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Feeding Wheat Germ Meal and Wheat Germ Oil Reduced Azoxymethane-induced Colon Tumors in Fisher 344 Male Rats

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Abstract: Wheat germ contains phytates, phenolics, ligands, phytosterols, vitamin E and B, which have been proposed to offer protection against chronic diseases such as colon cancer. Present aim was to determine the effects of feeding wheat germ (WGM) at 5 and 10% and Wheat Germ Oil (WGO) at 7 and 14% on Azoxymethane-induced colon carcinogenesis Fisher 344 male rats. Hepatic Glutathione-S-Transferase (GST), a crucial Phase II detoxification enzyme was determined. Following a 1 week period of acclimatization, rats were assigned to 6 groups and fed AIN-93G with 7% SBO and 14% SBO as controls. The rats fed WGO received AIN-93G with 7 and 14% WGO and the rats fed WGM received 5% WGM and 10% WGM. Rats were killed at 46 weeks by CO₂ asphyxiation. Reductions in tumor incidence in rats fed WGM (5%), WGM (10%), WGO (7%) and WGO (14%) compared to the control were 42, 58, 33.4 and 16.7, respectively. There were no significant ($p < 0.05$) differences in hepatic GST activity ($\mu\text{mol mg}^{-1}$) among rats fed (5% (29) and 10% (33)) WGM, however hepatic GST activity in the rats fed WGM was significantly ($p < 0.05$) higher than the control. Wheat germ products did offer protection against development of AOM-induced colon tumors. Although dietary fat is known to be a tumor promoter, type of fat may be as important as the amount consumed.

Key words: AOM, tumor, wheat germ, wheat germ oil, colon cancer

INTRODUCTION

The American Cancer Society estimates that there will be about 148,810 new cases of colorectal cases diagnosed in 2008 in the United States which makes it the second leading cause of death after heart disease. It is also estimated that about 49,980 deaths will have resulted due to colorectal cancer, making it the 3rd most common cause of cancer deaths (ACS, 2008). Adenomas are precursors of colorectal cancers and are appropriate biomarkers for assessing the effects of chemopreventative agents (Demasi *et al.*, 2009).

Adenomas are benign neoplasms of glandular epithelium in which there are atypia of various degrees. Benign epithelial tumors, which may be found throughout the colon, are clusters of cells and are still considered true neoplastic cells because they exhibit characteristics of true neoplasms such as enlarged, elongated and sometimes stratified nuclei (Cheuk and Chan, 2007).

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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 a rich source of tocopherols.

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 synergistic effects that go 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, increasing cecal weight by increased cell differentiation, increasing production of Short Chain Fatty Acids (SCFA) and by decreasing colonic pH which reduces pathogenic bacteria (Williams *et al.*, 2007; Boateng *et al.*, 2007; Michels *et al.*, 2005).

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 synergistic effects on diseases (Geber and Bowermen, 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; ACS, 2007). According to the ACS (2007), consuming a diet high in animal fat increases an individuals risk for cancer over a period of time. Consumption of fat promotes 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. The objective of the study was to determine the chemopreventative effects of wheat germ products as they contain important bioactive components that may play a significant role in colon carcinogenesis.

MATERIALS AND METHODS

Chemicals and Dietary Ingredients

All biochemicals excluding Azoxymethane (Midwestern Research Institute, NCI, Chemical Repository, Kansas City, MO) 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), rats were divided into six (6) groups (n = 12) and were assigned to 6 dietary treatments: 1) Control diets (7 and 14%) (WGO) 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 tumors all animals received two subcutaneous injections of azoxymethane (AOM) (NCI Chemical Repository, Kansas City MO) in saline at 16 mg kg⁻¹ body wt., one dose at seven weeks and another at eight weeks of age.

Collection of Colon Tumor Samples

At 46 weeks, all rats were killed using CO₂ asphyxiation. The colons from rats in each group were removed and flushed with PBS (0.1 M, pH 7.2). The number, size/volume location and tumors/tumor bearing rat were recorded (Shackelford *et al.*, 1983). Some colons (5/group) were split open longitudinally and the colonic mucosa scraped using a microscope slide. The colonic mucosal scrapings were stored in vials at -80°C until GST analysis.

Glutathione-S-Transferase Assay (GST)

GST in the liver and colonic mucosal samples was assayed by the procedure of Habig *et al.* (1974). Colonic mucosal scrapings or liver samples were homogenized in 10 volumes of potassium phosphate buffer (pH 7.0, 0.1 M) in Potter-Elvehjem homogenizer (10 strokes) at 4°C. The homogenate was centrifuged at 10,000 x g for 30 min. In the case of colonic mucosa, the supernatant was centrifuged for a second time at 10,000 x g for 5 min in order to obtain a clear supernatant, which was used for the assay. 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

Mineral Analysis

Five milliliter of 5% HCL was added to the ash in a crucible. The solution was heated on a hot plate and the resultant residue was dissolved in 2 mL HCl. The crucible was covered with a watch glass and subjected to a steam bath for 5 min. The watch glass was washed into a crucible with deionized water and the contents filtered into 100 mL volumetric flask. One hundred milliliter of sample was prepared by using 5% HCL.

Samples were diluted at the rate of 1:10 by using 5% HCL. After adequate dilutions, mineral concentrations were measured by induced coupled photospectrometer (ICP) at the following wavelengths: 422 nm (Ca); 690 nm (P); 285 nm (Mg); 248 nm (Fe) and 214 nm (Zn).

Femurs were excised from rats and weights of bones were noted. Length and width of bones were noted by using calipers. Bones were then cut into pieces and dried at 105°C for 12 h. The dried material was ashed and mineral content was determined by ICP by following the same procedure as fecal samples.

Fecal samples were freeze-dried, powdered and 3 to 5 g sample was weighed into an ashing crucible. Weight of the crucible (W_0) as well as weight of sample (W_1) were noted. The crucibles were covered and placed in a muffle furnace at 550-600°C and incinerated until a grey ash was obtained by adding 2 mL of concentrated nitric acid. Crucibles were allowed to cool to room temperature in a desiccators and weights were noted as (W_2).

$$\text{Calculation \% of ash} = \frac{W_2 - W_0}{W_1} \times 100$$

Apparent absorption of minerals was calculated by:

$$\text{Apparent absorption (\%)} = 100 \frac{\text{Total intake of mineral} - \text{Fecal excretion of mineral}}{\text{Total intake of mineral (AOAC, 1995)}}$$

Statistical Analysis

Data are expressed as Means \pm SEM. Differences were tested for statistical significance using two-way ANOVA. Differences among groups were determined using the Tukey's Studentized range test 9.0 (SAS, 2005 Cary, NC). A p-value of <0.05 was considered to indicate significant differences.

RESULTS

Weight Gain and Feed Intake

Weight gain and feed intake was highest in the rats fed WGM 10%. There were no significant ($p < 0.05$) differences in feed between the control and WGM (5%), however weight gain was significantly ($p < 0.05$) higher in rats fed WGM (5 and 10%) compared to the control. Rats consuming the WGM 10% diet had the significantly ($p < 0.05$) highest daily intake consuming about 18.15 g per day. Feed intake was Lowest in rats fed the control diet (SBO 7%) compared to the experimental groups (WGM 5 and 10%). Significantly ($p < 0.05$) lower daily feed intake was seen in the rats consuming the WGM 5% diet compared to their high fiber counterparts (WGM) 10%. (Table 1).

Weight gain was significantly ($p < 0.05$) higher in rats fed WGO (7 and 14%) compared to the control (SBO 7 and 14%). There were no significant differences seen in weight gain among the rats fed WBO (7 and 14 %), although weight gain in the rats fed SBO (7%) was the lowest among all the groups. There were no significant ($p < 0.05$) differences in feed intake among the groups. However, feed intake ranged from a high of 15.8 in the rats fed WGO (7%) to a low of 14.22 in rats fed the control (SBO 14%) (Table 1).

Tumor Incidence

In both the proximal and the distal colon, tumor incidence was highest in rats fed the control diet. Tumor incidence in the proximal colon was lower in rats fed WGM (10%)

Table 1: Weight gain and feed intakes in Fisher 344 male rats

Groups	Weight gain (g/41 week)	Feed intake (g/day)
C+SBO (7%)	291.77 \pm 7.20 ^a	15.26 \pm 0.53 ^b
C+SBO (14%)	309.22 \pm 8.90 ^a	14.22 \pm 0.46 ^a
C+ WGM (5%)	354.25 \pm 11.96 ^b	15.94 \pm 0.37 ^b
C+ WGM (10%)	399.00 \pm 6.85 ^a	18.15 \pm 0.70 ^a
C+ WGO (7%)	314.75 \pm 8.30 ^a	15.83 \pm 0.47 ^a
C+ WGO (14%)	322.16 \pm 8.08 ^a	15.00 \pm 0.66 ^a

Values are Mean \pm SEM, n = 12 (WGM) n=14 (Control). ^{ab}Means within columns without common letters differ ($p < 0.05$) (Tukey's studentized range test). SBO: Soybean oil; WGM: Wheat germ meal

Table 2: The effect of wheat germ on tumor incidence (%) and numbers in Fisher 344 male rats

Groups	N1/N2	Proximal incidence	Distal incidence	Total	Proximal No.	Distal No.	Total No.
C+ SBO (7%)	14/14	50.0	100.0	100.0	11	35	46
C+ SBO (14%)	14/14	80.0	100.0	100.0	28	50	78
C+ WGM (5%)	7/12	33.0	58.0	58.0	4	7	11
C+ WGM (10%)	5/12	0.0	42.0	42.0	0	5	5
C+ WGO (7%)	8/12	37.5	66.6	66.6	3	13	16
C+ WGO (14%)	10/12	50.0	83.3	83.3	5	25	30

Values are Mean±SEM, n = 12 (WGM) n = 14 (Control). N1/N2 (rats with tumors/number of rats in the group). SBO: Soybean oil; WGM: Wheat germ meal

compared to those fed WGM (5%). Rats fed WGM (10%) had a lower tumor incidence in the distal colon compared to WGM (5%) group. The reductions in tumor incidence (%) in the rats fed WGM (5%) and WGM (10%) compared to the control were 42 and 58, respectively (Table 2).

Rats fed SBO (7 and 14%) had a tumor incidence of 100%. In the proximal colon, highest (80%) tumor incidence was seen in the SBO (14%) fed group compared to those seen in rats fed WGO(14%) which had a tumor incidence of 50%. Tumor incidence (%) was 66.6 and 83.3 in the rats fed WGO (7%) and WGO (14%). Incidence (%) of tumors in the rats fed WGO (7%) and WGO (14%) was 33.4 and 16.7 lower compared to their SBO counterparts (Table 2).

Tumor Numbers

Rats in the control group had the highest number of tumors in both the distal (35) and proximal (11) sections of the colon. Tumor numbers were significantly ($p < 0.05$) higher in the control group compared to rats fed WGM (5 and 10%). Rats fed WGM (5%) had higher number of tumors compared to the WGM (10%) fed group in both the distal and proximal sections of the colon. Reductions in tumor numbers were 76% and 89%, in the groups fed WGM (5%) and WGM (10%) compared to the control (Table 2).

Tumors were seen in 8/12 rats fed WGO (7%), 10/12 rats fed WGO (14%) and 14/14 rats fed SBO (7 and 14%) as shown in Table 2. Rats fed SBO (14%) had a higher number of tumors (28) in the proximal colon compared to rats fed WGO (14%)(5).

Number of tumors in the distal colon was higher in the rats fed SBO (7%) compared to rats fed WGO (7 and 14%). The total number of tumors in the rats fed WGO (7%) was 65% lower compared to its SBO (7%) counterpart. There was a 61.5% reduction in tumor numbers in the group fed WGO (14%) compared to the SBO (14%) fed group. The total number of colon tumors was highest in rats fed SBO (14%) and lowest in rats fed WGO (7%). The rats fed WGO (14%) (30) had lower tumor numbers compared to the SBO (7%) fed group (46) (Table 2). WGO therefore seemed to offer protection against the development of tumors even at the high fat level (14%) compared to SBO.

Tumor Size

Largest tumors were seen in the rats fed the control diet. Among the rats fed WGM, smaller tumors were seen in rats fed WGM (10%). Rats fed the control diet had larger tumors in the proximal colon compared to the WGM (5%). No proximal tumors were seen in rats fed WGM (10%). Tumors in the distal region were significantly ($p < 0.05$) larger in rats fed the control diet compared to the WGM (10%). There were no significant differences in tumor size (mm) among rats fed control and WGM (5%) or WGM (5%) and WGM (10%). Tumor size (mm) in the rats fed WGM (5%) and WGM (10%) were 21 and 51% smaller compared to the control group (Fig. 1).

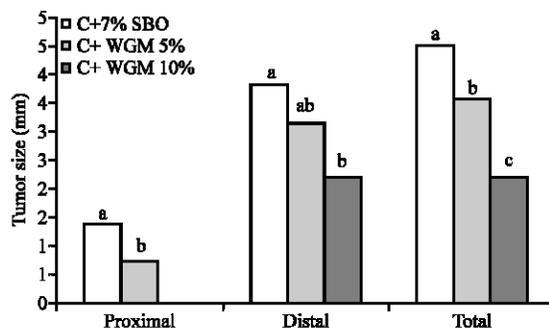


Fig. 1: The effects of Wheat Germ meal on tumor size (mm) in Fisher 344 male rats. ^{abc}Bars with different superscripts are significantly different ($p < 0.05$) using Tukey's studentized range test. SBO: Soybean oil; WGM: Wheat germ meal

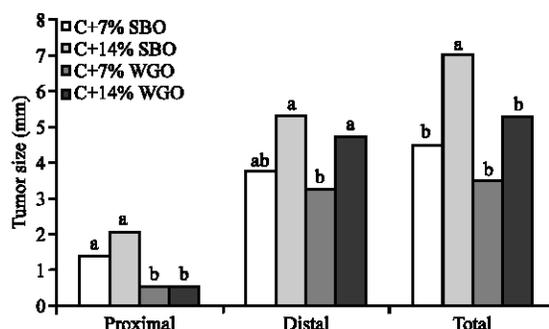


Fig. 2: The effects of wheat germ oil on tumor size (mm) in Fisher 344 male rats. ^{ab}Bars with different superscripts are significantly different ($p < 0.05$) using Tukey's studentized range test. SBO: Soybean oil; WGO: Wheat germ oil

Rats fed SBO (14%) had significantly ($p < 0.05$) larger tumors (7 mm) compared to the other 3 groups. There was a 50% reduction seen in tumor size (mm) in rats fed WGO (7%) compared to the SBO (14%). WGO (14%) fed rats had larger tumors (5.33 mm) compared to the WGO (7%) (3.5 mm) (Fig. 2).

Tumors/ Tumor Bearing Rat Ratio (TBR)

TBR ratio was significantly ($p < 0.05$) higher in rats fed the control diet compared to rats fed WGM (5 and 10%) diets. The TBR ratios were higher in the rats fed WGM (5%) (1.5) compared to the rats fed WGM (10%) (1.0). The reductions (%) in TBR ratios in the WGM (5%) and WGM (10%), compared to the control group were 54 and 69, respectively (Table 3).

TBR ratio ranged from a low of 2 (WGO 7%) to a high of 5.8 in the groups fed SBO (7 and 14%). Rats fed SBO (7%) (3.25) had a lower TBR ratio compared to the SBO (14%) (5.80) group (Table 6). The groups fed WGO had a lower TBR ratio compared to the SBO (14%) fed group. The TBR ratio was 44% higher in the rats fed SBO (14%) compared to their normal fat (SBO 7%) counterpart (Table 3).

Glutathione S-transferase Activity

There were no significant ($p < 0.05$) differences in hepatic GST activity ($\mu\text{mol mg}^{-1}$) among the rats fed WGM (5 and 10%), however hepatic GST activity in the rats fed WGM

Table 3: The effects of wheat germ on tumor per tumor bearing rat ratio (TBR) in Fisher 344 male rats

Groups	N1/N2	T/TBR
C+SBO (7%)	14/14	3.25
C+SBO (14%)	14/14	5.80
C+ WGM (5%)	7/12	1.50
C+ WGM 10%	5/12	1.00
C+WGO (7%)	8/12	2.00
C+ WGO (14%)	10/12	3.00

Values are Mean±SEM, n = 12 (WGM) n = 14 (Control). N1/N2 (rats with tumors/number of rats in the group). SBO: Soybean oil; WGM: Wheat germ meal

Table 4: Hepatic and colonic Glutathione S-transferase activity in the Fisher 344 male rats fed wheat germ

Groups	Hepatic GST activity ($\mu\text{mol mg}^{-1}$)	Colonic GST activity ($\mu\text{mol mg}^{-1}$)
C+SBO (7%)	15.86±1.04 ^b	0.38±0.04 ^b
C+SBO(14%)	12.42±1.02 ^b	0.49±0.08 ^b
C+WGM (5%)	28.72±1.92 ^a	7.23±0.55 ^a
C+WGM (10%)	33.30±2.24 ^a	6.38±0.85 ^a
C+WGO (7%)	28.20±0.55 ^a	9.14±1.12 ^a
C+WGO (14%)	30.39±1.12 ^a	10.42±1.23 ^a

Values are Mean±SEM, n=12 (WGM) n=14 (Control). ^{a,b}Means within columns with different letters are significantly different ($p<0.05$) (Tukey's studentized range test). SBO: Soybean oil; WGM: Wheat germ meal

(5 and 10%) was significantly ($p<0.05$) higher than that of the control (SBO 7%). GST activity ($\mu\text{mol mg}^{-1}$) in the rats fed WGM (5%) and WGM (10%) were 45 and 52% higher compared to the control (SBO 7%).

GST activity in the colon was significantly higher in rats fed WGM (5 and 10%) compared to the rats fed SBO (7%). There were no significant ($p<0.05$) differences in GST activity (CMS) in rats fed WGM (5 and 10%), although rats consuming WGM (5%) had a numerically higher GST activity (CMS) compared to the WGM (10%) fed group (Table 4).

GST activity ($\mu\text{mol mg}^{-1}$) was significantly ($p<0.05$) higher in the rats fed WGO (7 and 14%) compared to the rats fed the control diet (SBO 7 and 14%). GST activity ($\mu\text{mol mg}^{-1}$) was lower in the rats fed WGO (7%) compared to high fat counterpart (WGO 14%). GST activity ($\mu\text{mol mg}^{-1}$) was significantly ($p<0.05$) lower in the SBO (7 and 14%) fed groups compared to the WGO (7 and 14%) fed group. Hepatic GST activity ($\mu\text{mol mg}^{-1}$) was 43 and 55% higher in the WGO (7%) fed group compared to their respective control SBO (7 and 14%) (Table 4).

Colonic GST activity ($\mu\text{mol mg}^{-1}$) was significantly ($p<0.05$) higher in the rats fed WBO (7 and 14%) compared to the rats fed (SBO 7 and 14%). GST activity ($\mu\text{mol mg}^{-1}$) was 12% higher in the rats fed WGO (14%) compared to its low fat counterpart (WGO 7%). A 95% increase in GST activity ($\mu\text{mol mg}^{-1}$) was seen in the rats fed WGO (7%) compared to the rats fed SBO (7%). A similar trend was also seen in the rats fed high fat diets with WGO (14%) fed rats having a 95% higher colonic GST activity ($\mu\text{mol mg}^{-1}$) compared to the SBO (14%) group (Table 4).

Physical Parameters

The femur weight ranged from a low of 0.821 g in the rats fed WGM (10%) to a high of 1 g in the control to a low of The volume of the femurs in the rats fed WGM (10%) was significantly lower compared to the WGM (5%) fed rats. The rats fed the control diet (0.71) had significantly higher bone volume compared to the WGM (5%) fed group. The rats fed the control diet (4.70 cm) had significantly ($p<0.05$) longer femurs compared to the rats fed WGM (5%) and WGM (10%). The femurs weights in the rats fed WGM (5%) and WGM (10%) were 17 and 19% lower compared to the control fed rats (Table 5).

Table 5: Physical parameters of femurs of Fisher 344 male rats

	Control SBO (7%)	C + WGM (5%)	C + WGM (10%)
Bone weight (g)	1.01 ^a	0.84 ^a	0.82 ^a
Volume (mL)	0.71 ^a	0.62 ^b	0.49 ^b
Length (cm)	4.70 ^a	3.49 ^b	3.59 ^b

Values are Mean±SEM, n=12 (WGM) n=14 (Control). ^{abc}Means within the columns with different letters are significantly different (p<0.05) (Tukey's studentized range test). SBO: Soybean oil; WGM: Wheat germ meal

Table 6: Daily mineral intake in Fisher 344 male rats (mg)

Groups	Feed intake	Ca	P	Mg	Zn	Fe
C+SBO (7%)	15.26	168.88	92.71	11.35	0.78	2.90
C + WGM (5%)	15.94	176.48	96.90	11.86	0.81	3.00
C + WGM (10%)	18.15	200.86	110.27	13.50	0.92	3.41

SBO: Soybean oil; WGM: Wheat germ meal

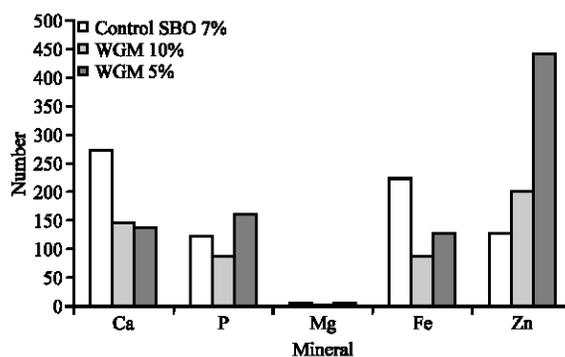


Fig. 3: Bone mineral number (mg g⁻¹) in femurs of Fisher 344 male rats

Bone Mineralization

The bone minerals measured were Ca, P, Mg, Zn and Fe. Bone Ca (mg g⁻¹) was significantly (p<0.05) higher in rats fed the control diet compared to the rats fed WGM (5 and 10%). The group fed WGM (10%) had significantly (p<0.05) higher bone P compared to the groups fed the control diet and WGM (5%). There were no significant differences in bone Mg between the rats fed control and WGM (5%). The rats fed WGM (5 and 10%) had significantly higher Zn (mg g⁻¹) compared to the control fed rats. However Fe levels in the bone were significantly (p<0.05) lower in the WGM (5 and 10%) fed groups (Fig. 3).

Fecal Mineral Excretion

The excretion of Ca (mg g⁻¹) was higher in the rats fed WGM (10%) compared to the control, however fecal excretion of P, Mg, Zn and Fe was higher in the control group compared to the rats fed WGM (5 and 10%). The fecal Ca excretion was higher in the rats fed WGM (5%) compared to the WGM (10%). However, the fecal P, Mg, Zn and Fe excretion was higher in the rats fed WGM (10%) compared to the WGM (5%). Fecal Ca excretion (mg g⁻¹) ranged from a low of 57.26 (control) to a high of 63.09 (WGM 5%) group. The rats fed WGM (10%) had a lower Ca excretion compared to the WGM (5%) (Fig. 4).

Mineral Intake and Apparent Absorption

Rats fed WGM (10%) had significantly (p<0.05) higher intakes of Ca, P, Mg and Fe compared to the WGM (5%) and control groups (Table 6). Apparent absorption of C, P, Zn and Fe were similar among all the groups with mineral absorption ranging between 70 and 85 (Fig. 5). However, Mg absorption was 25% higher in the rats fed WGM (5 and 10%).

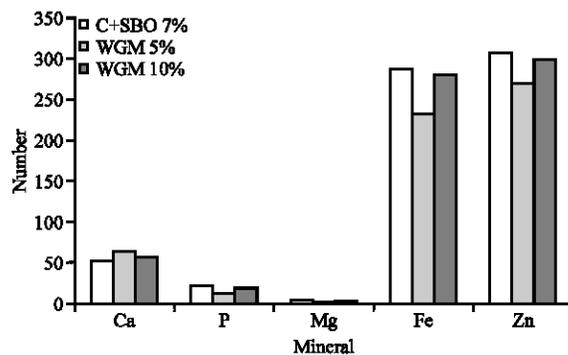


Fig. 4: Fecal mineral number (mg g⁻¹) in Fisher 344 male rats

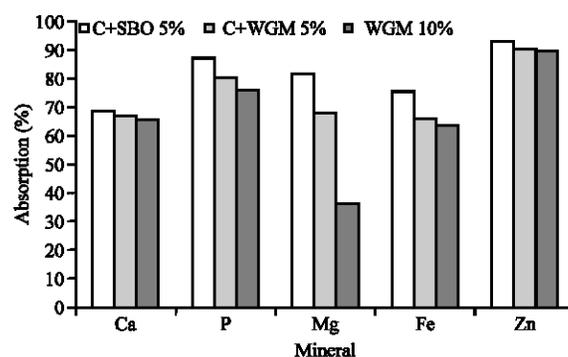


Fig. 5: The effects of wheat germ Meal on mineral absorption in Fisher 344 male rats

DISCUSSION

The objective of this study was to determine the long term effects of feeding wheat germ on colon tumorigenesis in azoxymethane-induced Fisher 344 male rats. To our knowledge this is the first animal model study comparing the chemopreventative/antitumorigenic effects of wheat germ compared to wheat germ oil. In previous studies, vitamin B and E and omega-3 fatty acids, which are all constitutions of wheat germ have been show to reduce the risk of colon cancer (Williams *et al.*, 2007).

In this study weight gain (g) ranged from a high of 399 in the WGM (10%) fed group to a low of 291.77 in the control SBO (7%) fed rats. Because the rats fed WGM (10%) consumed more fiber (100 g compared to 50 g/100 g) we anticipated that the rats would have lower body weights compared to the other groups. This did not occur because the rats fed SBO (7%) had the lowest weight gain which may be due to the higher tumor burden compared to the WGM fed groups. However, WGM (10%) fed rats consumed 2.89 g higher feed (daily) than the control group (SBO 7%). An increase in soluble to insoluble fiber has the ability to increase laxation and increase motility causing an increase in fecal contents thereby increasing the feeling of hunger (Bes-Rastrollo *et al.*, 2006).

Results from this study showed that consumption of WGM and WGO did offer protection against colon tumors. Rats consuming WGM and WGO had significantly ($p < 0.05$) lower tumor incidence, tumor number and tumor size and TBR ratio compared to the control

fed (SBO) male rats. Rats consuming the WGM diets (5 and 10%) had a tumor incidence of 58 and 42%, respectively. Our results are consistent with research conducted by McIntosh *et al.* (2001) where rats fed a fiber rich diet of whole wheat had a tumor incidence of 44% with a 25% reduction in tumor number.

High fiber diets such as those containing chick peas (McIntosh *et al.*, 2001) dry beans (Hughes *et al.*, 1997), barley (Dongowski *et al.*, 2002) and flax seed (Williams *et al.*, 2007) have shown to reduce tumor incidence, number and size in rat models.

The fermentation of dietary fiber in the intestine aids in the production of SCFAs and organic acids, which are important in maintenance of a healthy colon. SCFAs such as butyrate are rapidly absorbed by the colonic mucosa and act as a signal metabolite which stimulates cell migration and cell differentiation thereby reducing tumor formation (Dongowski *et al.*, 2002). Fibers, such as those found in wheat are believed to protect against colon cancer by adsorbing carcinogens found in the gastrointestinal tract. Once absorbed these carcinogens are then carried out of the body bound to insoluble dietary fiber (Klurfeld and Bull, 1997).

Studies by Zalatnai *et al.* (2001) found that wheat germ extracts may decrease ACF and tumor formation by acting as an immunomodulator. In animal studies it was found that wheat germ extracts caused an increase in macrophage activity thereby exposing tumor cells to natural killer cell activity (Zalatnai *et al.*, 2001).

One characteristic of tumor cells is their over expression of COX-2 which indicates an increased capacity to produce 2-series prostaglandins (PGE₂) in the presence of arachidonic acid. Wheat germ oil has a higher ratio of n-3 compared to n-6. A n-3 PUFA-rich diet, compared to high fat or a n-6 PUFA-rich diet, decreases the activities of phospholipase A₂ and COX-2 in colonocytes, causing a depletion in arachidonic acid and prostaglandin E₂ synthesis (Rao *et al.*, 1996).

Fermentation of soluble fiber (such as those found in wheat germ) by intestinal microflora leads to the production of SCFAs (acetic, propionic and butyric acids) and organic acids resulting in a lower intestinal pH. An acidic environment in the intestine facilitates the absorption of minerals due to increased solubility of minerals, enlargement of absorptive surfaces due to enterocyte proliferation, enhanced mineral binding, protein expression and improvement of gut health. The excretion of P, Mg, Zn and Fe was significantly ($p < 0.05$) higher in the control compared to the treatment groups, however the rats fed WGM (10%) had significantly higher P, Mg, Zn and Fe excretion compared to the WGM (5%) fed group.

Increased fiber in the diet may reduce absorption of minerals as it may chelate minerals, reducing their availability. Foods especially rich in insoluble fiber without being a significant source of soluble fiber may chelate minerals such as Ca, P, Mg, Zn and Fe, thereby increasing fecal excretion and reducing absorption and bone mineralization. Fermentation of soluble fiber however may reduce the antinutritive property of fiber resulting in reduced excretion and increased absorption.

The results of this study suggest that dietary wheat germ meal (WGM) and Wheat Germ Oil (WGO) suppressed Azoxymethane (AOM) induced colon tumors in Fisher 344 male rats. As diseases such as obesity, diabetes, heart disease and colon cancer continue to be prevalent in western societies, it is necessary for the food industry to increase incorporation of consumable products such as wheat germ and wheat germ oil into various food products. Some of the chemopreventative properties of wheat germ may be attributed to fiber, minerals and phytochemicals such as Vitamin E content, which may have played a beneficial role in reducing the tumor-promoting effects of fat. Human clinical trials are needed to further evaluate the effectiveness of wheat germ products as chemopreventive agents.

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REFERENCES

- ACS., 2007. Cancer facts and figures. American Cancer Society. http://www.cancer.org/docroot/STT/content/STT_1x_Cancer_Facts_Figures_2007.asp.
- AOAC, 1995. Official Methods of Analysis of the Association of Official Analytical Chemist. 16th Edn., AOAC International, Arlington, VA., USA.
- Bes-Rastrollo, M., M.A. Martínez-González, A. Sánchez-Villegas, C. de la Fuente Arrillaga and J.A. Martínez, 2006. Association of fiber intake and fruit/vegetable consumption with weight gain in a Mediterranean population. *Nutrition*, 22: 540-511.
- 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: 3-13.
- 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.
- Cheuk, W. and J.K. Chan, 2007. Advances in salivary gland pathology. *Histopathology*, 51: 1-20.
- Demasi, A., C. Furuse, S. Soares, A. Altemani and V. Araújo, 2009. Peroxiredoxin I, platelet-derived growth factor A and platelet-derived growth factor receptor a are overexpressed in carcinoma ex pleomorphic adenoma: Association with malignant transformation. *Hum. Pathol.*, 40: 390-397.
- 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.
- Geber, D. and S. Bowerman, 2001. Applying science to changing dietary patterns. *J. Nutr.* (Review), 131: 3078S-3081S.
- 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.
- Hughes, J.S., C. Ganthavorn and S. Wilson-Sanders, 1997. Dry beans inhibit azoxymethane-induced colon carcinogenesis in F344 rats. *J. Nutr.*, 127: 2328-2333.
- 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.
- 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 Epidemiol.*, 10: 17-23.
- 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 coronary 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.

- Liu, R.H., 2007. Whole grain Phytochemical and Health. *J. Cereal Sci.*, 46: 207-219.
- McIntosh, G., P. Royle and G. Pointing, 2001. Wheat aleurone flour increases cecal b-glucuronidase activity and butyrate concentration and reduces colon adenoma burden in azoxymethane-treated rats. *J. Nutr.*, 131: 127-131.
- 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.
- Rao, C., B. Simi, T. Wynn, K. Garr and B. Reddy, 1996. Modulating effect of amount and types of dietary fat on colonic mucosal phospholipase A2, phosphatidylinositol-specific phospholipase C activities and cyclooxygenase metabolite formation during different stages of colon tumor promotion in male F344 rats. *Cancer Res.*, 56: 532-537.
- Shackelford, L.A., D.R. Rao, C.B. Chawan and S.R. Pulusani, 1983. Effect of feeding fermented milk on the incidence of chemically-induced colon tumors in rats. *Nutr. Cancer*, 5: 159-164.
- Williams, D., M. Verghese, L.T. Walker, J. Botang, L.T. Shackelford and C.B. Chawan, 2007. Flax seed oil and flax seed meal reduce the formation of aberrant crypt foci (ACF) in azoxymethan-induced colon cancer in Fisher 344 male rats. *Food Chem. Toxicol.*, 45: 153-159.
- Wong, R., A. Dalton, J. Barlow, J. Erickson, K. Boucher and R. Burt, 2004. Relationship of colon adenomas to diet and exercise in subjects at high-risk of colon cancer based on family history. *Gastrointestinal Epidemiol.*, 59: 278-278.
- Zalatnai, A., K. Lapos, B. Szende, E. Raso, A. Telekes, A. Resetar and M. Hidvegi, 2001. Wheat germ extract inhibits experimental colon carcinogenesis in F344 rats. *Carinogenesis*, 22: 1649-1652.
- Zhang, K., P. Wong and P. Chow, 2003. Conjugation of chlorambucil with GSH by GST purified from human colon adenocarcinoma cells and its inhibition by plant polyphenols. *Life Sci.*, 72: 2629-2640.