Consumption of Black Beans and Navy Beans \textit{(Phaseolus vulgaris)} Reduced Azoxymethane-Induced Colon Cancer in Rats

L. Hangen and M. R. Bennink

\textbf{Abstract:} Beans \textit{(Phaseolus vulgaris)} are an important food staple in many traditional diets. There is limited evidence to suggest an inverse relationship between bean consumption and colon cancer. The objective of this study was to determine whether consumption of black beans and/or navy beans would reduce colon carcinogenesis in rats. Rats were fed a modified AIN-93G diet (control) or diets containing 75\% black beans or 75\% navy beans for 4 wk, and then colon cancer was initiated by administration of two injections of azoxymethane 1 wk apart. At 31 wk after the second injection, the incidence of colon adenocarcinomas was significantly lower \((P < 0.05)\) in rats fed the black bean (9\%) and navy bean (14\%) diets than in rats fed the control diet (36\%). Total tumor multiplicity was also significantly lower in rats fed the black bean (1.1) and navy bean (1.0) diets than in rats fed the control diet (2.2). The 44–75\% reduction in colon carcinogenesis in rats fed beans was attributed to 1) more controlled appetites, leading to significantly less body fat, and 2) much greater concentrations of butyrate in the distal colon. It was concluded that eating black beans and navy beans significantly lowered colon cancer incidence and multiplicity.

\textbf{Introduction}

It is estimated that 70\% of the colorectal cancer deaths in the United States could be avoided by changing dietary patterns (1), and there is evidence to suggest that increased consumption of common beans \textit{(Phaseolus vulgaris)} could help reduce mortality from colon cancer. Correa (2) found an inverse correlation \((-0.68)\) between bean consumption and colon cancer mortality. In addition, Hughes et al. (3) demonstrated that eating pinto beans significantly reduced chemically induced colon cancer in rats. The World Cancer Relief Fund/American Institute for Cancer Research Committee recognized the potential of pulse (legume) consumption to decrease cancer risk and emphasized the need for additional research in this area (4).

Properties of \textit{P. vulgaris} that may relate to anticancer action include the presence of bioactive microconstituents and the physiochemical properties of bean starch. Protease inhibitors, saponins, phytosterols, and phytate are putative anticarcinogens in soybeans (5), and these compounds are present in significant quantities in common edible beans also. Beans contain a considerable amount of resistant starch (starch that escapes digestion and absorption in the small intestine) (6,7). A combination of resistant starch and slowly fermented fiber leads to increased concentrations of short-chain fatty acids in the distal colon (8,9). There is considerable interest in increasing the amount of butyrate produced in the distal colon, where most tumors develop, because of the putative anticancer activity of butyrate. Butyrate has been shown to induce growth arrest, apoptosis, and differentiation in several colon cancer cell lines (10–12). Butyrate may exert its effects through histone hyperacetylation, up-regulation of p21\textsuperscript{WAF1}, and down-regulation of the epidermal growth factor receptor (13,14). Finally, bean starch is digested slowly, produces a low glycemic index (15), and attenuates the postprandial insulin response (6). Hyperglycemia and hyperinsulinemia are hypothesized to increase the risk of colon cancer (16).

This study was designed to determine whether consuming beans \textit{(P. vulgaris)} other than pinto beans would reduce azoxymethane-induced colon carcinogenesis in an initiation-promotion study. Preference for beans of a particular seed coat color varies with geographical region and cultural background. Pinto beans are preferred in Mexico and the southwestern United States, but Central and South American and African populations eat mostly colored beans and very little pinto beans. Because pinto beans are not as widely consumed as the other market classes of dry beans, the anticancer potential of two other classes of dry beans was evaluated. Tannins (condensed polyphenols), anthocyanins, and flavonoids have been suggested to have anticancer activity. Therefore, two cultivars representative of the navy bean and black bean market classes were chosen, because black beans have significantly more tannins, anthocyanins, and flavonoids (17–19). If tannins, anthocyanins, quercetin glycosides, or kaempferol glycosides (flavonoids) have anticancer properties, then rats eating black beans should have...
the lowest colon cancer incidence and rats eating navy beans should have an intermediate incidence.

**Materials and Methods**

**Animals and Housing**

Male Fischer 344 rats (21 days old) were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed three per plastic cage with sawdust bedding and assigned to treatment groups by weight. The animal room was temperature (22–24°C) and humidity (40–70%) controlled with a 12:12-h light-dark cycle. The rats were given free access to the diet and distilled water throughout the study. This study was approved by the Michigan State University Committee on Animal Use and Care.

**Diets**

Navy beans and black beans, grown in Michigan, were soaked overnight in distilled water at 4°C and cooked in open kettles until soft. They were then dried in a convection oven at 50–60°C. The dry beans were ground to pass through a screen with 1.6-mm-diameter holes before they were mixed with other diet ingredients. Table 1 shows the composition of the diets. In a preliminary experiment, apparent protein digestibility was 66% for black beans, 76% for navy beans, and 95% for casein. The diets listed in Table 1 were formulated to provide 12.69 g of digestible protein per 100 g of diet. All diets contained the same amount of total fat (16%). The composition of fat in the beans is similar to that of corn oil, so an equal amount of corn oil was added to the control diet. Total fat and the ratios of saturated, polyunsaturated, and monounsaturated fatty acids were based on the typical American diet (20). The bean diets were supplemented with cysteine and methionine to meet the amino acid needs of growing rats (21). Cysteine was added to the control diet to make casein a complete protein.

**Administration of Carcinogen**

After the rats were fed the diets for 1 mo, the colon carcinogen azoxymethane (Ash-Stevens, Detroit, MI) was injected (15 mg/kg body wt sc) into the flank. Two injections were administered, with 1 wk between injections.

**Food Consumption**

To measure food consumption, groups of three rats were housed in stainless steel hanging wire cages for collection of spilled food and feces. Food consumption was measured over a 6-day period, and results are presented as the average food intake per rat. Eighteen rats from each treatment were used to determine food consumption every 2 mo throughout the study.

**Necropsy and Histology**

The diets were fed for a total of 36 wk (31 wk after the 2nd azoxymethane injection), and then the rats were sacrificed using CO₂ inhalation and exsanguination. The entire abdominal cavity was examined visually for tumors. Then the colon was removed, cut open longitudinally, rinsed with warm tap water, pinned to cardboard, and fixed in 4% buffered formaldehyde (pH 7.4). All suspected tumors were excised, weighed, and processed using routine histological procedures. The embedded tissues were sectioned, stained with hematoxylin and eosin, and classified by a pathologist who was blinded to the treatments. Adenocarcinomas are reported separately, whereas adenomas plus adenocarcinomas are reported as total tumors.

**Body Composition**

Fat was extracted from 15 randomly selected rats per treatment. The carcasses were digested with ethanolic NaOH (35% 3 M NaOH-65% ethanol). The carcasses were heated at ~70°C until all soft tissue was dissolved. When the carcasses were completely solubilized, the mixture was placed in a separatory funnel and acidified with concentrated HCl, and lipids were extracted with hexane. The hexane was evaporated, the residue was heated at 60°C until a constant weight was achieved, and the weight of the lipid was determined.

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Table 1. Diet Compositions

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Black bean</th>
<th>Navy bean</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beans</td>
<td>75.0</td>
<td>75.0</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.138</td>
<td>0.121</td>
<td>0.218</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.100</td>
<td>0.121</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>Tallow</td>
<td>11.2</td>
<td>11.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>2.3</td>
<td>4.9</td>
<td>51.5</td>
</tr>
<tr>
<td>Fiber (cellulose)</td>
<td></td>
<td></td>
<td>12.7</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Minerals²</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamins²</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>BHT</td>
<td>0.0032</td>
<td>0.0032</td>
<td>0.0032</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

a: Values are presented per 100 g of diet. All diets contained 12.69% digestible protein, 16% fat, and 12.75% fiber. Black beans contained 21.1% protein, navy beans contained 22.3% protein, and casein contained 92.0% protein. BHT, butylated hydroxytoluene.

b: Mineral mix (AIN-93G MX) (23); mineral mix did not contain calcium.

c: Vitamin mix (AIN-93G VX).
Resistant Starch Analysis

Ileal contents were collected from 15 rats per treatment at time of sacrifice and stored at −20°C for starch analyses. Starch remaining in the ileal contents is defined as “resistant starch,” because it had escaped digestion and absorption. Ileal contents were weighed, and 2 N NaOH was added in an amount equal to three times the weight of the sample. The NaOH was added to dissolve the starch in the sample. After 1 h, the mixture was centrifuged at 13,000 g for 15 min, and the supernatant was collected and weighed. An equal weight of 2 N HCl was added to neutralize the sample. Then the pH was adjusted to 4.5 by addition of a 1 M sodium acetate-0.1 M CaCl₂ buffer in an amount equal to twice the volume of the HCl added previously. The mixture (490:1 dilution) was combined with a 10:1 dilution of an enzyme solution containing 3 enzyme units of amyloglucosidase (from Aspergillus niger). The test tubes were capped and heated at 60°C overnight to complete starch hydrolysis. The amyloglucosidase digest was analyzed for glucose with a diagnostic kit (catalog no. 510A, Sigma, St. Louis, MO). Starch was calculated as 0.9 (glucose).

Short-Chain Fatty Acid Analysis

Distal colon contents were collected from 15 rats per treatment at time of sacrifice and stored at −20°C for short-chain fatty acid analyses. The samples were mixed with 2 vol of double-distilled water and 1 vol of 25% (wt/wt) metaphosphoric acid diluted 1:1:2 in water. The samples were centrifuged at 13,000 g for 15 min, and the supernatant was used to measure short-chain fatty acids by gas chromatography with flame ionization detection. The short-chain fatty acids were separated on a 1-m long × 3-mm-ID glass column packed with 15% SP-1220 and 1% H₃PO₄ on 100/120 Chromosorb WAW. Nitrogen was the carrier gas. Initial column temperature was held at 80°C for 3 min, and then the temperature was increased 3°C/min until it reached 98°C. Acetate, propionate, isobutyrate, butyrate, valerate, and iso-valerate in the samples were determined by the external standard technique.

Statistical Analysis

The SPSS statistical software was used for the statistical analysis of all data. One-way analysis of variance was used to compare means for weight gain, food consumption, short-chain fatty acids, resistant starch, body fat, lean tissue mass, and tumor multiplicity. When analysis of variance indicated a significant treatment effect, post hoc analysis was done by Fisher’s least significant difference multiple comparison test to identify treatment differences. The χ² test was used to assess differences in tumor incidence. Because the variance for tumor weight per rat was nonhomogeneous, the tumor burden was analyzed by the Wilcoxon rank sum procedure. Values are means ± SE; α = 0.05 was used to determine statistical significance.

Results

Growth and Food Consumption

There was a significant difference in weight gain among treatments, with rats consuming the two bean diets gaining significantly less weight than the control group (Table 2). There were significant differences in food consumption for all treatments. The quantity of diet consumed was as follows: control > black bean > navy bean (Table 2).

Body Composition

Body composition was affected by dietary treatment (Table 2). There was a significant difference in percent body fat among all treatments. Body fat was lowest in rats fed navy beans, and rats fed the black bean diet had an intermediate percent body fat compared with control rats.

Resistant Starch

Rats eating the bean diets had significantly (P < 0.05) more starch in their ileal contents than rats fed the control diet (233, 221, and 14 mg/g dry ileal contents for black bean, navy bean, and control diets, respectively). There was no difference in the amount of resistant starch in ileal contents between the two bean diets. These data show that significantly more starch escapes digestion in the small intestine when beans are consumed.

Short-Chain Fatty Acid Concentrations

The concentrations of short-chain fatty acids in the distal colon contents are shown in Table 3. There were significant treatment effects in several short-chain fatty acid concentrations. There was significantly more acetate in colon contents from rats consuming bean diets than in colon contents from rats consuming the control diet. The navy bean diet produced the greatest acetate concentrations. The differences for propionate were minor, even though there were statistical differences. The butyrate concentrations were much greater (9

Table 2. Weight Gain, Food Consumption, and Body Fat for Rats Fed Bean or Control Dietsa,b

<table>
<thead>
<tr>
<th>Diets</th>
<th>n</th>
<th>Weight Gain, g</th>
<th>Food Consumption, g/day/rat</th>
<th>Body Fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black beans</td>
<td>32</td>
<td>338 ± 4†</td>
<td>11.9 ± 0.3†</td>
<td>19.7 ± 0.4†</td>
</tr>
<tr>
<td>Navy beans</td>
<td>28</td>
<td>334 ± 4*</td>
<td>10.4 ± 0.2*</td>
<td>18.0 ± 0.8*</td>
</tr>
<tr>
<td>Control</td>
<td>28</td>
<td>387 ± 5†</td>
<td>13.0 ± 0.4†</td>
<td>23.6 ± 0.6†</td>
</tr>
</tbody>
</table>

a: Values are means ± SE; n, number of rats.

b: Values in a column with different symbols (*, †, ‡) are significantly different (P < 0.05).
times) in the colon contents from rats fed the two bean diets than in the colon contents from rats fed the control diet. The concentrations of isobutyrate, isovalerate, and valerate were very small; therefore, the results are expressed as a range.

### Tumor Parameters

Tumor data are shown in Table 4. Colon tumor incidence (percentage of animals that had ≥1 tumor) was significantly lower in rats fed the diets containing beans. Likewise, the incidence of colon adenocarcinomas was significantly lower in rats fed the diets containing beans. In addition, colon tumor multiplicity (number of tumors/tumor-bearing rat) was significantly lower in rats fed the bean diets. There was no difference in the incidence of colon adenomas or adenocarcinomas or in tumor multiplicity between rats fed diets containing black beans and rats fed diets containing navy beans. Three percent of rats fed black beans, 18% of rats fed navy beans, and 46% of rats fed the control diet had one or more tumors in the small intestine. Tumor incidence in the small intestine paralleled adenocarcinoma incidence in the colon. There were no dietary treatment effects (P > 0.05) on tumor burden (g tumor/tumor-bearing rat, data not shown).

### Discussion

The main objective of this study was to determine whether consumption of black and/or navy beans would reduce colon carcinogenesis in rats. The data in Table 4 show that eating black beans and navy beans had a profound impact on azoxymethane-induced colon carcinogenesis. Eating black beans decreased total tumor incidence by 54% and adenocarcinoma incidence by 75%, whereas eating navy beans reduced total tumor incidence by 59% and adenocarcinoma incidence by 44%. Similarly, total tumor multiplicity was decreased by 50% and 55% by feeding black beans and navy beans, respectively. The only other study that has examined the effect of feeding edible dry beans on colon carcinogenesis was done by Hughes et al. (3). They demonstrated that consumption of pinto beans reduced azoxymethane-induced total colon tumor incidence from 50% for the casein control to 24% for the pinto bean group. The results in this study are similar to those reported by Hughes et al.

It is well known that food or energy restriction reduces cancer. Kumar et al. (22) restricted energy intake and measured azoxymethane-induced colon cancer incidence. They found that 10% energy restriction reduced weight gain by 16%, but tumor incidence was not significantly reduced. However, 20% and 30% energy restriction reduced weight gain by 23% and 38% while reducing tumor incidence by 34% and 39%, respectively. In the present study, rats eating beans weighed 12.4% less than the control group but had a 54–59% reduction in total tumor incidence and a 44–75% reduction in adenocarcinoma incidence. The reduced food intake by the rats eating the bean diets most likely accounted for only a portion of the dramatic reduction in colon cancer incidence. All three diets were equivalent for digestible protein, minerals, vitamins, fat, fiber, and energy content. All essential amino acids were present in sufficient amounts, and normal growth occurred for all three diets. However, the control group ate more, grew faster, and had a higher percent body fat than the rats fed the navy bean or the black bean diet. The association of greater body fat with greater colon tumor incidence in the control rats is compatible with the observations that excess weight and obesity in adult humans increase the risk of colon cancer (23).

The majority of tumors in humans and in chemically induced animal models develop in the distal colon. However, fermentation of resistant starch and soluble fiber occurs more proximal in the colon, making the concentrations of short-chain fatty acids high in the proximal colon and progressively lower toward the distal colon. Thus the potential protection that could be derived from butyrate (10–12) does not occur, because starch does not reach the distal colon, where most of the tumors develop, to produce butyrate.

### Table 3. Short-Chain Fatty Acid Concentrations in Distal Colon Contents

<table>
<thead>
<tr>
<th>Diets</th>
<th>Fatty Acids, µmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
</tr>
<tr>
<td>Black beans</td>
<td>32 ± 2.8†</td>
</tr>
<tr>
<td>Navy beans</td>
<td>47 ± 5.3†</td>
</tr>
<tr>
<td>Control</td>
<td>7 ± 0.7*</td>
</tr>
</tbody>
</table>

* Values are means ± SE, except for isobutyrate, isovalerate, and valerate, where values are presented as a range from T (trace) to the highest amount.

### Table 4. Influence of Bean and Control Diets on Colon Tumor and Cancer Incidence and Tumor Multiplicity

<table>
<thead>
<tr>
<th>Diets</th>
<th>n</th>
<th>Tumor Incidence, %</th>
<th>Adenocarcinoma Incidence, %</th>
<th>Tumor Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>32</td>
<td>28*</td>
<td>9*</td>
<td>1.1 ± 0.33*</td>
</tr>
<tr>
<td>Navy</td>
<td>28</td>
<td>25*</td>
<td>14*</td>
<td>1.0 ± 0.4*</td>
</tr>
<tr>
<td>Control</td>
<td>28</td>
<td>61†</td>
<td>36†</td>
<td>2.2 ± 1.18†</td>
</tr>
</tbody>
</table>

* Values in a column with different symbols (*, †) are significantly different (P < 0.05).
Govers et al. (8) and Morita et al. (9) manipulated the site of fermentation by mixing resistant starch with wheat bran or psyllium to shift the site of fermentation and increase the concentrations of short-chain fatty acids in the distal colon. Beans characteristically contain high amounts of resistant starch (6,7) as well as high amounts of soluble and insoluble fibers. We expected the fiber in beans to carry the resistant starch toward the distal colon, where fermentation of resistant starch and fiber would be more beneficial. There was ~15 times more starch in the ileal contents from rats eating beans than in control rats, which shows that greater amounts of resistant starch entered the colons of rat fed beans. Table 3 shows that greater concentrations of butyrate were present in distal colon contents of rats fed the bean diets.

Beans contain potentially bioactive microconstituents, such as phenolic compounds (flavonoids, tannins, and anthocyanin), protease inhibitors, phytic acid, and saponins. The last three have demonstrated anti-colon cancer activity. Kennedy (24) demonstrated that protease inhibitors, particularly chymotrypsin inhibitors, exert anticancer activity at several points in the multistep cancer process. Dry beans contain 1–2% phytate, and phytate has been proposed to inhibit colon cancer (25). Dry beans contain saponins (26), and saponins have been shown to inhibit the development of aberrant crypt foci (27) in the colon. It is not possible from this study to determine the relative contribution of the bioactive microconstituents to the overall anticancer action elicited by eating beans. It is likely that the protease inhibitors, phytate, and saponins in beans were in part responsible for the inhibition of colon cancer.

Conclusions

In this type of research when foods (as opposed to chemicals) are used, it is not possible to identify a specific component of beans that is responsible for the observed reduction in colon cancer. The reduction in colon carcinogenesis in rats fed beans was attributed to 1) more controlled appetites, leading to less body fat, 2) much greater concentrations of butyrate in the distal colon, and 3) the presence of anticancer microconstituents in beans. The key finding from this research is that eating black beans and navy beans significantly lowered colon cancer incidence and tumor multiplicity.

Acknowledgments and Notes

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References


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