Independent Effects of Fiber and Protein on Colonic Luminal Ammonia Concentration[1]

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ABSTRACT The potential interactive effects of protein and fiber on cecal and colonic surface areas, colonic luminal ammonia concentrations, luminal pH and blood indices of nitrogen metabolism were tested using two levels of protein (8% and 24%) and two types of fiber (8% pectin or cellulose). Pectin supplementation resulted in larger cecal surface areas and longer large intestines than those of rats fed fiber-free or cellulose-supplemented diets. All high protein diets resulted in total large bowel luminal ammonia ($NH_3 + NH_4^+$) concentrations that were twice as high as their low protein counterparts ($P < 0.05$). The effect of fiber on ammonia concentration depended on the fiber type. In the distal colon, pectin-fed animals had three times the ammonia concentration of the fiber-free animals, and 4–5 times the ammonia concentration of the cellulose-fed animals ($P < 0.001$). Blood urea nitrogen values were higher in the high protein than in the low protein groups ($P < 0.05$), and highest in the high protein/pectin animals ($P < 0.01$). This study clearly demonstrates that luminal ammonia concentration is dependent upon both protein level and fiber type, and that a fermentable fiber (pectin), rather than decreasing colonic ammonia concentrations, actually increases them several-fold. *J Nutr.* 119:235–241, 1989.

INDEXING KEY WORDS:

- *dietary fiber* • *ammonia* • *pectin*
- *cellulose* • *cecal surface area*

Of all the cancers, colon cancer appears to be the most affected by diet (2), with dietary fat considered promotive and certain dietary fibers protective against this disease (3). Protein is also positively correlated with colon cancer incidence (3) yet it has received less attention, in part because fat and protein are often found together in foods, making their effects hard to separate. However, when fat is kept constant and protein level varied, experimental colon cancer studies show greater numbers of tumors with higher levels of protein (4).

Although the mechanism by which protein may enhance colon tumorigenesis is not known, one hypothesis is that increased dietary protein results in increased colonic luminal ammonia concentrations and ammonia promotes tumorigenesis by stimulating cell proliferation (5). Dietary fiber may protect against colon cancer by diluting luminal ammonia in a bulky stool (6), or speeding its passage through the colon (7). In addition, fermentable fibers may lower colonic pH (8), and since ammonia absorption from the large intestine is primarily by nonionic diffusion (9), the more acidic the colonic contents the less absorption of ammonia should occur. Finally, given a suitable energy source (such as dietary fiber), bacteria are thought to scavenge ammonia nitrogen for protein synthesis thus acting as a "nitrogen sink" (10). In theory, then, it may be possible to counteract the potentially negative effects of a high protein diet by the addition of the appropriate type and amount of dietary fiber. This study tests for potential interactions between protein and fiber on in vivo ammonia concentration and compares the results to those of feeding a fiber-free control diet.

MATERIALS AND METHODS

Animals and diets. Sixty male Sprague-Dawley rats (Harlan Sprague-Dawley, Houston, TX), approximately 114 d old and weighing 280–300 g were randomly and equally assigned to one of six diets (10 animals per diet) and housed individually in suspended, wire mesh-bottomed cages. They were maintained in temperature and humidity-controlled animal facilities on a daily photoperiod of 12 h light and dark. The basal fiber-free low and high protein diets are shown in **Table 1**. An iso-

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TABLE 1

Composition of basal fiber-free control diets

Ingredient[1]	Low Protein	High Protein
	%	
Glucose	75.2	59.2
Casein	8.0	24.0
L-Methionine	0.4	0.4
American Blend Fat[2]	8.0	8.0
Mineral mix	6.0	6.0
Vitamin mix	2.0	2.0
Choline bitartrate	0.4	0.4
Total	100.0	100.0

[1]The source of each diet component was: D-(+)anhydrous dextrose (glucose), #14535, U.S. Biochemical, Cleveland, OH; Vitamin-Free test casein, #160040, Teklad, Madison, WI; crystalline L-methionine, #18910, U. S. Biochemical; American Blend Fat, supplied by the Institute of Shortening and Edible Oils, gift of Proctor & Gamble, Cincinnati, OH; AIN-76A mineral mixture, (11), #905455, ICN Nutritional Biochemicals, Cleveland OH; AIN-76A vitamin mixture (11,12), #80001, BioServ, Frenchtown, NJ; choline bitartrate, #0166H, BioServ.

[2]The composition of the American Blend Fat is as follows: tallow, 27%; butterfat, 15%; lard, 13%; partially hydrogenated soybean and palm oils (Crisco shortening) 27%; partially hydrogenated soybean oil (Crisco oil), 9%; cottonseed oil, 3%; peanut oil, 5%; corn oil, 1%.

energetic substitution was made of casein at the expense of glucose; hence, the only difference between the two basal diets was the protein/carbohydrate ratio. The fiber-supplemented diets (**Table 2**) were prepared by uniformly diluting each basal fiber-free diet by the addition of 8% cellulose or pectin. The diets were designed by this uniform dilution technique so that if rats had equivalent energy intakes, they would have equivalent amounts of all nutrients except fiber at each protein level. Food and water were allowed ad libitum. Food intakes and body weights were recorded weekly, 24-h fecal output was measured twice during the study. Diets were provided for 4 wk.

Blood collection and analyses. After receiving its respective diet for 4 wk, each non-fasted animal was anesthetized with ether. An incision was made along the linea alba to expose the cecum and the intestines. The intestines were placed on a sterile gauze pad moistened with 0.85% saline. Three ml blood was drawn from the vena cava 1 cm distal to the hepatic artery using a 22 gauge needle and a 3-cc syringe. The blood was refrigerated overnight and centrifuged at 1000 × *g* for 20 min. The serum was pipetted into a second tube, spun again to separate any contaminating red blood cells, and frozen until analysis. Serum albumin, creatinine, total serum protein and blood urea nitrogen were measured as indices of nitrogen metabolism by an automated method (Technicon SMA 12/60 Biochemical Analyzer, Technicon Industrial Systems, Tarrytown, NY).

TABLE 2

Composition of the experimental diets

Component[1]	Basal diets	Fiber supplement
	%	
Fiber-free	100	0
Cellulose	92	8
Pectin	92	8

[1]The source of each fiber supplement was: Avicel microcrystalline cellulose type pH-105, a gift from FMC, Philadelphia, PA; citrus pectin, #0312H, BioServ, Frenchtown, NJ.

In vivo pH measurements. In vivo pH measurements of intestinal contents were taken as previously described (8). A glass electrode (MI-710 tiny combination pH probe, Microelectrodes, Londonderry, NH) was inserted through a 5-mm incision in the bowel wall in the cecum 1 cm distal to the cecal-proximal colon junction (called proximal colon) and at the pelvic ridge (called distal colon). Readings were recorded using a pH meter (Orion 811, Orion Research, Cambridge, MA). The measurements were made within 1 min while the animal was alive. After recording the measurements, the animal was killed by bilateral cutting of the diaphragm. All animals in this experiment were killed between 0900 and 1300 h in order to minimize any major artifacts arising from diurnal variation.

Cecal measurements and large intestine lengths. The cecum and the large intestine were quickly removed. The large intestine was measured and divided in half (called proximal and distal colon). Ceca were resected at the ileocecal valve and the cecal/proximal colon junction, flushed clean with oxygenated ice-cold phosphate buffered saline (NaH_2PO_4, 1.9 mM; Na_2HPO, 8.4 mM; Nacl, 145.4 mM, pH 7.4) opened along the greater curvature and pinned flat on dental wax. The perimeter was marked on the wax and transferred to tracing paper. These tracings were analyzed using a digitizing tablet (Videoplan IV, Carl Zeiss, Thornwood, NY).

Ammonia analyses. The contents of the cecum and of the proximal and distal colon were placed in individually labeled vials with tightly fitting lids. For each analysis, 0.5 g of sample was used. To each 0.5 g sample, 15 ml of 0.1 M HCl was added. The vials were vigorously shaken for 20 sec to extract the ammonium ions. Due to the great amount of undissolved material in the cecal solutions, these were filtered through fast filter paper. Each solution was poured into a 20 ml beaker. With constant stirring, the pH was raised to between 10.6 and 11.0 using 10 *N* NaOH. At this pH, 94% of the ammonium ions would have been deionized to the ammonia form, yet protein should not have been deaminated (13). Readings were taken with an ammonia electrode (Orion 95-12, Orion Research, Cambridge, MA) interfaced to a pH meter (Orion 811 pH meter, Orion Research, Cambridge, MA). When the value was stabilized (usually at 2 min) the change in electrode potential was recorded. Values were compared to appropriate calibration curves, established daily.

Data analysis. The data were analyzed by a one-way analysis of variance technique, keeping results from different animals distinct. When F values were significant ($P < 0.05$), homogeneous subgroups were identified using Duncan's Multiple-Range Test. Protein/fiber interactions were tested using a Chi Square Test of Independence.

RESULTS

Food intake and weight gain. **Table 3** shows final body weights and food and energy intake data recorded the week the animals were killed. The cellulose/high protein animals ate a greater weight of food than the fiber-free/high protein animals. However, when the energy density of the diet was considered (cellulose and pectin 15.56 kJ/g, fiber-free 16.90 kJ/g) there were no differences in energy intake. There were no significant differences in rat body weight at any weighing with final body weights shown in Table 3.

Fecal output. There were highly significant differences in fecal output among the diet treatments (**Fig. 1**), with cellulose resulting in 24-h fecal collections approximately 400% heavier than the fiber-free/high protein group.

Cecal size and large intestine length. **Table 4** shows the effect of fiber and protein on cecal size and large intestine length. There was an interactive effect between protein and fiber on cecal surface area, with cecal surface areas from rats fed pectin/high and low protein diets being larger than those from rats fed fiber-free or cellulose-supplemented diets. In addition, pectin/high protein resulted in larger cecal surface areas than pectin/low protein. Pectin supplementation also resulted in longer large intestines than those from rats receiving the fiber-free low protein diet.

TABLE 3

Effect of fiber type and protein level on food and energy intake and body weight[1]

Diet	24-h Food Intake Final Week	24-h Energy Intake Final Week	Final Body Weight
	g	*kJ*	*g*
Fiber-free/ low protein	22.4 ± 1.1[a,b]	386 ± 19[a]	349 ± 5[a]
Fiber-free/ high protein	19.7 ± 0.9[a]	338 ± 16[a]	361 ± 4[a]
Cellulose/ low protein	22.2 ± 0.9[a,b]	350 ± 15[a]	351 ± 6[a]
Cellulose/ high protein	23.9 ± 1.2[b]	375 ± 26[a]	356 ± 6[a]
Pectin/ low protein	22.4 ± 1.3[a,b]	354 ± 20[a]	349 ± 7[a]
Pectin/ high protein	22.4 ± 1.5[a,b]	359 ± 18[a]	356 ± 7[a]

[1]Results are expressed as means ± SEM on a sample size of 10 rats/group. Energy density of each diet is described in the text. In each column, means not sharing the same letter are significantly different ($P < 0.05$).

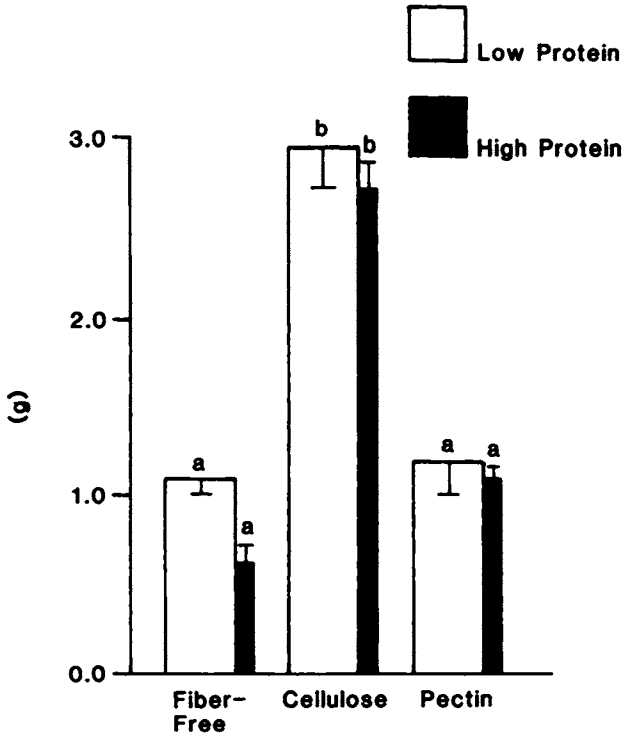

FIGURE 1 Effect of protein level and fiber type on the wet weight of a 24-h fecal collection, taken after rats had received their diets for 3 wk. Each bar represents the mean for 10 animals. Small vertical bars indicate SEM. Means not sharing the same superscript are statistically different from each other ($P < 0.05$).

Luminal pH. **Figure 2** shows the effect of diet on luminal pH throughout the large intestine. In the cecum, the two pectin diets had a significantly lower pH than the others. This remained true in the proximal colon, except that pectin was no longer significantly lower than the fiber-free high protein diet. There were no significant differences in pH values among diets in the distal colon.

Serum indices of nitrogen metabolism. Although serum albumin, creatinine and protein levels were similar with all diets, protein level had a highly significant effect on blood urea nitrogen (BUN) (**Table 5**). This was particularly striking with the two pectin groups, where pectin/high protein had BUN values that were five times greater than pectin/low protein ($P < 0.001$).

Luminal ammonia. **Figure 3** shows the effect of protein and fiber on ammonia + ammonium. Since the ratio of ionized to unionized ammonia is pH dependent and the pK of ammonia is 9.26, at the mean in vivo pH of 7.2 found in this study (see Fig. 2) 99% would be in the ionized form. At the highest pH values of 7.8 (found in the distal colon) approximately 97% would be ionized, and with the lowest pH values, found with certain diets, in the cecum and proximal colon, more than 99% of ammonia + ammonium would exist as the ammonium ion.

TABLE 4

Effect of fiber type and protein level on cecal size and large intestine length[1]

Diet	Cecal Surface Area	Large Intestine Length
	mm^3	*cm*
Fiber-free/ low protein	1041 ± 103[a]	17.0 ± 0.5[a]
Fiber-free/ high protein	1118 ± 110[a]	18.1 ± 0.7[a,b]
Cellulose/ low protein	1070 ± 72[a]	18.4 ± 0.6[b,c]
Cellulose/ high protein	1099 ± 47[a]	18.3 ± 0.5[a,b]
Pectin/ low protein	1483 ± 139[b]	19.8 ± 0.5[c]
Pectin/ high protein	1995 ± 128[c]	19.1 ± 0.2[b,c]

[1]Results are expressed as means ± SEM on a sample size of 10 rats/group. In each column, means not sharing the same letter are significantly different ($P < 0.05$).

Both protein and fiber independently affected luminal ammonia + ammonium values, with all high protein diets resulting in concentrations greater than that of their low protein counterparts, at every site. Of the fibers, the highly fermentable pectin resulted in the most striking effects. In the cecum, both pectin diets lowered ammonia, with the pectin/high protein diet resulting in similar values to those from the fiber-free/low protein and cellulose/low protein diets. The pectin/low protein diet produced an ammonia concentration significantly lower than all other treatments at this site. However, this was not the case in the proximal colon. At this site, the pectin/high protein group had higher ammonia concentrations than all other diets except fiber-free/high protein. The pectin/low protein diet produced higher ammonia concentrations than the other low protein treatments. Pectin/low protein was similar to cellulose/high protein in its effect on luminal ammonia. In the distal colon, both pectin diets (high and low protein) resulted in ammonia concentrations that were higher than all other treatments. At each protein level, the pectin-supplemented animals had almost three times greater ammonia concentrations than the fiber-free group, and five times greater concentrations as compared to those supplemented with cellulose.

DISCUSSION

This study clearly demonstrates that both protein level and fiber type affect the concentration of ammonia in colonic contents. The rats fed high protein diets had about twice the ammonia concentration in the large intestine as their low protein–fed counterparts. This was not unexpected since several studies

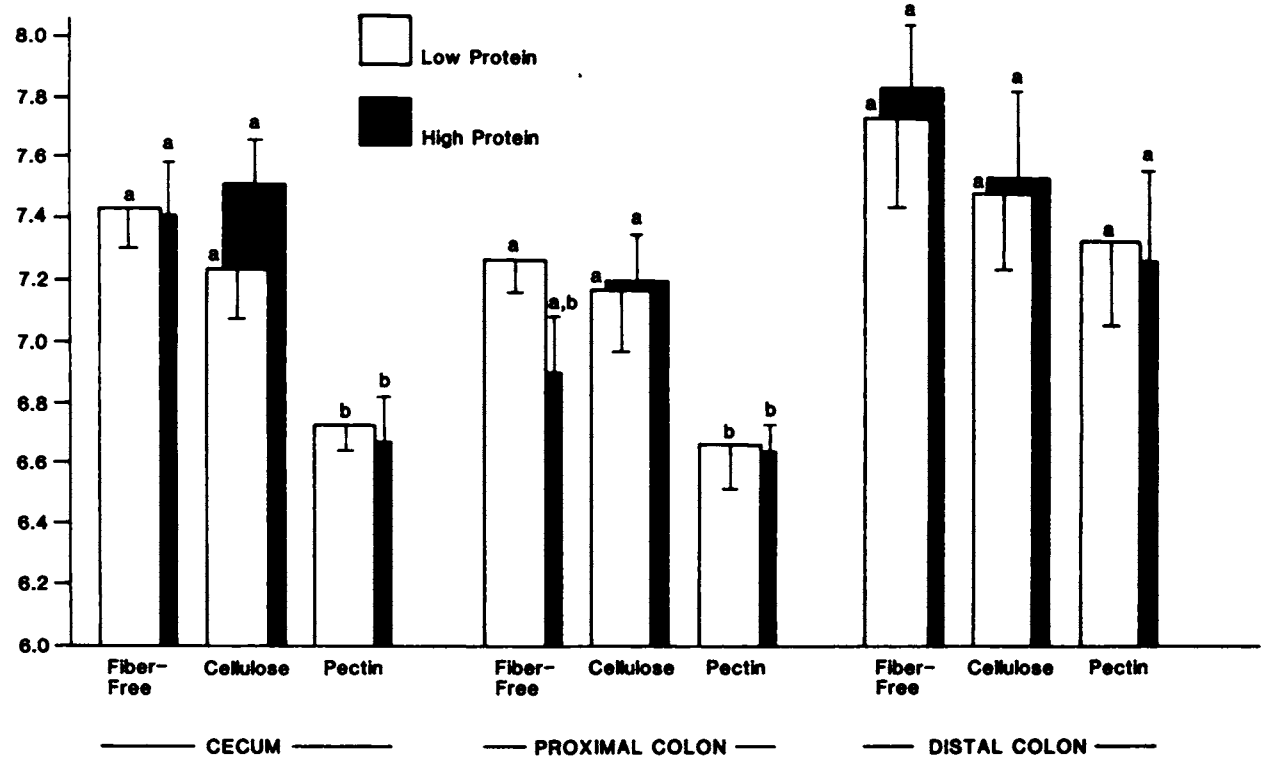

FIGURE 2 Effect of protein level and fiber type on the pH of large bowel contents in the cecum, proximal and distal colon. In vivo pH measurements were taken as described in the text. Each bar represents the mean for 10 animals. Small vertical bars indicate SEM. At each anatomical site, means not sharing the same superscript are statistically different from each other ($P < 0.05$).

TABLE 5

Effect of fiber type and protein level on serum indices of nitrogen metabolism[1]

Diet	Albumin	Creatinine	Protein	Blood Urea Nitrogen
	g/100 ml	*mg/100 ml*	*g/100 ml*	*mg/100 ml*
Fiber-free/low protein	3.9 ± 0.1[a]	0.7 ± 0.1[a]	6.2 ± 0.2[a]	7 ± 1[a,b]
Fiber-free/high protein	4.1 ± 0.1[a]	0.7 ± 0.1[a]	6.4 ± 0.1[a]	15 ± 4[c,d]
Cellulose/low protein	4.1 ± 0.1[a]	0.7 ± 0.1[a]	6.2 ± 0.2[a]	7 ± 2[a,b]
Cellulose/high protein	4.1 ± 0.2[a]	0.6 ± 0.1[a]	6.5 ± 0.2[a]	13 ± 4[b,c]
Pectin/low protein	4.2 ± 0.1[a]	0.5 ± 0.1[a]	6.2 ± 0.1[a]	4 ± 1[a]
Pectin/high protein	4.1 ± 0.1[a]	0.7 ± 0.1[a]	6.5 ± 0.1[a]	21 ± 1[d]

[1]Results are expressed as means ± SEM on a sample size of 10 rats/group. In each column, means not sharing the same letter are significantly different ($P < 0.05$).

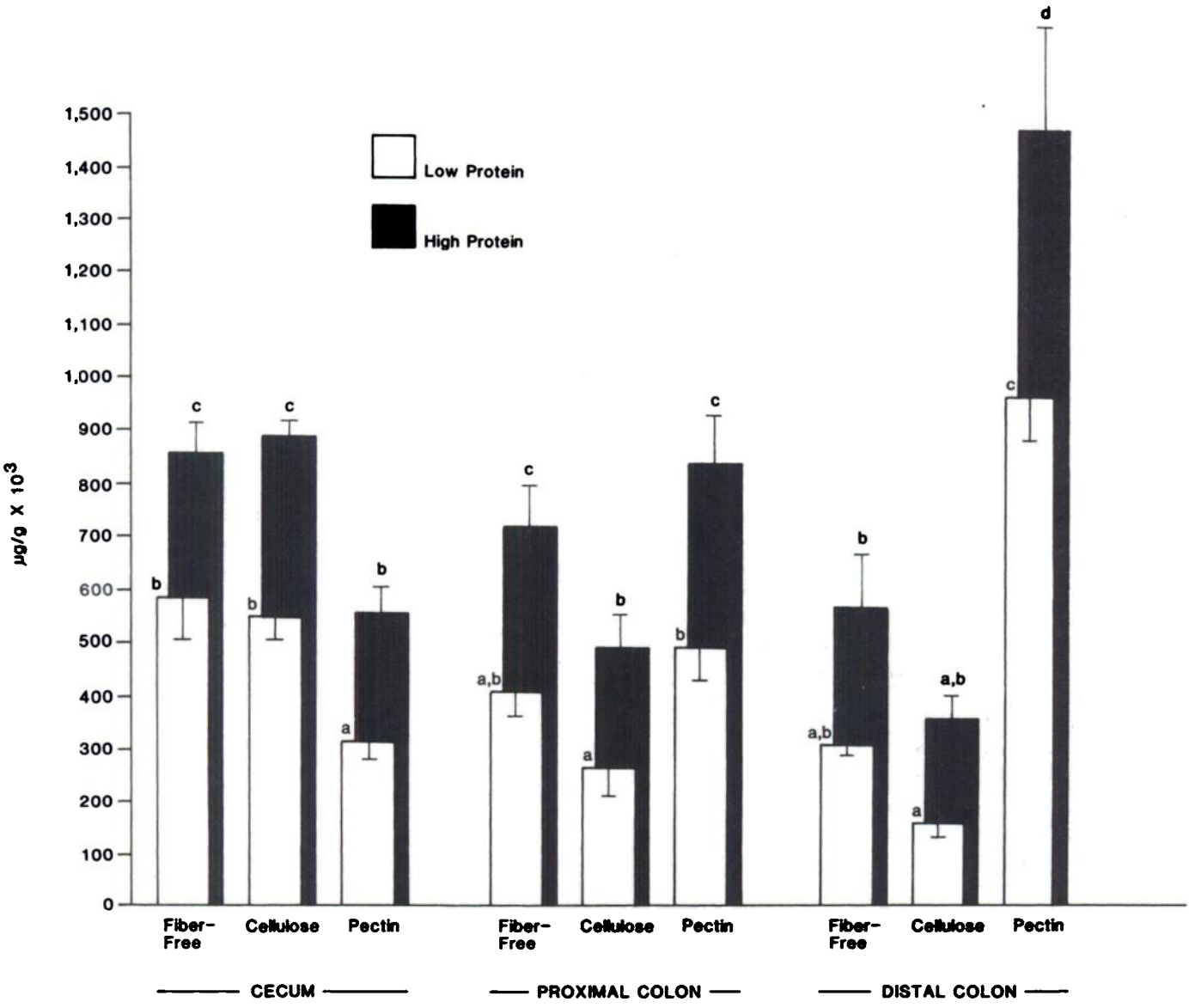

FIGURE 3 Effect of protein level and fiber type on ammonia + ammonium/g fecal material in large bowel contents in the cecum, proximal and distal colon. Ammonia/ammonium measurements were taken as described in the text. Each bar represents the mean for 10 animals. Small vertical bars indicate SEM. At each anatomical site, means not sharing the same superscript are statistically different from each other ($P < 0.05$).

have shown that the quantity and quality of protein in the diet will determine the quantity of protein in the large bowel (14) which is then available for deamination by the colonic microflora.

What was unexpected, however, was the effect of the fermentable fiber, pectin, on colonic ammonia. The one study reporting on cecal ammonia with fiber supplementation found, as we did, that fermentable fiber lowered ammonia concentrations in the cecum (15). From this, they and others (10) have suggested that the addition of fermentable fiber to the diet should lower in vivo ammonia by providing substrate for bacterial growth. By incorporating ammonia into bacterial protein, the colonic microflora would act as a "nitrogen sink." However, it is clear from this study that pectin does not lower ammonia in the colon. In the distal colon, at each protein level the pectin-fed animals had almost three times greater ammonia concentrations than the fiber-free group, and five times greater concentrations than the group fed poorly fermentable cellulose.

Part of the increase in large bowel ammonia with pectin supplementation may be due to decreased protein absorption from the small intestine and thus a greater amount of protein reaching the large intestine (16). Pectin may also affect ammonia concentration by increasing the numbers of colonic microorganisms. The microorganisms deaminate protein and also produce urease for the hydrolysis of urea to ammonia/ammonium. Studies comparing conventional and germ-free animals show that those with a microflora hydrolyze more urea (17) and have greater amounts of ammonia in the portal blood (18) than their germ-free counterparts. Thus, an increase in the bacterial population with pectin supplementation should result in increased ammonia due to increased deamination of dietary protein and increased urease activity. The pectin/high protein diet also resulted in higher levels of BUN than all other diets except the fiber-free/high protein diet (Table 5). This was presumably due to a combination of urea synthesized in the liver from deaminated proteins and from ammonia produced in the large intestine and transported to the liver via the portal vein.

While pectin-supplemented diets resulted in higher colonic ammonia concentrations than controls, cellulose had the opposite effect. This can be explained because cellulose, unlike pectin, should not increase bacterial mass, since it is poorly fermented. In addition, cellulose, unlike pectin, is an excellent bulking agent (6), and in this study produced 24-h fecal outputs greater than fiber-free controls or pectin-supplemented animals (Figure 1) which should result in a dilution of luminal ammonia.

The question remains as to why pectin decreased ammonia in the cecum but increased it in the colon. One possible explanation is the effect pectin had on cecal surface area (Table 4). Both the low and high protein pectin-supplemented diets increased cecal surface area (143% and 178% respectively) over their fiber-free counterparts. One consequence of increased surface area is increased absorptive capacity which could mean that ammonia produced in the cecum would be rapidly absorbed.

If colonic luminal ammonia concentrations are correlated with large bowel tumorigenesis, then this study suggests that the most protective diet would be one low in protein and high in cellulose. In studies testing the effect of protein level on experimentally-induced colon cancer, low protein diets have been shown to exert a protective effect (4, 19). Likewise, poorly fermented fibers such as cellulose appear to lower tumor incidence, whereas in most studies using pectin, tumor enhancement has occurred (19, 21). Our data are therefore consistent with the experimental colon cancer literature. Whether or not this is simply a correlation or ammonia stimulates colonic mucosal cell proliferation and thus promotes tumorigenesis remains to be tested.

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