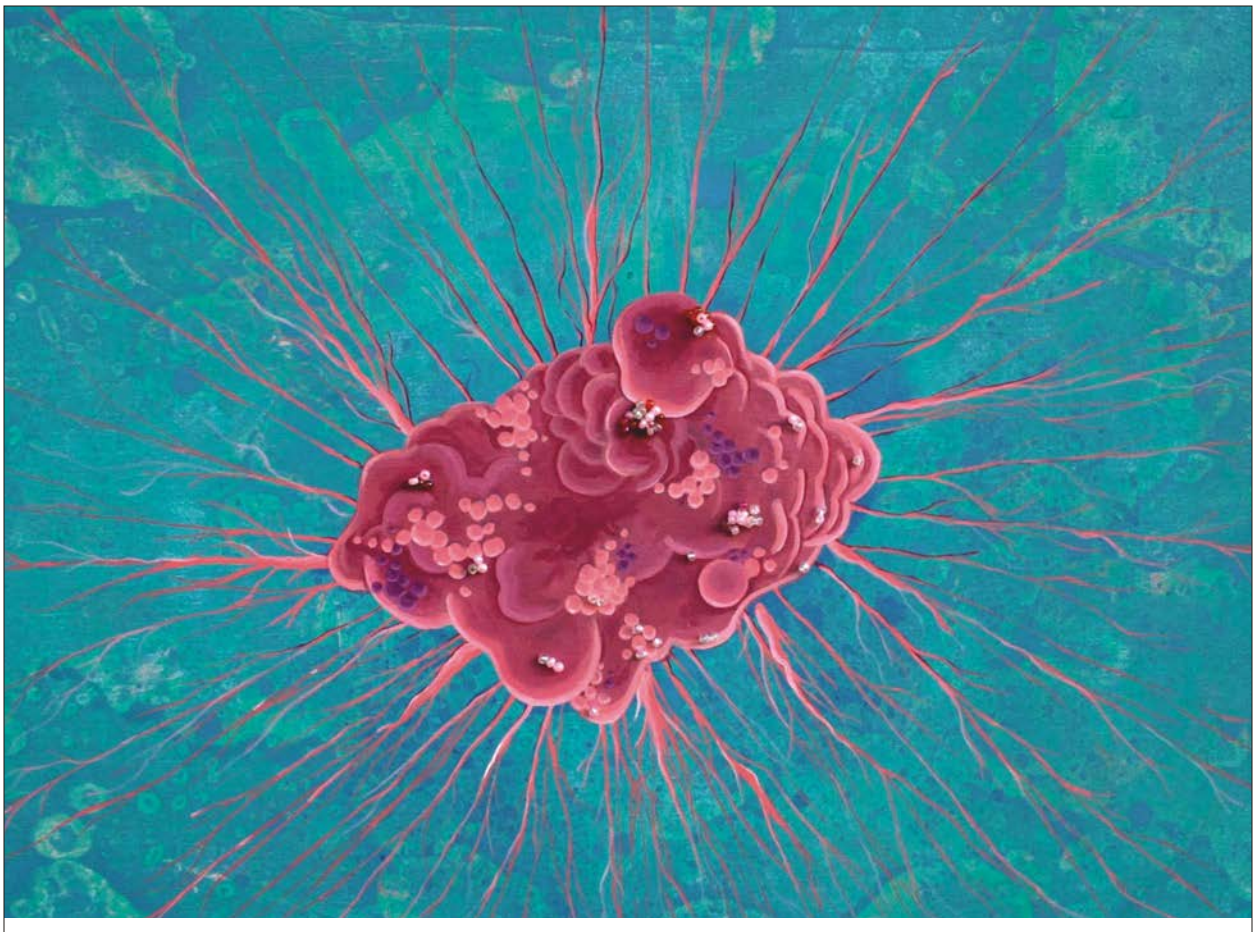

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Feeding Wheat Germ Meal and Wheat Germ Oil Reduced Azoxymethane-Induced Aberrant Crypt Foci in Fisher 344 Male Rats

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Abstract: The aim of present study was to determine the effects of feeding Wheat Germ Meal (WGM) at 5 and 10% and Wheat Germ Oil (WGO) at 7% (normal fat) and 14% (high fat) on azoxymethane (AOM) induced aberrant crypt foci in Fisher 344 male rats. Following a 1 week period of acclimatization, rats were assigned to 6 groups and fed American Institute of Nutrition 93-Growth diet (AIN-93G) with 7% (C1) and 14% (C2) soybean oil (SBO), AIN-93G diet with 7 and 14% WGO (instead of SBO) and Wheat Germ Meal (WGM) C1+ 5 and 10% WGM. At 7 and 8 week of age all rats received subcutaneous injections of azoxymethane (AOM) at 16 mg kg^{-1} body weight and were killed at 17 week of age by CO_2 asphyxiation. AOM-induced Aberrant Crypt Foci (ACF) were counted. Results showed that ACF formation in distal colons in rats fed 7 SBO, 14 SBO, 7 WGO and 14% WGO (Means±SEM) were 99.2±15.9, 126.0±17.0, 68.2±0.4 and 79.7±1.9 respectively. In rats fed 5% WGM and 10% WGM the number of ACF were 23.75±1.11, 18.6±1.0, 75.2±2.39 and 49.6±2.2 in proximal and distal colon. Total ACF reductions (%) compared to control in rats fed 5% WGM and 10% WGM were 25% and 47%, respectively. Glutathione S-transferase (GST) activities were significantly ($p<0.05$) higher in treatment groups (5 and 10% WGM) compared to control (7 and 14% SBO). These results indicated that wheat germ and wheat germ oil significantly ($p< 0.05$) reduced AOM induced ACF. Regular consumption of wheat germ and wheat germ oil may have health implications.

Key words: Soybean oil, wheat germ meal, aberrant crypt foci, glutathione s-transferase

INTRODUCTION

Wheat is an important source of vitamins, minerals, dietary fiber and phytochemicals. The oil is a rich source of topherols and tocotrienols. The germ is the most nutritious portion of the wheat kernel and it makes up about 2.5% of the weight. During the milling process the germ is separated from the bran and starch. Wheat germ is a rich source of B complex vitamins, with wheat germ oil being the richest source of tocopherols. These nutrients and phytochemicals may have significant implications in chemoprevention (Liu, 2007; Jensen *et al*., 2004).

The American Cancer Society estimates that there will be about 153,760 new cases of colorectal cancer diagnosed in 2007 in the United States which makes it the second leading cause of death after heart disease. It is also estimated that about 52,180 deaths will have resulted due to colorectal cancer, making it the 3rd most common cause of cancer deaths (Levin *et al*., 2008). Colorectal cancer development is a slow multi step process that is initiated by mutations in the DNA, such as *K-ras*, *p53* and *APC* gene, resulting in aberrations in colonocytes, know as ACF (aberrant crypt foci) (Stevens *et al*., 2007).

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Several modifiable and non modifiable factors have been implicated with increased risk of developing colorectal cancer which include, smoking, hereditary, physical activity and diet (Emmons *et al*., 2005). Of these modifiable factors researchers have turned their focus to diet and its implication in colon cancer development. New developments in nutrogenomic research are now able to identify and develop biomarkers that may assist in the prevention of cancer through dietary intervention (Williams *et al*., 2007).

Whole grains, such as wheat germ are rich sources of dietary fiber, vitamins, minerals and phytochemicals including phenolics, carotenoids, vitamin E, lignans, β-glycan, inulin, resistant starch, sterols and phytates (Liu, 2007; Jensen *et al*., 2004). Plant based foods contain significant amounts of bioactive phytochemicals, which when consumed together may have a synergistic affect that goes beyond the basic individual function of each single component in combating diseases (Liu, 2007).

Despite efforts made by Burkitt (1971), the exact role of dietary fiber on colon cancer is yet to be completely understood. In regions of Africa, in natives who consumed a large amount of fiber, colon cancer prevalence was low (Negri *et al*., 1998). It is suggested that high fiber intake may reduce the risk of colon cancer by decreasing fecal transit time thereby decreasing the time carcinogens are in contact with the colonic epithelium, increasing cecal weight by increased cell differentiation, increased production of Short Chain Fatty Acids (SCFA) such as butyrate which act as a fuel to the colonic cells and by decreasing colonic pH which reduces pathogenic bacteria (Williams *et al*., 2007; Boateng *et al*., 2007; Michels *et al*., 2005).

The germ is the most nutritious portion of the wheat kernel and it makes up about 2.5% of the weight. During the milling process the germ is separated from the bran and starch. Wheat germ is a rich source of B complex vitamins, with wheat germ oil being the richest source of tocopherols.

When incorporated into the diet wheat bran was shown to be more effective in lowering biomarkers such as fecal mutagens and secondary bile acids, compared to corn and oat bran (Reddy *et al*., 2000).

Glutathione S-transferases (GST) are phase II multifunctional detoxification enzymes that catalyze the conjugation between glutathione and electrophilic xenobiotics such as carcinogens (AOM) and anticancer drugs with sulfhydryl moiety of glutathione (Laffon *et al*., 2003). This conjugation between glutathione and the reactive species produce less toxic water soluble compounds that are then able to be excreted via the urine (Zhang *et al*., 2003).

Single dietary components found in plant foods are not exclusively responsible for the health benefits of foods rather; dietary plant compounds have a synergistic effect on disease (Geber, 2001). This notion that food synergies play a key role in disease prevention is continuously being evaluated. Food synergy has been defined as additive or more than additive influences of foods and food constituents on health (Jacobs and Steffen, 2003).

A major risk factor contributing to the onset of colon cancer is the consumption of a high fat diet (Oba *et al*., 2006; Wong *et al*., 2004). According to Oba *et al*. (2006), consuming a diet high in animal fat increases an individuals risk for cancer over a period of time. Consumption of fat promotes the increased body weight gain which normally results in a more sedentary lifestyle (Klurfeld and Bull, 1997). As of 2006, 37.7% of American adults are overweight, with 22.1% being obese with about 24.4% reporting little or no physical activity.

Recent studies have shown that a fiber rich diet reduces or causes a delay in fat digestion, impedes the absorption of cholesterol and fat in the intestine, reduces cholesterol synthesis by volatile fatty acids produced during fermentation and alters lipoprotein metabolism (Cara *et al*., 1992). A study conducted by Boateng *et al*. (2007) concluded that dietary fat, depending on the source, quantity, fatty acid composition may have implications in the incidence of colon cancer. Hence it would be beneficial to determine the chemopreventive effects of wheat germ oil at 7% which represents a normal fat diet and 14% representing a high fat diet and wheat germ meal both containing substantial bioactive

components that have shown to play a significant role in colon carcinogenesis. Therefore, the overall goal of this project is to study the chemopreventive potential of wheat germ. The specific objectives of the study were; to determine the effect of wheat germ (5 and 10%) and wheat germ oil (7 and 14%) on azoxymethane-induced aberrant crypt foci in Fisher 344 male weanling rats and to determine the effect of feeding wheat germ (5 and 10%) and wheat germ oil (7 and 14%) on liver and colonic Gluthathione S-Transferase (GST) activity.

MATERIALS AND METHODS

Chemicals and Dietary Ingredient

All biochemicals excluding Azoxymethane were obtained from Sigma Chemical Company (St. Louis, MO). Dietary ingredients were obtained from MP Biomedicals (Costa Mesa, CA.).

Animals and Housing

Male Fisher 344 weanling rats were obtained from Harlan, IN and housed in stainless steel wire cages at two rats per cage. Beginning at four weeks of age (January, 2006) the rats were divided into six (6) groups (n = 6) and were assigned to 6 dietary treatments: 1) Control diets (7 and 14% SBO) and four treatment diets consisting of wheat germ (5 and 10%) and wheat germ oil (7 and 14%). The temperature and relative humidity were maintained at 21°C and 50%, respectively. Light and dark cycles were 12 h each. Feed and water were provided *ad libitum*. All diets were prepared at intervals of four weeks or less and stored at refrigeration temperature (4°C). Daily feed intake and weekly body weights were recorded. All protocols were approved by the Institutional Animal Care and Use Committee of Alabama A and M University, Normal AL 35762.

Carcinogen Injection

For induction of colonic aberrant crypt foci all animals received two subcutaneous injections of azoxymethane (AOM) (NCI Chemical Repository, Kansas City MO) in saline @ 16 mg kg^{-1} body wt., one dose at seven weeks and another at eight weeks of age.

Sample Collection

At 17 weeks of age rats were killed using CO_2 asphyxiation after an overnight's fast. Colons of rats were removed and flushed with potassium phosphate buffer (0.1 M, pH 7.2). Livers were excised and rinsed in PBS and frozen and stored at -80°C for glutathione s-transferase activity.

Enumeration of Aberrant Crypt Foci (ACF)

Colons were split open longitudinally and placed on a filter paper with their luminal surface open and exposed, another filter paper was placed on top of the luminal surface. The colons were fixed overnight using 10% buffered formalin. Total number of ACF and the number of crypts per focus were counted as described by Bird (1987).

Glutathione-S-Transferase Assay (GST)

GST in the liver and colonic mucosal samples was assayed by the procedure of Habig *et al.* (1974). Liver samples were homogenized in 10 volumes of potassium phosphate buffer (pH 7.0, 0.1 M) in Potter-Elvejem homogenizer (10 strokes) at 4°C. The homogenate was centrifuged at 10,000 x g for 30 min. The assay mixture (1 mL) consisted of potassium phosphate buffer (0.1 M, pH 6.5), 1, Chloro 2, 4- dinitrobenzene (1 mM) and glutathione (1 mM). Reaction was started by the addition of 50-100 μL of sample and change in absorbance at 340 nm as a function of time was monitored in a Cary dual beam spectrophotometer.

Statistical Analysis

Data are expressed as Means±SEM. Differences were tested for statistical significance using two-way ANOVA. Statistical analysis was conducted using SAS 9.0 and mean were separated using Tukey's Studentized range test. A $p \leq 0.05$ was considered to indicate significant differences.

RESULTS

Weight Gain and Feed Intake

Feed intake was significantly ($p<0.05$) lower in rats fed control soy bean oil (SBO 7%) compared to the treatment diet (Table 1). There were no significant ($p<0.05$) differences in weight gain between the C+SBO 7% and Wheat Germ Meal (WGM) 5 and 10%. Feed intake was significantly ($p<0.05$) higher in the rats fed WGM (5 and 10%) compared to the control.

Weight gain was significantly ($p<0.05$) lower in the control (SBO 7%) group compared to the Wheat Germ Oil (WGO) (7%) group. Rats fed WGO (14%) had a significantly ($p<0.05$) higher weight gain compared to the control (SBO 14%). Among the rats fed WGO (7 and14%), weight gain was significantly ($p<0.05$) higher in the rats fed the high fat diet (WGO 14%). A similar trend was observed in those rats fed SBO at 7 and 14%.

Feed intake was significantly ($p<0.05$) lower in the rats fed SBO (7%) compared to the rats fed WGO (7%). Daily feed intake in the rats fed WGO (14%) was significantly ($p<0.05$) higher than the rats fed the control (SBO 14%) diet. There were no significant ($p>0.05$) differences seen in feed intake between rats fed WGO (7 and 14%), although rats fed WGO (14%) were seen to consume more feed compared to the WGO (7%) group (Table 2).

Cecal Weight and Cecal pH

Cecal weight (g) in the rats ranged from a high of 2.24 in WGM (10%) to a low of 1.14 in WGM (5%). Cecal weight was significantly ($p<0.05$) lower in the control group compared to the WGM (10%) fed group. The rats fed WGM (10%) had a significantly ($p<0.05$) lower cecal pH compared to the rats fed WGM (5%) and control diets. There were no significant ($p>0.05$) differences in cecal weight and pH between the rats fed normal and high fat (7 and 14%) soybean oil (SBO) and wheat germ oil diet (Table 2).

Aberrant Crypt Foci

The number of Aberrant Crypt Foci (ACF) in the proximal colon was significantly ($p<0.05$) lower than the distal colon. Total ACF was significantly ($p<0.05$) higher in the control compared to the WGM (5 and 10%) groups. Among the rats fed WGM, total ACF was significantly ($p<0.05$) lower in the rats fed WGM (10%) compared to the group fed WGM (5%). There were over 25% and 50% reductions in ACF in rats fed WGM (5 and 10%) compared to the control fed group (Table 3).

Table 1: Weight gain and feed intakes, cecal weight and cecal pH in Fisher 344 male rats

Groups	Weight gain (g)	Feed intake (g)	Cecal weight (g)	Cecal pH
Control (SBO 7%)	207.4±2.5^{a}	12.6±0.7^{b}	1.5±0.1^{b}	7.5±1.2^{b}
C+WGM (5%)	203.6±17.4^{a}	17.5±1.1^{a}	1.1±0.1^{b}	7.0±0.1^{b}
C+WGM (10%)	210.8±4.2^{a}	18.3±2.4^{a}	2.2±1.0^{a}	6.4±1.3^{a}

Values are Mean±SEM, (n = 6), ^{ab} Means within columns without common letter(s) differ ($p<0.05$) (Tukey's studentized range test), Abbreviations: SBO = Soybean oil; WGM = Wheat germ meal

Table 2: Weight gain and feed intakes, cecal weight and cecal pH in Fisher 344 male rats

Groups	Weight gain (g)	Feed intake (g)	Cecal weight (g)	Cecal pH
Control (SBO 7%)	207.4±2.5^{d}	12.6±0.7^{b}	1.5±0.1	7.5±1.2
Control (SBO 14%)	253.4±5.1^{b}	13.1±0.9^{b}	1.6±0.1	7.8±1.4
C+WGO (7%)	233.6±19.6^{c}	19.8±0.0^{a}	1.1±0.1	7.5±0.5
C+WGO (14%)	270.3±18.2^{a}	22.3±2.0^{a}	1.2±0.1	7.6±0.1

Values are Mean±SEM, (n = 6), ^{abcd} Means within columns without common letter(s) differ ($p<0.05$) (Tukey's studentized range test), Abbreviations: SBO = Soybean oil; WGO = Wheat germ oil

The incidence of ACF in the distal colon in the WGO (7%) fed rats was about 50% lower compared to the SBO (14%) group. Total ACF was significantly ($p<0.05$) higher in the high fat (14%) fed groups compared to their normal fat (7%) counterparts. The highest ACF numbers were seen in the SBO (14%) group, whereas the lowest incidence of ACF was seen in the rats fed WGO (7%). Increasing fat from 7 to 14% resulted in an increased incidence of total ACF in the SBO and WGO group by 20% and 19%, respectively. The number of ACF in the WGO (7%) group was 29% lower than the SBO (7%) group. ACF numbers in the WGO (14%) fed group was 29.4% lower than its SBO (14%) counterpart. Feeding WGO at both 7 and 14% levels did offer some protection against ACF induction in Fisher 344 male rats (Table 4) compared to feeding diet with SBO (normal and high fat) diet.

Number of Crypts/Focus or Crypt Multiplicity

The rats fed the control diet had significantly ($p<0.05$) higher number of ACF containing 3, 4 and 5 crypts/foci compared to rats fed WGM (5 and 10%) (Fig. 1) and WGO (7 and 14%) (Fig. 2). Feeding WGM at 5 and 10% and WGO at 7 and 14% significantly ($p<0.05$) reduced ACF with 3, 4 and 5 crypts which is an important factor since those ACF are more likely to develop into tumors over time. The number of ACF with 1 and 2 crypts/focus were similar in rats fed control and WGM (10%). There were no significant ($p>0.05$) differences in the ACF with 4 and >5 crypts/focus in the rats fed WGM (5 and 10%). There were no significant differences in ACF with 1, 2, 3, 4 and 5 crypts between rats fed WGO (7 and 14%) diets. The number of ACF with higher number of crypts were significantly ($p<0.05$) lower in rats fed wheat germ products which is significant as those with ACF >3 crypt/foci are more likely to become tumors over time, although the ACF with <3 crypts/foci may dissolve over time and disappear.

Table 3: Wheat germ meal on aberrant crypt foci in Fisher 344 male rats

Groups	Proximal	Distal	Total
Control (SBO 7%)	$43.7\pm13.9^{a,c}$	$99.2\pm15.9^{a,d}$	143^{a}
C+WGM (5%)	$23.7\pm1.1^{b,c}$	$75.2\pm2.3^{b,d}$	96^{b}
C+WGM (10%)	$18.6\pm1.0^{b,c}$	$49.6\pm2.2^{c,d}$	68^{c}

Values are Means±SEM (n = 6), [abc]Means within columns without common letters differ ($p<0.05$) (Tukey's studentized range test), [dc]Means within rows without common letters differ ($p<0.05$), Abbreviations: SBO = Soybean oil; WGM = Wheat germ meal

Table 4: Wheat germ oil on aberrant crypt foci in Fisher male 344 rats

Groups	Proximal	Distal	Total
Control (SBO 7%)	43.7 ± 13.9^{b}	99.2 ± 15.9^{b}	143.0^{b}
Control (SBO 14%)	53.0 ± 10.8^{a}	126.0 ± 17.0^{a}	178.0^{a}
C+WGO (7%)	33.3 ± 1.3^{c}	68.2 ± 0.4^{c}	101.5^{d}
C+WGO (14%)	45.7 ± 1.6^{ab}	79.7 ± 1.9^{d}	125.5^{c}

Values are Mean±SEM, (n = 6), [abcd]Means in a column without common letters differ ($p<0.05$) (Tukey's studentized range test), Abbreviations: SBO = Soybean oil; WGO = Wheat germ oil

Table 5: Hepatic Glutathione S-Transferase (GST) activity in Fisher 344 male rats

Groups	GST activity (μmol mg^{-1})
Control SBO (7%)	14.6 ± 1.4^{b}
Control (SBO 14%)	12.0 ± 1.0^{c}
C+WGM (5%)	29.8 ± 1.9^{a}
C+WGM (10%)	32.2 ± 2.2^{a}
C+WGO (7%)	28.9 ± 0.5^{b}
C+WGO (14%)	34.0 ± 2.7^{a}

Values are Means±SEM, (n = 6), [abc]Means within the columns with different letters are significantly, different ($p<0.05$) (Tukey's studentized range test), Abbreviations: SBO = Soybean oil; WGM = Wheat germ meal WGO = Wheat germ oil

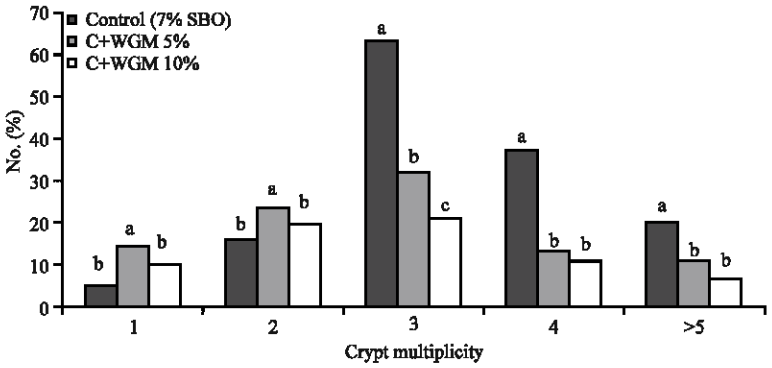

Fig. 1: Crypt multiplicity in rats fed wheat germ meal, [abc]Bars with different superscripts are significantly different ($p<0.05$) using Tukey's studentized range test, Abbreviations: SBO = Soybean oil; WGM = Wheat germ meal

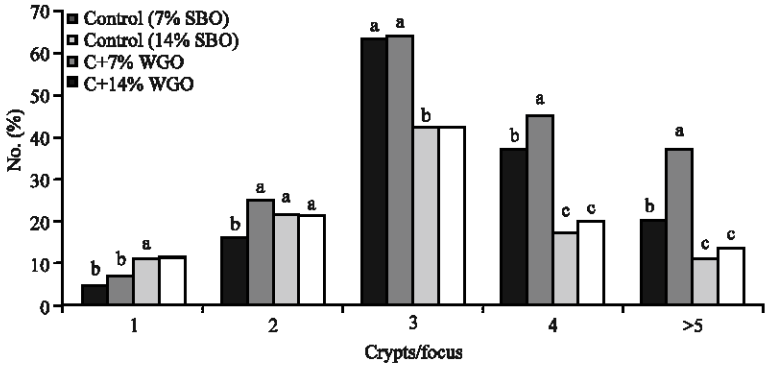

Fig. 2: Crypt multiplicity in rats fed wheat germ oil, [abc]Bars with different superscripts are significantly different ($p<0.05$) using Tukey's studentized range test, Abbreviations: SBO = Soybean oil; WGO = Wheat germ oil

Glutathione S-transferase (GST) Activity

There were no significant differences in GST activity (μmol mg^{-1}) among the rats fed WGM. GST activity was significantly ($p<0.05$) higher in rats fed WGM (5 and 10%) compared to rats fed control (SBO 7%). The GST activity (μmol mg^{-1}) was 50% lower in control rats compared to WGM (5 and 10%) fed rats. GST activity (μmol mg^{-1}) was significantly ($p<0.05$) lower in the control (7 and 14% SBO) groups compared to WGO (7 and 14%) fed groups. GST activity (μmol mg^{-1}) was lower in the control high fat fed group (SBO 14%) compared to the normal fat control (SBO 7%) fed group, although rats fed WGO (7%) had a significantly ($p<0.05$) lower GST activity compared to WGO (14%). GST activity was significantly ($p<0.05$) lower in the SBO (7 and 14%) fed groups compared to the rats fed WGO (7%). GST activity was also significantly ($p<0.05$) higher in rats fed WGO (14%) compared to rats fed SBO (7% and 14%). GST activity was 15% higher in the WGO (14%) compared to its low fat counterpart (WGO 7%). The rats fed WGO (7%) had a 49% higher GST activity compared to its SBO counterpart. GST activity was 64% in WGO (14%) fed rats compared to its SBO (14%) counterpart (Table 5).

DISCUSSION

The aim of this study was to determine the effects of feeding wheat germ, which is a rich source of fiber (Takahashi *et al*., 1999), in AOM induced colon carcininogenesis in Fisher 344 rats.

The results showed that the weight gain in the rats fed the WGM diet was not significantly higher compared to the rats fed the control di*et al*though rats fed the WGM (10%) diet did consume significantly ($p<0.05$) higher amounts of feed. Similar trends were seen in studies by Dongowski *et al*. (2002) where rats fed a fiber rich diet consumed more than the control fed rats but there were no significant differences ($p>0.05$) seen in weight gain. Fiber has been suggested to affect weight gain through insulin sensitivity, greater satiety sensation, blunting prostprandial glycemic and insulinemic responses in the small intestine (Rastrollo *et al*., 2006).

Rats fed the WGM diet (10%) had a lower cecal weight and cecal pH compared to the control fed rats. Cereal fiber added to the diet resulted in similar effects on the cecal weight and pH (Bird, 1995). Short Chain Fatty Acids (SCFA) such as acetate, propionate and butyrate and organic acids are end products of bacterial fermentation in the colon (Lupton, 2004). The decrease in cecal pH observed may be due to the ability of SCFA to inhibit dehydroxylation of bile acids thereby causing a decrease in colonic pH (Grasten *et al*., 2000). A study where barley products was fed, reported the ability of fiber to cause a significant increase in cecal weight (Dongowski *et al*., 2002). Rats fed WGO had similar cecal weight and pH compared to the control fed rats.

Aberrant crypt foci are good predictors of tumor outcome because they can be detected in the early stages of carcinogenesis (Ishizuka *et al*., 2003). There were 25 and 50% reductions in ACF in rats fed WGM (5 and 10%) compared to the rats fed the control diet. Total crypts were also significantly ($p<0.05$) lower in the rats fed the treatment diets (WGM 5 and 10%) compared to control diet (SBO 7%). Present results are consistent with a study conducted by Ferguson and Harris (1998) in which they saw a 33% reduction in ACF in rats fed a 5% wheat bran diet. Similar findings were also seen in research reported by Ishizuka *et al*. (2003) where they saw a significant ($p<0.05$) reduction in ACF in rats fed a polydextrose diet compared to rats fed a fiber free diet.

One of the proposed mechanisms by which wheat germ may decrease ACF formation may be due to a decrease in mean retention time (MRT). In the colon, fiber has the ability to bind water and increase fecal bulk therefore resulting in an increase in fecal viscosity and decrease in MRT (Wenk, 2001). In a diet where wheat bran accounted for 35.8% of the total dietary fiber, a decrease in transit time by three hours was seen (Ferguson and Harris, 1998). A decrease in MRT decreases the time that carcinogens and other toxins are exposed to the colonocytes thereby decreasing ACF formation and the risk of developing colon cancer.

Wheat germ is a good source of vitamin E which has been shown to offer protection to cells from carcinogens through synergistic interactions with inhibitors of prostaglandin synthesis (Weitberg, 1987).

Diet studies conducted on humans using wheat bran have shown that fiber supplementation increases the fecal bulk thereby diluting potential carcinogens and tumor promoters in the colon which causes a decrease in formation of colonic mutagens and secondary bile acids. Another mechanism proposed was that being water soluble, fiber has the ability to delay starch absorption, which causes a reduction in the glycemic load and postprandial hyperinsulinemia (Reddy *et al*., 1997).

Feeding WGO at 7 and 14% did significantly ($p<0.05$) reduce the number of ACF in Fisher 344 male rats compared to those rats fed SBO at 7 and 14% (Table 3). Results from this study are comparable to other studies conducted in our laboratory where red palm oil (7 and 14%) (Boateng *et al*., 2007) and flax seed oil (7 and 14%) (Williams *et al*., 2007) which are also rich sources of carotenoids, vitamin E and omega 3 fatty acids and other phytonutrients, significantly ($p<0.05$) reduced the number of ACF and total crypts in Fisher 344 male rats. The mechanisms by which

vitamin E may have reduced the number of ACF and total crypts maybe by neutralizing reactive oxygen species and other free radicals that may cause DNA damage (Jacobs *et al*., 2001) and also by possibly inhibiting Cox-2 activity (O'Leary *et al*., 2004).

Research has shown that crypts with≥3 crypt/foci have a greater propensity to develop into tumors whereas ACF with < 3 crypts/foci may disappear or dissolve over time (Bird, 1987, 1995; Boateng *et al*., 2007; Shirtliff and Bird, 1996). As the number of crypts/foci increase, there is an increase in tumor incidence, with ACF containing≥3 crypts/foci correlating to a >50% tumor incidence and <3 crypts/foci correlating to <30% tumor incidence (Alabaster *et al*., 1996).

Production of SCFA such as butyrate may be attributed to the induction of apoptosis leading to the reduction in ACF with >3 crypts/foci. In this study we saw a decrease in ACF containing≥2 crypts/foci in the rats fed the wheat germ diet compared to the control. Fermentation of dietary fiber leads to an increased production of butyric acid. Studies have shown that butyric acid has the ability to stimulate apoptosis and increase differentiation in colonic cells (Pirman *et al*., 2007). Consumption of Vitamin E rich wheat germ oil significantly reduced ACF's with >3 crypts/foci. One of the mechanism by which consumption of WGO may have been able to reduce ACF with >3 crypt/foci may have been due to the vitamin E induced apoptosis, which has been reported by several authors (Boateng *et al*., 2007; Ramanathan *et al*., 2005)

In this study GST (μmol mg^{-1}) activity ranged from a low of 14.62 in control to a high of 32.29 in the rats fed WGM 10% (Table 5). An inverse relationship between GST activity (μmoL mg^{-1}) and the number of ACF was seen, possibly linking GST activity as one of the possible mechanisms in reducing colonic ACF. A similar trend was also reported by Williams *et al*. (2007) and Boateng *et al*. (2007), where a decrease in ACF in rats fed flaxseed oil and flaxseed meal and selected fruits, was also related with an increase in GST activity (μmoL mg^{-1}). An induction in GST increases the conjugation of glutathione with carcinogens such as AOM and ROS produced leading to their excretion.

The results of this study suggest that dietary Wheat Germ Meal (WGM) and Wheat Germ Oil (WGO) suppressed Azoxymethane (AOM) induced ACF in Fisher 344 male rats. Although dietary fat is known to be a tumor promoter, the type of fat may be as important as the amount. Wheat germ oil, a significant source of phytochemicals such as Vitamin E may have played a beneficial role in reducing the tumor-promoting effects of fat. Long term studies are needed to further evaluate the effectiveness of wheat germ products as chemopreventive agents.

REFERENCES

Alabaster, O., Z. Tang and N. Shivapukar, 1996. Dietary fiber and chemopreventive modulation of colon carcinogenesis. Mutat. Res., 350: 185-197.

Bird, R., 1987. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett., 37: 147-151.

Bird, R., 1995. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett., 93: 55-71.

Boateng, J., M. Verghese, L. Shackelford, L.T. Walker and J. Khatiwada *et al*., 2007. Selected fruits reduce azoxymethane (AOM)-induced Aberrant Crypt Foci (ACF) in Fisher 344 male rats. Food Chem. Toxicol., 45: 725-732.

Burkitt, D.P., 1971. Epidemiology of cancer of the colon and rectum. Cancer, 28: 3-3.

Cara, L., C. Dubois, P. Borel, M. Armand and M. Senft *et al*., 1992. Effects of oat bran, rice bran, wheat fiber, and wheat germ on postprandial lipdemia in healthy adults. Am. J. Clin. Nutr., 55: 81-88.

Dongowski, G., M. Huth, E. Gerbhardt and W. Flamme, 2002. Dietary fiber-rich barley beneficially affects intestinal tract of rats. J. Nutr., 132: 3701-3714.

Emmons, K.M., C.M. Mcbride, E. Puleo, K.I. Pollak and B.H. Marcus *et al*., 2005. Prevalence and predictory of multiple behavior risk factors for colon cancers. Preventative Med., 40: 527-534.

Ferguson, L. and P. Harris, 1998. Suberized plant cell walls suppress formation of heterocyclic amine-induced aberrant crypts in a rat model. Chemico-Biol. Interact., 114: 191-209.

Geber, D. and S. Bowerman, 2001. Applying science to changing dietary patterns. J. Nutr. (Review), 131: 3078S-3081S.

Grasten, S.M., K.S. Juntunen, K.S. Poutanen, H.K. Gylling, T.A. Miettinen and H.M. Mykkanen, 2000. Rye bread improves bowel function and decreases the concentrations of some compounds that are putative colon cancer risk markers in middle-aged women and men. J. Nutr., 130: 2215-2221.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Ishizuka, S., T. Nagai and H. Hara, 2003. Reduction of aberrant crypt foci by ingestion of polydextrose in the rat colorectum. Nutr. Res., 23: 117-122.

Jacobs, E., C. Connell, P. Chao, C. Rodriguez and J. Seymour *et al*., 2001. Vitamin C and vitamin E supplementation and colon cancer mortality in a large American society cohort. Cancer Epidemol., 10: 17-23.

Jacobs, D.R. and L.M. Steffen, 2003. Nutrients, foods, and dietary patterns as exposures in research: A framework for food synergy. Am. J. Clin. Nutr., 78: 508S-513S.

Jensen, M.K., P. Koh-Banerjee, F.B. Hu, M. Franz and L. Sampson *et al*., 2004. Intake of whole grains, bran and germ and the risk of cornary heart disease in men. Am. J. Clin. Nutr., 80: 1492-1499.

Klurfeld, D.M. and A.W. Bull, 1997. Fatty acids and colon cancer in experimental models. Am. J. Clin. Nutr., 66: 1530S-1538S.

Laffon, B., B. Perez-Cadahia, E. Pasaro and J. Mendez, 2003. Effects of epoxide hydrolase and glutathione S-transferase genotypes on the induction of micronuclei and DNA damage by styrene-7, 8-oxide *in vitro*. Mutat. Res., 536: 49-59.

Levin, B., D. Lieberman, B. McFarland, K.S. Andrews and D. Brooks *et al*., 2008. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American cancer society, the us multi-society task force on colorectal cancer and the American college of radiology. Gastroenterology, 134: 1570-1595.

Liu, R.H., 2007. Whole grain phytochemical and health. J. Cereal Sci., 46: 207-219.

Lupton, J.R., 2004. Microbial degradation products influence colon cancer risk: The butyrate controversy. J. Nutr., 134: 479-482.

Michels, K., C. Fuchs, E. Giovannucci, G. Colditz, D. Hunter, M. Stampfer and W. Willett, 2005. Fiber intake and Incidence of colorectal cancer among 76,947 women and 47,279 men. Cancer Epidemiol. Biomarkers Prev., 14: 842-849.

Negri, E., S. Franceschi, M. Parpineal and C. Vecchai, 1998. Fiber intake and risk of colorectal cancer. Cancer Epidemiol. Biomarker Prev., 7: 667-671.

Oba, S., N. Shimizu, C. Nagata, H. Shimizu and M. Kametani *et al*., 2006. The relationship between the consumption of meat, fat and coffee and the risk of colon cancer: A prospective study in Japan. Cancer Lett., 244: 260-267.

O'Leary, K., S. Pascual-Tereasa, P. Needs, Y. Bao, N. O'Brien and G. Williamson, 2004. Effects of flavonoids and Vitamin E on cyclooxygenases-2 (COX-2) transcription. Mutat. Res., 551: 245-254.

Pirman, T., M.C. Ribeyre, L. Mosoni, D. Remond and M. Vrecl *et al*., 2007. Dietary pectin stimulate protein metabolism in the digestive tract. Nutrition, 23: 69-67.

Ramanathan, K., M. Anusuyadevi, S. Shila and C. Panneerselvam, 2005. Ascorbic acid and α-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicol. Lett., 156: 297-306.

Rastrollo, M., M. Martínez-González, A. Sánchez-Villegas, C. Arrillaga and J. Martínez, 2006. Association of fiber intake and fruit/vegetable consumption with weight gain in a Mediterranean population. Nutrition, 22: 540-511.

Reddy, B.S., R. Hamid and C. Rao, 1997. Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. Carcinogenesis, 18: 1371-1374.

Reddy, B.S., Y. Hirose, L.A. Cohen, B. Simi, I. Cooma and C.V. Rao, 2000. Preventive potential of wheat bran fraction against experimental colon carcinogenesis: Implications for human colon cancer prevention. Cancer Res., 60: 4792-4797.

Shirtliff, N. and R.P. Bird, 1996. Growth features of aberrant crypt foci that resist modulation by cholic acid. Carcinogenesis, 17: 2093-2096.

Stevens, R., H. Swede and D. Rosenberg, 2007. Epidemiology of colonic aberrant crypt foci: Review and analysis of existing studies. Cancer Lett., 252: 171-183.

Takahashi, T., M. Satou, N. Watanabe, Y. Sakaitani and A. Takagi *et al*., 1999. Inhibitory effect of microfibril wheat bran on azoxymethane-induced colon carcinogenesis in CF1 mice. Cancer Lett., 141: 139-146.

Weitberg, A.B., 1987. Effects of inhibitors of arachidonic acid metabolism and vitamin E on oxygen radical induced sister chromatid exchanges. Carcinogenesis, 8: 1619-1620.