight gain, cecal wall weight and cecal pH of the control and treatment groups. Differences were attributed to the production of SCFAs that ultimately lead to the reduction of VLDL by decreased lipogenesis in the liver. The present results suggest that limiting the growth of pathogenic bacteria through decreased

pH may not be the primary mechanism for eliminating ACF formation. Other possible mechanism for action against colon cancer includes anti-genotoxicity (Pool-Zobel *et al.*, 1996), inhibition of colonic enzyme activity (Reddy, 1999) and immune system stimulation (Matsuzaki and Chin, 2000). One study suggests a possible mechanism of action against carcinogenesis as decreased levels of ornithine decarboxylase activity which may inhibit the ability of the colonic mucosal cells to proliferate (Reddy, 1999).

In this study, the ACF incidence was lowest among the treatment groups incorporating *Bifidobacterium longum* singly and in the various combinations of soybean flour, flaxseed meal and *Bifidobacterium longum*. Interestingly the groups Bl singly, SF+FM+Bl (5%: 10%) and (10%: 5%) had similar results, indicating that there was lower synergistic effects from the combination at the 0.25% usage level. In contrast, a study utilizing inulin and *B. longum* at 5 and 1.7%, respectively showed significant synergies against the control for ACF formation (Rowland *et al.*, 1998). This suggests that the usage level in the current study may not have been adequate to see a significant and compounding difference in the amount of ACF formed. As with the results of the current study, another study concluded that feeding flaxseed meal at 10 and 20% resulted in a reduced incidence of ACF when compared to the control (Williams *et al.*, 2007). Total crypt formation is often used as an indicator of chemopreventive potential of various bioactive components in foods. In a related study, treatment groups utilizing soybean meal at 5% and a combination of Synergy1® (10%)+soybean meal (5%) exhibited total crypt reductions compared to control of 49.54 and 77.70%, respectively (Gourineni *et al.*, 2011b). The present study showed similar results, with the lowest reductions seen in the treatment groups fed various combinations of SF+FM+Bl. In contrast to the enumeration of ACFs there may be more synergistic effects that exist in the addition of Bl with SF+FM, allowing greater anti-proliferative effects.

Glutathione-S-Transferase (GST) is an important phase II enzyme responsible for the detoxification of carcinogens, thus increased activity of this enzyme has been seen as beneficial in cancer prevention (Hayes and Pulford, 1995). The current study shows elevated hepatic GST activity among all of the treatment groups when compared to the control suggesting that this may be a potential mechanism against carcinogenesis. Although not compounding, synergistic effects of the rats fed the combination of all 3 ingredients were seen in the groups fed (10%: 10%), (5%: 10%) and (10%: 10%). As mentioned previously, flaxseed is a major source of lignans, specifically secoisolariciresinol diglycoside (SDG) which is known to have antioxidative effects by scavenging ROS (Bassett *et al.*, 2009). Superoxide dismutase (SOD) and catalase (CAT) enzyme activity were also determined to be significantly higher in the treatment groups than the control. This may be due to the increased levels of antioxidants found in the treatment diets. In a previous feeding study conducted, similar results were achieved in increasing the hepatic antioxidant enzyme status of rats fed flaxseed or its lignans (Yuan *et al.*, 1999). Another study by Yeh and Yen (2006) using Sprague-Dawley rats indicated the administration of various phenolic compounds increased the induction of specific hepatic enzyme activities, i.e., SOD, catalase and GPx (Yeh and Yen, 2006).

Soy flour, flaxseed and *B. longum* each have various mechanisms for potential chemopreventive effects. Soy specifically has proven to have antiproliferative properties such as inhibition of tyrosine kinase and induction of differentiation and apoptosis (Setchell, 1998). When combined with other ingredients such as flaxseed and a probiotic, the expectation is that the various mechanisms of action for chemopreventive potential will work synergistically to provide a dramatic effect against the onset of colon cancer. Although results displayed significant differences in the treatments when compared to the control, only minor differences were exhibited when comparing the treatments utilizing Bl. and SF+FM+Bl.

CONCLUSION

In summary, there is a necessity for increased research to be conducted regarding the relationship between dietary consumption patterns and disease. The level and action of the metabolism of various bioactive compounds may differ based on the source of the component. Soy flour and flaxseed meal contain phenolic compounds that are essential in up-regulating detoxification enzymes. The results of the current study suggest that various combinations of soy and flax, *Bifidobacterium longum* singly and soy, flax and *Bifidobacterium longum* have antiproliferative and antioxidative properties. The incorporation of these ingredients significantly decreased the development of ACF and also reduced total crypts. There is no published research that defines the synergistic effects of soy flour, flaxseed meal and *Bifidobacterium longum*. The results of this study prove that synergies exist through the combination of the ingredients in diets. Dietary ingredients such as flaxseed meal and soybean flour have been used individually in food products for their health benefits. Thus, in the food industry, development of food products with combinations of ingredients with functional and synergistic effects is always critical. This study established that although not compounding, there is a synergistic relationship with the inclusion of probiotics in diets rich in soy and flaxseed.

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