Nutrient Requirements and Interactions

Feeding Diets Containing High Levels of Milk Products or Cellulose Decrease Urease Activity and Ammonia Production in Rat Intestine[1,2,3]

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ABSTRACT Three studies were done to determine the effect of feeding diets containing high levels of a readily fermentable carbohydrate (lactose in milk or yogurt, or pure lactose) or an undigestible, unfermentable diluent (α-cellulose) on urease (EC 3.5.1.5) activity and net ammonia production in the rat gastrointestinal (GI) contents. Rats (170–200 g) were fed a control diet or diets containing 55% dried milk or 55% dried yogurt, 25% lactose or 10% α-cellulose. Feeding diets containing milk or yogurt decreased urease activity to ~11% of the control value in the small intestine (on the basis of grams of collected contents or total contents), and to 50% in the large intestine (only on the basis of grams of collected contents). Feeding the diet containing 25% lactose also decreased urease activity (on the basis of grams of collected contents or total contents) to about 20% of the control value in the small intestine, but not ($P > 0.05$) in the large intestine. Net ammonia production rate was correlated ($r^2 = 0.98$) with urease activity in the large intestinal contents, and the rate of ammonia production from ureolysis represented about two thirds of the total. Feeding the cellulose diet decreased ($P < 0.05$) both urease activity and net ammonia production in the large intestine to ~30% of the control value. Weights of tissue and contents of the large intestine were much higher ($P < 0.01$) in rats fed diets containing milk products or lactose than in the control rats, but were not affected by consumption of the cellulose diet. Results of our studies indicate that feeding diets containing high levels of milk products (lactose) or cellulose reduces urease activity and net ammonia production in the rat intestine, and thus may be beneficial for improving animal and human health. J. Nutr. 128: 1186–1191, 1998.

KEY WORDS: • *rats* • *urease activity* • *ammonia production* • *milk products* • *cellulose*

Ammonia (including ammonium ion) produced from the amino acid degradation in the body is converted to urea in the mammalian liver, 20–25% of which is excreted into the gastrointestinal (GI) tract in humans and rats, and hydrolyzed into ammonia by microbial urease (Wrong et al. 1981). This ammonia, together with that produced by bacteria acting on other nitrogenous substrates, may be used for microbial protein synthesis or may enter the blood stream.

Many intestinal bacteria (e.g., bacteroides, clostridia, *Proteus* spp. and *Klebsiella* spp.) produce urease, whereas others (e.g., *Escherichia coli*) produce no urease but other ammonia-generating enzymes. However, most *lactobacillus* strains produce little urease or other ammonia-generating enzymes (Macbeth et al. 1965).

Urease-producing bacteria inhabiting the GI tract are important in both their nutritional and pathological aspects because they are involved in nitrogen recycling, and the resulting product, ammonia, can be harmful to animal health. The growth-promoting effects of subtherapeutic-level antibiotics (see the review by Visek 1978a) or probiotics (Yeo and Kim 1997) used in animal feeds as growth promotants have been ascribed to suppression of urea hydrolysis and subsequently reduced ammonia production in the GI tract. Urease immunization (Visek 1978a) has also been suggested as a means of improving the growth of farm animals.

Lactulose and other fermentable substrates also can decrease ammonia production in a fecal incubation (Vince et al. 1978) and thus have been suggested for use in the treatment of hepatic encephalopathy (Weber 1979). Undigested lactose in the small intestine becomes subject to microbial fermentation in the lower part of the intestine, producing mainly lactic acid and short-chain fatty acids, and in turn lowering the pH of the contents in nonruminants (Kim et al. 1979). Lowered pH reduces ammonia production by intestinal bacteria (Vince et al. 1978).

No studies have shown that dietary milk products or cellulose decreases urease activity in the GI tract of nonruminant animals. Furthermore, most studies of urease activity measured ammonia production in the feces or in the contents of the GI tract and overlooked quantitative relationships between urease

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TABLE 1

Composition of experimental diets

Ingredient	Control[1]	Exp. 1 Milk	Exp. 1 Yogurt	Exp. 2 Lactose	Exp. 3 Cellulose
			g/kg		
Ingredient					
Casein (vitamin-free)[2]	200	—	—	200	200
Milk (lyophilized)[3]	—	550	—	—	—
Yogurt (lyophilized)[3]	—	—	550	—	—
L-Methionine[2]	3	3	3	3	3
Corn oil[4]	50	50	50	50	50
Cornstarch[5]	690	310	240	440	590
Lactose[6]	—	30	100	250	—
α-Cellulose[7]	—	—	—	—	100
Vitamin mix[8]	5	5	5	5	5
Choline chloride[9]	2	2	2	2	2
Salt mix[8]	50	50	50	50	50
Chemical composition					
Crude protein,[10] %	17.6	17.6	16.5	17.6	17.6
Gross energy,[10] *MJ/kg*	15.9	15.9	15.9	15.9	15.9

[1] The diet was used as the control for Experiments 1, 2 and 3.
[2] U.S. Biochemical, Cleveland, OH.
[3] Produced from 2%-fat milk at the University of Wisconsin Dairy Plant, Madison, WI.
[4] Archer Daniels Midland, Decatur, IL (Exps. 1 and 2) or Jeil Jedang, Seoul, Korea (Exp. 3).
[5] Harlan Teklad, Madison, WI (Exps. 1 and 2) or Sunil Pododang, Seoul, Korea (Exp. 3).
[6] Mallincrodt, St. Louis, MO.
[7] Sigma Chemical, St. Louis, MO.
[8] AIN 76 (AIN 1977).
[9] Fisher Scientific, Fair Lawn, NJ.
[10] Calculated.

activity and ammonia production. Taking these considerations into account, we assessed the effect of feeding diets containing a high level of fermentable carbohydrate (lactose in the form of milk, yogurt or pure lactose) or an undigestible, unfermentable diluent (α-cellulose) on urease activity and ammonia production in the GI contents of adult rats.

MATERIALS AND METHODS

Animals and diets. In Experiment 1, a group of five Holtzman (Harlan Sprague Dawley, Madison, WI) male rats (mean weight, 170 g) was assigned to each of the following diets: a control diet, or diets containing 55% dried milk or 55% dried yogurt (both diets were formulated to contain 25% lactose). In Experiment 2, a group of six Holtzman male rats (170 g) was assigned to a control diet, or a diet containing 25% lactose as a source of readily fermentable carbohydrate. For Experiment 3, a group of six Sprague-Dawley (Korea Institute of Chemistry, Tae-Jun, Korea) male rats (200 g) was assigned to a control diet or a diet containing 10% α-cellulose as a bulking agent. All of the diets were formulated to be isonitrogenous and isocaloric (gross energy); their composition is shown in **Table 1.** Rats were housed individually in suspended wire cages in a room maintained at 23°C with a 12-h light (0600–1800 h) and 12-h dark cycle (1800–0600 h). Rats had free access to water and diets for at least 7 d before being killed. All animal management and sampling procedures were in accord with the NIH guidelines (NRC 1985).

Incubation of the GI contents. After the experimental diets were fed, two rats from different treatments were killed daily between 1100 and 1500 h until all of the rats were used. The contents of the stomach, small intestine and large intestine (cecum + colon) were collected (only large intestine in Experiment 3) in 50-mL centrifuge tubes, weighed and diluted 1:2 (wt/v) with 0.2 mol/L sodium phosphate buffer (pH 6.5). For collection of small intestinal contents, the intestine was slit and homogenized with a Potter-Elvehjem homogenizer in ice; the homogenate (excluding a small piece of serosal residue) was used to recover bacteria attached to the mucosal layer as well as those in the contents.

If enough samples were available, two 3-mL samples of diluted contents were transferred into 15-mL centrifuge tubes, and 1 mL of 0.4 mol/L urea containing 3.7 kBq of [^{14}C]urea (74 MBq/mmol; New England Nuclear, Boston, MA) was added to one sample. (Added urea concentration in the 4-mL final volume was 100 mmol/L.) This sample was then incubated in a shaking water bath at 37°C for 30 min while being flushed with N_2 for the first 2 min; each unit was then sealed with clamps. Incubation of the contents from the large intestine, small intestine and stomach was initiated within ~10, 15 and 20 min after rats were killed, respectively.

To the other sample was added 1 mL of 0.4 mol/L urea containing no [^{14}C]urea; the mixture was inactivated with 0.4 mL of 3 mol/L H_2SO_4 before incubation and served as blank for determination of ammonia production. In a preliminary study, negligible radioactivity was recovered in CO_2 from the blank to which [^{14}C]urea was added; therefore we made no attempt to recover CO_2 from blanks during incubation. When the sample volumes were insufficient, only 1.5 mL of diluted samples was used as a blank after 0.5 mL of 0.4 mol/L urea and 0.2 mL of 3 mol/L H_2SO_4 were added.

At the end of the 30-min incubation, an air stream was pulled through the reaction chamber and past a CO_2 trap containing 5 mL of a mixture of ethanolamine and ethylene glycol monomethyl ether (1:2, v/v). Over a 20-min period, CO_2 released was trapped by use of a gas dispersion tube while 0.4 mL of 3 mol/L H_2SO_4 was added to the inlet tube of the reaction chamber to stop the reaction and release CO_2. This technique allowed >98% recovery of radioactivity of $Na_2{}^{14}CO_3$, which was added to the reaction chamber and acidified. During the 30-min incubation, CO_2 production from urea was linear.

The acid-inactivated samples (blanks and incubated samples) from Experiments 2 and 3 were centrifuged at 15,000 × g for 10 min; the supernatants were collected into plastic vials and stored at 4°C for later analysis of ammonia.

Determination of urease (EC 3.5.1.5) activity. Radioactivity in the 1-mL CO_2 trap was determined in 15 mL of Bio-Safe II (Research

TABLE 2

Effect of feeding diets containing casein, dried milk or dried yogurt on weight of gastrointestinal tract and urease activity in the GI contents of rats (Experiment 1)[1]

Diet	Control	Milk	Yogurt
Stomach			
Tissue, *g*	1.24 ± 0.05	1.43 ± 0.05	1.37 ± 0.05
Contents,[2] *g*	1.55 ± 0.42[b]	2.70 ± 0.90[b]	4.43 ± 0.54[a]
Urease activity, units[3]/			
g Collected contents	15.2 ± 4.3	20.7 ± 5.1	6.8 ± 1.8
Total contents	29.3 ± 10.8	47.1 ± 16.5	30.8 ± 9.8
Small intestine[4]			
Serosal tissue,[5] *g*	1.36 ± 0.18	1.67 ± 0.10	1.49 ± 0.11
Contents,[6] *g*	4.34 ± 0.20[b]	6.33 ± 0.48[a]	7.10 ± 0.14[a]
Urease activity, units/			
g Collected contents[2]	17.1 ± 5.1[a]	1.4 ± 0.2[b]	1.1 ± 0.4[b]
Total contents[2]	88.2 ± 28.2[a]	10.0 ± 1.4[b]	8.4 ± 3.2[b]
Large intestine			
Tissue,[6] *g*	0.99 ± 0.06[b]	1.60 ± 0.05[a]	1.52 ± 0.12[a]
Contents,[6] *g*	2.39 ± 0.13[b]	4.87 ± 0.39[a]	4.65 ± 0.18[a]
Urease activity, units/			
g Collected contents[2]	112.6 ± 17.3[a]	50.6 ± 11.0[b]	39.5 ± 15.3[b]
Total contents	272.2 ± 43.2	260.4 ± 68.6	182.8 ± 74.0

[1] Values are means ± SEM, n = 4 or 5.
[2] Values within the same row bearing different superscript letters differ ($P < 0.05$).
[3] A unit of urease activity was defined as μmol urea hydrolyzed/30 min at 37°C.
[4] Small intestinal contents included mucosa (see Materials and Methods for details).
[5] Tissue residue after the slit intestine was homogenized.
[6] Values within the same row bearing different superscript letters differ ($P < 0.01$).

Products International, Mount Prospect, IL) by using a Liquid Scintillation Counter (Tricarb 4000, Packard Instrument, Ltd, Downers Grove, IL or model 1220, Quantulus, Wallac, Turku, Finland). Total radioactivity in the 5-mL CO_2 trap was calculated. Urease activity (μmol/30 min per gram collected contents or in the total contents) was calculated by dividing radioactivity (Bq) recovered in CO_2 during the 30-min incubation by specific radioactivity (Bq/μmol) of urea added to samples, assuming that no significant amount of urea was present in the contents (Combe et al. 1965). This calculated urease activity was divided by 0.9 to correct for the unrecovered CO_2 (~10% on average, ranging from 8 to 13%) after the 30-min incubation. The unrecovered CO_2 (10%) was observed in a preliminary experiment when $Na_2{}^{14}CO_3$ was added to large intestinal content samples and incubated for 30 min. This unrecovered CO_2 was presumably used for microbial metabolism.

Determination of ammonia production. Net ammonia production during the 30-min incubation was calculated from the difference between the amounts of ammonia in the blank and the incubated sample in Experiments 2 and 3. Ammonia-N (including ammonium ion) concentrations in the 15,000 × *g* supernatants of both blanks and incubated samples were determined by using a modified method of Weatherburn (1967).

Statistical analysis. Data were analyzed by ANOVA with the General Linear Models procedures (SAS 1988). The main source of variation for all variables was dietary treatment in the model. When the F-value was significant ($P < 0.05$), the Newman-Keuls test was applied to compare individual means (Snedecor and Cochran 1980). The linear regression procedure of SAS (1986) was used to establish a relationship between net ammonia production and urease activity in Experiment 2.

RESULTS

Effect of feeding diets containing 20% casein, 55% dried milk or 55% dried yogurt on urease activity in the stomach, small and large intestine of rats. *Experiment 1.* Urease activity in the stomach contents was not influenced by diets (**Table 2**) but activity in the small intestinal contents that include mucosa was significantly ($P < 0.01$) lower in rats fed diets containing milk or yogurt compared with that in rats fed the control diet containing 20% casein. Urease activity in the large intestinal contents was lower ($P < 0.05$) in rats fed diets containing the milk products than in control rats when expressed per gram of collected contents, but urease activity in the total contents was not significantly different ($P = 0.08$) due to a greater weight of the contents in rats fed the milk or yogurt diet. Weights of tissue and contents of the large intestine were much higher ($P < 0.01$) in rats fed the milk or yogurt diet than in control rats. Weights of the stomach and small intestinal contents were also greater in rats fed diets containing milk or yogurt compared with those in control rats.

Effect of feeding a diet containing 25% lactose on urease activity and ammonia production in the stomach, small and large intestine of rats. *Experiment 2.* Urease activity in the small intestinal contents was significantly ($P < 0.05$) lower in rats fed the diet containing 25% lactose compared with that found in control rats (**Table 3**). Urease activity (Table 3) and net ammonia production (**Table 4**) in the large intestinal contents (on the basis of grams of collected contents) of rats fed diet containing lactose was approximately half that found in control rats, although the difference was not significant ($P > 0.05$). On the basis of total large intestinal contents, urease activity was higher in rats fed the lactose diet than in control rats because of a greater weight of the contents in rats fed the lactose diet.

Net ammonia production rates (y), expressed as μmol/30 min per gram of collected contents of the large intestine, were correlated with 2 × urease activity[5] (x): $y = 1.63x + 33.3$ ($r^2 = 0.99$) and $y = 1.34x + 36.8$ ($r^2 = 0.98$) for control rats and rats fed diet containing lactose, respectively. Similar to rats fed diets containing milk or yogurt, those fed the lactose diet

[5] Urease activity was multiplied by the factor two because one mole of urea produces two moles of ammonia upon hydrolysis.

TABLE 3

Effect of feeding diet containing 25% lactose on weight of gastrointestinal tract (GIT) and urease activity in the GI contents of rats (Experiment 2)[1]

Diet	Control	Lactose
Stomach		
Tissue, *g*	1.37 ± 0.03	1.33 ± 0.03
Contents, *g*	2.48 ± 0.35	3.40 ± 0.70
Urease activity, units[2]/		
g Collected contents	3.14 ± 1.23	2.11 ± 0.71
Total contents	8.76 ± 3.20	7.42 ± 2.92
Small intestine[3]		
Serosal tissue,[4] *g*	1.78 ± 0.16	1.82 ± 0.18
Contents, *g*	4.74 ± 0.27	5.84 ± 0.39
Urease activity, units/		
g Collected contents	3.94 ± 1.70	0.78 ± 0.23*
Total contents	18.3 ± 7.9	4.05 ± 1.20*
Large intestine		
Tissue, *g*	1.03 ± 0.06	1.78 ± 0.07*
Contents, *g*	2.17 ± 0.13	5.36 ± 0.46**
Urease activity, units/		
g Collected contents	54.0 ± 16.1	28.5 ± 10.7
Total contents	112.1 ± 28.5	162.2 ± 69.3

[1] Values are means ± SEM, $n = 6$.
[2] A unit of urease activity was defined as μmol urea hydrolyzed/30 min at 37°C.
[3] See footnote 4 to Table 2.
[4] Tissue residue after the slit intestine was homogenized.
* Values differ ($P < 0.05$) between the two diets.
** Values differ ($P < 0.01$) between the two diets.

had greater weights of large intestine and its contents ($P < 0.01$) than did control rats.

Effect of feeding a diet containing 10% α-cellulose on urease activity and ammonia production in the large intestine of rats. *Experiment* 3. Urease activity and net ammonia production (on the basis of grams of collected contents or total contents) in the large intestine of rats fed a diet containing 10% α-cellulose was approximately one third of the control value ($P < 0.05$; **Tables 5 and 6**), indicating that a high level of dietary cellulose is a potent suppressor of urease activity and ammonia production. Weights of tissue and contents of the large intestine were not affected by dietary cellulose (Table 5).

TABLE 4

Effect of feeding diet containing 25% lactose on ammonia production in the gastrointestinal (GI) contents of rats (Experiment 2)[1]

Diet	Control	Lactose
	μmol ammonia/g collected contents	
Stomach		
Before incubation	32.1 ± 5.0	35.5 ± 5.0
After incubation	49.1 ± 7.8	55.3 ± 15.5
Net production[2]	8.5 ± 3.6	5.2 ± 2.0
Small intestine		
Before incubation	15.4 ± 2.4	12.8 ± 2.8
After incubation	39.8 ± 8.1	35.4 ± 5.1
Net production[2]	24.7 ± 8.4	22.6 ± 5.6
Large intestine		
Before incubation	44.7 ± 4.4	42.9 ± 7.8
After incubation	254 ± 51	170 ± 40
Net production[2]	209 ± 53	125 ± 32

[1] Values are means ± SEM, $n = 6$.
[2] After − before 30-min incubation.

TABLE 5

Effect of feeding diet containing 10% α-cellulose on urease activity in the large intestinal contents of rats (Experiment 3)[1]

Diet	Control	Cellulose
Tissue, *g*	2.13 ± 0.21	2.18 ± 0.30
Contents, *g*	2.37 ± 0.19	2.62 ± 0.32
Urease activity, units[2]/		
g Collected contents	48.5 ± 8.2	14.0 ± 1.9*
Total contents	106.8 ± 15.8	37.0 ± 7.9*

[1] Values are means ± SEM, $n = 6$.
[2] A unit of urease activity was defined as μmol urea hydrolyzed/30 min at 37°C.
* Values differ ($P < 0.05$) between the two diets.

DISCUSSION

Consumption of milk or milk products has long been related to health and longevity in humans, and dietary fiber is also been known to play an important role in the health of the GI tract, especially the large intestine. One aspect of the beneficial effects of those components is their interaction with microbes in the GI tract. In these studies, we explored relationships between dietary milk products or cellulose, and microbial activity (urease activity and ammonia production) in the GI tract of rats.

Results of Experiment 1 (Table 2) suggest that part of lactose in the milk or yogurt diet passes through the small intestine undigested and enters the large intestine, as shown in our early study (Kim et al. 1978), in which we found that ~30–40% of lactose in a 30% lactose diet consumed by rats entered the large intestine undigested. This fermentable substrate promotes the growth of *lactobacilli*, most of which produce no urease or other ammonia-generating enzymes (Macbeth et al. 1965).

The ability of dietary milk products to decrease urease activity was remarkable in the small intestine, suggesting an additional benefit of consuming milk and yogurt, which promote the growth of *lactobacilli* (Hill 1983). Interestingly, an appreciable amount of urease activity was found in the stomach contents (Tables 2 and 3), even though the pH was low. Bacteria from coprophagy are thought to be responsible for urease activity in the stomach. Our earlier study (Kim et al. 1978) showed

TABLE 6

Effect of feeding diet containing 10% α-cellulose on ammonia production in the large intestinal contents of rats (Experiment 3)[1]

Diet	Control	Cellulose
	μmol ammonia/g collected contents	
Before incubation	58.5 ± 15.2	42.8 ± 4.6
After incubation	269.0 ± 31.3	129.1 ± 9.0*
Net production[2]	210.5 ± 29.8	86.3 ± 6.3*

[1] Values are means ± SEM, $n = 6$.
[2] After − before 30-min incubation.
* Values differ ($P < 0.05$) between the two diets.

that ~20% of the undigestible marker Cr-EDTA in the stomach of rats originated from coprophagy, indicating that a substantial amount of fecal material comprises the stomach contents. In that study (Kim et al. 1978), lactose hydrolytic activity was not found in the stomach. These findings of our studies indicate that urease producers survive in the stomach better than lactase producers.

Urease has been known to play an essential role in the pathogenesis of gastritis induced by *Helicobacter pylori*, and a urease-negative strain did not cause gastritis in gnotobiotic piglets (Eaton et al. 1991). Similarly, the generation of ammonia in rat stomach after instillation of urea in the presence of urease resulted in deleterious influences on the gastric mucosa, including stasis of microcirculation, disruption of the surface epithelial cells and necrosis of the mucosa (Murakami et al. 1990). These reports support our contention that ammonia that is locally produced by ureolysis in the intestinal mucosa can damage the surface cells. Therefore, decreasing urease activity and ammonia production may be beneficial for improving animal and human health.

Urease activity determined in rats used in Experiment 1 was more than twice that found in the other experiments (cf. data in Table 2 with those in Tables 3 and 5). Urease activity found in some rats was five times as high as that found in others in the same group, indicating a large variation among individuals. Our data suggest that the balance of the microbial population in the GI tract is unstable and can vary with environment, stress or infection as well as diet.

Findings of Experiment 2 (Table 3) are consistent with the hypothesis arising from Experiment 1 that part of the lactose in the milk or yogurt diet enters the large intestine unhydrolyzed (Kim et al. 1978), is fermented mainly into lactic acid and short-chain fatty acids (Kim et al. 1979) and promotes the growth of *lactobacillus* organisms (Hill 1983). However, suppression of urease activity in the large intestinal contents was less evident when pure lactose rather than milk or yogurt was used. The large intestinal contents were more diluted (cathartic) when rats were fed a diet containing pure lactose than when they were fed diets containing milk or yogurt (cf. the data in Tables 2 and 3). Lactose intolerance is more pronounced with pure lactose than with milk or yogurt when compared on an equimolar lactose basis (Kolars et al. 1984); thus, large intestinal contents are expected to be more variable in texture, volume and possibly microbial make-up among rats fed a diet containing pure lactose than among those fed diets containing milk or yogurt.

In a fecal incubation system, Vince et al. (1978) found that lactulose or other fermentable substrates (glucose, mannitol and sorbitol) in a medium decreased ammonia concentration. They attributed the decrease to either an increased microbial use of ammonia or a reduction in the deamination of nitrogenous compounds in conjunction with a lower pH, which reduces the growth of bacteria, especially ammonia producers.

Results of Experiment 2 showed that 39% ($100 \times 0.63/1.63$) and 25% ($100 \times 0.34/1.34$) of the net ammonia production in the large intestinal contents was not accounted for by ureolysis in control rats and rats fed the lactose diet, respectively. This ammonia production that was not accounted for by ureolysis perhaps resulted in part from hydrolysis of endogenous urea in the sample and also from deamination of non-urea nitrogen sources. In contrast to the report by Combe et al. (1965), a substantial amount of urea was detected in the contents of the small intestine and cecum of rats in our separate study (Kim, K. I., unpublished results).

The lower percentage of ammonia production that was not accounted for by ureolysis in rats fed the lactose diet than in control rats, as shown above, might be due to dilution of endogenous urea and non-urea nitrogen sources in the large intestine of rats fed the lactose diet. (Note that the added urea concentration in the incubations of both groups was the same.) Dietary lactose usually increases the volume of water in the large intestine because of the fermentation products, which increase osmolarity (Kim et al. 1978).

Rats used in our studies consumed ~30 mmol nitrogen/d (15 g of diet containing 17% protein). If 70% (21 mmol/d) of the nitrogen intake is metabolized via the urea cycle [a comparable value, 2.5 μmol/(min · 100 g body weight), was reported by Buttrose et al. 1987], and 26% (5.5 mmol/d) of urea-nitrogen is excreted into the GI tract as reported by Holtzman and Visek (1965), ammonia production from ureolysis would be 5.5 mmol/d. Although the amount is relatively small, ammonia produced from ureolysis can be highly concentrated in close proximity to the intestinal surface cells because urea may be hydrolyzed by bacteria adhering to the mucosal layer before it diffuses out into the lumen as Wolpert et al. (1971) suggested. Thus this highly concentrated ammonia may affect the surface cells as discussed above.

As expected, weights of tissue and contents of the large intestine were greater ($P < 0.01$) in rats fed diets containing milk, yogurt or lactose compared with those found in control rats. Fermentation products of lactose, such as small-chain fatty acids and lactic acid, are responsible for the greater osmolarity of the contents and thus the tissue growth (Fischer 1957, Kim et al. 1978).

The lower urease activity in rats fed cellulose, as shown in Experiment 3 (Table 5), may be due in part to smaller microbial populations in the presence of undigestible, unfermentable cellulose. In addition to the reduced microbial density, dilution of the diet with cellulose may have increased nitrogen flowing into the large intestine (Isaksson et al. 1983); hence more nitrogen was available for microbial protein synthesis, requiring less nitrogen from ureolysis. Indeed, many urease-producing bacteria (in pure culture) did not produce urease when growth media contained available non-urea nitrogen sources (Wonzy et al. 1977). The mechanisms of reducing urease activity and net ammonia production of lactose and cellulose in the intestinal contents may be different: the former promotes the growth of non-urease and non-ammonia producers, such as *lactobacilli*, but the latter dilutes the microbial population.

Lupton and Marchant (1989) found that feeding rats a diet containing 8% cellulose decreased colonic ammonia concentration (by 50%), but not cecal ammonia concentration, compared with feeding a control diet, whereas feeding a diet containing 8% pectin decreased cecal ammonia concentration (by 50%) but increased colonic ammonia concentration (by 200%). They suggested that the increased ammonia concentration in the colon of rats fed the pectin diet might be due to a greater amount of protein reaching the large intestine and an increased number of microorganisms, which increase deamination of protein and urease activity. This suggestion, however, does not explain why cecal ammonia concentration was decreased by feeding the pectin diet.

Rémésy and Demigné (1989) found that cecal ammonia concentration was significantly decreased by feeding rats diets containing 10% pectin as well as 10% guar gum, 25% or 50% amylomaize starch, but was significantly increased by feeding a diet containing 10% lactulose. In our study, ammonia concentration in the large intestinal contents (as determined in blanks) was not significantly affected by diets, although net ammonia production was influenced by diets (Tables 4 and 6). Results of our studies as well as those mentioned above suggest that luminal ammonia concentration should not be directly

related to microbial activity in the GI tract because ammonia concentration is dictated by the balance between its production and utilization by bacteria along with its absorption into the blood stream.

Epidemiologic studies (Burkitt 1971) have shown that dietary fiber intakes are inversely related to prevalence rates of colon cancer. Ammonia has been implicated in carcinogenesis because of its ability to reduce colonic epithelial cell life span, alter DNA synthesis, disrupt intermediary metabolism and increase mucosal cell turnover rates (Lin and Visek 1991, Visek 1978b and 1978c).

Depressed net ammonia production in the large intestine (shown in Experiment 3) and increased nitrogen excretion in the feces (Cummings et al. 1976, Isaksson et al. 1983) may play a part in lowering colorectal carcinogenesis with consumption of high fiber diets, as suggested by others (Clinton et al. 1988). It is also interesting to consider that in addition to urea resynthesis, the increased mucosal cell turnover (or protein turnover) by ammonia produced in the GI tract is a contributing factor to the specific dynamic effect, especially when animals consume excess amounts of protein (Visek, W. J., College of Medicine, University of Illinois, personal communication).

Collectively, results of our studies in conjunction with those of others suggest that urease activity and net ammonia production in the GI tract can be reduced by dietary fermentable carbohydrates or cellulose. These indicators may be used as a measure of a health-promoting potential, (which is not always reflected in growth and feed efficiency), of dietary ingredients or dietary additives that act on animals as well as humans through the modification of intestinal microflora.

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LITERATURE CITED

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