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Research Communications

A blend of dietary fibers increases urea disposal in the large intestine and lowers urinary nitrogen excretion in rats fed a low protein diet

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The presence of rapidly fermented indigestible carbohydrates in the diet has been shown to influence intestinal fermentations and the route of excretion of urea nitrogen. The objective of the present study was to determine the effects of a dietary fiber blend on nitrogen excretion in the rat. The dietary fiber blend studied (the "oligo/fiber blend") was comprised of 41.2% fructooligosaccharides, 17.7% insoluble oat fiber, 13.0% soy polysaccharide, 10.3% gum arabic, and 4.4% carboxymethylcellulose, which provided a diversified source of soluble and insoluble fibers. Two levels (4% and 8%) of the oligo/fiber blend were added to diets containing low (8%) and normal (14%) protein levels. Plasma urea levels were reduced by another 37% when 8% oligo/fiber was added to the low protein diet. The reduction of plasma urea levels can be explained by nitrogen metabolism in the cecum. Feeding the oligo/fiber mix elicited a bacterial proliferation characterized by short chain fatty acid production, decreased cecal pH and marked cecal hypertrophy. At the 8% oligo/fiber level, urea nitrogen uptake from blood into the cecum was increased by over 2 fold. There was a compensatory increase in ammonia nitrogen flux from gut lumen to blood at the higher oligo/fiber levels, but on balance the direction of nitrogen flux remained strongly in the direction of the cecal lumen in the oligo/fiber fed rats. As a function of total nitrogen excretion, fecal nitrogen excretion was over 80% greater in rats fed the low protein diet than in rats fed the normal protein diet. With the addition of the oligo/fiber blend, the percentage of N in the feces was increased even further. In rats fed the 8% casein/8% oligo/fiber diet, fecal N excretion was nearly the same as urinary nitrogen excretion (≈50% of total nitrogen excretion by each route). In conclusion, by decreasing dietary protein and increasing the level of a dietary fiber blend within nutritionally acceptable ranges, plasma urea was decreased and the contribution of the kidneys to nitrogen excretion would be reduced. (J. Nutr. Biochem. 7:474–480, 1996.)

Keywords: dietary fiber; urea; nitrogen excretion; dietary protein; rats

Introduction

In man, urea produced by the liver is mainly excreted in the urine (70 to 80%), the remaining 20 to 30% being hydrolyzed in the intestine.[1,2] The rate of intestinal urea clearance depends of the capacity of bacterial hydrolysis, particularly in the large bowel.[3,4] In a previous work,[5] it was observed that the consumption of indigestible carbohydrates (nonfermentable fiber, fermentable fiber, fermentable oligosaccharides) results in a greater rate of urea transfer from blood to the cecal lumen of rats, and therefore, in a higher fecal nitrogen excretion (coupled with a lowering of urinary nitrogen excretion) compared with rats fed a fiber-free diet. In parallel, blood urea level was decreased by 20 to 30%.

To obtain an optimal physiological result, feeding a com-

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plex mixture of oligosaccharides and polysaccharides, with different solubilities and fermentabilities, may be necessary. Oligosaccharides or soluble fibers promote the proliferation of bacteria in the proximal large intestine and less fermentable fibers should help to limit proteolysis and deamination of nitrogen end products in the distal colon. In addition, some nonfermentable fibers such as insoluble oat fiber may accelerate intestinal transit[6] and prevent prolonged exposure of the large intestine to the products of nitrogen catabolism.

The effect of indigestible carbohydrates on fecal nitrogen excretion depends on the dietary protein level.[7,8] Levrat et al.[9] have reported that, in rats fed high protein level diets (45%), the percentage of nitrogen excreted in the feces was lower than 10% even in the presence of an oligosaccharide (inulin), which favors bacterial proliferation in the large intestine. With a more physiological dietary level (15% casein), this percentage may be as high as 29% when inulin was present in the diet. Another study[10] on resistant starch also showed that when a high protein level (26% casein) was fed, the increase in fecal excretion was not sufficient to noticeably shift the route of nitrogen excretion towards intestinal elimination (15% of total N excretion). In contrast, with a moderate dietary protein level (13% casein), the percentage of nitrogen excreted in the feces reached 50%. These results thus suggested a possible usefulness of combining fermentable carbohydrates with a reduction of protein intake to lower the renal excretion of nitrogen.

Low protein diets increase the efficiency of urea disposal in the cecum and decrease ammonia recycling.[11] The question arises also whether fermentable carbohydrates could further enhance urea disposal and limit ammonia accumulation in digestive contents, even when protein levels are restricted. It is possible that limiting protein to less than the 10 to 15% that is typically fed would also limit the bacterial fermentation of fiber, because systemic urea governs, at least in part, the flux of urea N to the large intestine. The aim of the present work was to determine whether a balanced fiber blend at one of 2 levels (4% and 8%) can support useful bacterial fermentations in the cecum even at a lower level of protein intake (8%), and thus optimize the digestive disposal of urea.

Methods and materials

Animals and diets

Sixty male Wistar rats (IFFA-CREDO, l'Arbresle, France) weighing 160 g were randomly assigned to one of six treatments (10 rats/treatment) (*Table 1*). The oligo/fiber blend was substituted for wheat starch in the diet. The animals were allowed free access to water and purified diets and the dark period was from 08.00 to 20.00 h. Rats were fed for a total of 18 days. During the period of adaptation (10 days), rats were housed two per cage in a temperature-controlled room (22°C). After the adaptation phase, rats were housed individually for an additional 8 days in metabolic cages fitted with urine/feces separators to collect feces and urine (experimental phase). The animals were maintained and handled according to the standard operating procedures of the Institutional Ethics Committee of the University of Clermont-Ferrand.

Daily food consumption and body weight were recorded every 3 days during the adaptation phase, then daily during the 8-day experimental phase. Urine (acidified by HCl, final concentration of 5 mmol/L) and feces were collected during the last 5 days of the experiment for determination of nitrogen excretion.

Sampling procedure

Rats were sampled just after the dark period (between 08.00 and 09.00 h). The procedure of blood sampling from anesthetized animals (40 mg sodium pentobarbital/kg body weight) and for measurement of arteriovenous differences across the cecum has been described previously.[12] For blood flow measurement, bromosulfophtalein in saline (4.7 mmol/L) was infused at a rate of 50 μL/min into the small afferent vein on the internal curvature of the cecum. Dilution of the marker in the vein draining the whole cecum allows determination of the cecal blood flow.

After blood sampling, the cecum, complete with contents, was removed and weighed (total cecal weight). Duplicate samples of cecal contents was transferred to 2 mL microfuge tubes that were immediately stored at −20°C. The cecal wall was flushed clean with ice-cold saline, blotted on filter paper and weighed (cecal wall weight). Water in the cecal contents was determined by difference between wet weight and dry weight on aliquots of cecal contents that were dried (at 100°C) to constant weight. Supernatants of the digestive contents were obtained by centrifuging the microfuge tubes at 20,000 × g for 10 min at 4°C.

Table 1 Composition of the experimental diets (amount per 100 g of diet)

	Low protein			High protein		
Ingredient	Control (g)	Low fiber (g)	High fiber (g)	Control (g)	Low fiber (g)	High fiber (g)
Casein	8	8	8	14	14	14
Wheat starch	78.7	74.7	70.7	72.7	68.7	64.7
Oligosaccharide/fiber blend[1]	0	4	8	0	4	8
Corn oil	5	5	5	5	5	5
Mineral mixture[2]	7	7	7	7	7	7
Vitamin mixture[2]	1	1	1	1	1	1
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3

[1]Oligosaccharide/fiber blend ("Oligo/Fibe"): 41.2% fructooligosaccharide, 26.4% oat fiber, 17.7% soy polysaccharide, 10.3% gum arabic, and 4.4% carboxymethyl cellulose. This product was supplied by ROSS Laboratories, Columbus, USA.
[2]Vitamins and minerals supplied in mg/Kg (except as noted) of diet: thiamin, 20; riboflavin, 15; pyridoxin, 10; nicotinamide, 100; calcium panthotenate, 70; folic acid, 5; biotin, 0.3; cyanocobalamin, 0.05; retinyl palmitate, 1.5; DL-alpha-tocopheryl acetate, 125; cholecalciferol, 0.15; menadione, 1.5; ascorbic acid, 50; myo-inositol, 100; choline, 1.36 g; $CaHPO_4$ 15 g; K_2HPO_4, 2.5 g; KCl, 5 g; NaCl, 5 g; $MgCl_2$, 2.5 g; Fe_2O_3, 2.5; $MnSO_4$, 125; $CuSO_4.7H_2O$, 0.2; $ZnSO_4.7H_2O$, 100; K1, 0.4. Purchased from UAR (Villemoisson, Epinay-sur-orge, France).

Analytical procedures

Short chain fatty acids (SCFA) were measured by gas-liquid chromatography on an aliquot of supernatants from the cecal contents.[13] Ammonia and urea were determined enzymatically on neutralized perchloric acid extracts of plasma.[14,15] Nitrogen in cecal contents, urine and feces was determined by the Kjeldahl method: homogenized samples (0.5 to 1 g of cecal contents or feces, 1 mL of urine) were treated with 6 mL of 36 M sulfuric acid in the presence of catalyst (K_2SO_4, 1.5 g; Se, 7.5 mg); finally, the ammonia extracted and trapped in a solution of boric acid was determined by direct titration with phosphoric acid.

Statistical analysis

Values are given as the means ± SEM and, where appropriate, significance of differences between mean values were determined by analysis of variance using the analysis of variance (Super ANOVA) package (ABACUS, Berkeley, CA, USA). When necessary, data were normalized by log or reciprocal transformations before analysis of variance. Values of $P < 0.05$ were considered significant.

Results

As shown in *Table 2*, there was a significant effect of dietary protein level on final body weights. The animals fed the higher protein level were 11% heavier on average than their counterparts fed a lower level of protein. In contrast, the oligo/fiber blend had no noticeable effect on weight gain. The addition to the diet of the oligo/fiber blend did lead to an enlargement of the cecum and an hypertrophy of the cecal wall that was roughly proportional to the percentage of fiber that was added to the diet. In parallel with cecal development, cecal blood flow was significantly increased by feeding the oligo/fiber blend. At the 8% casein level, mean values were 0.90 ± 0.1 mL/min in control rats vs. 1.20 ± 0.13 and 1.44 ± 0.15 mL/min in rats fed 4% and 8% oligo/fiber blend respectively. At the 14% casein level the values were 1.05 ± 0.14 in control rats vs. 1.30 ± 0.14 and 1.60 ± 0.15 mL/min in rats fed the two levels of oligo/fiber blend. The cecal contents were markedly acidified by the presence of fermentable carbohydrates, with the lowest pH value (<6.5) coinciding with the greatest accumulation of SCFA (>150 mmol/L). As shown in *Figure 1*, the oligo/fiber blend elicited very large increases in the absolute levels of cecal SCFA and altered the molar ratio of cecal SCFA, with a decrease in acetate relative to propionate and butyrate.

As shown in *Figure 2*, the primary determinant of plasma urea concentration was the casein level in the diet: urea levels were only one third as high in animals fed 8% casein as in those fed 14% casein. The oligo/fiber blend further decreased plasma urea levels by over 30% at both levels of protein. The very lowest level of plasma urea (0.75 mmol/L) was achieved in rats fed the low level of protein and the high level of oligo/fiber blend.

The cecal ammonia concentration was 33% higher in rats fed the 14% casein diet than in those fed the 8% casein diet (*Figure 3, panel 1*). At either protein level, the ammonia concentration was markedly depressed by 50% or more by feeding the oligo/fiber blend. Nevertheless, cecal ammonia pools were not markedly affected by the presence of fiber in the diet (*Figure 3, panel 2*) because the addition of the oligo/fiber blend caused large increases in the volume of cecal contents, as shown in *Table 2*. Total cecal nitrogen, including all bacterial nitrogen and soluble nitrogen in the cecum, was approximately 20 to 30% higher in the rats fed the higher protein level than in those fed the lower level. Regardless of protein level, total cecal nitrogen was increased by over 50% at the lower level of oligo/fiber and over 100% at the higher level of oligo/fiber.

Figure 4 summarizes cecal N flux. In all groups, there was a net flux of urea into the cecum (namely a negative [cecal vein]-[artery] difference for plasma urea, as shown in *Figure 4, panel 1*). Urea flux into the cecum was approximately 2 fold higher in the rats adapted to the 14% casein diet than in those on the 8% casein diet. The greatest flux of all groups (≈1,3 μmol/min) was registered in animals fed the 14% casein/8% oligo/fiber diet. But even when plasma

Table 2 Effect of diets on final body weight, food intake, and cecal development and fermentations[1]

Diet	Final body weight (g)	Daily food intake (g/rat/day)	Total cecal weight (g)	Cecal wall weight (g)	pH	SCFA concentration (mmol/L)	SCFA molar ratio (Ac/Pr/Bu)
Casein 8%							
Control	259 ± 6^a	23.4 ± 0.8	2.17 ± 0.08^a	0.59 ± 0.03^a	7.16 ± 0.03^a	84 ± 2^a	67/22/11
Oligo/fiber 4%	262 ± 4^a	25.7 ± 0.7	3.55 ± 0.20^b	0.77 ± 0.04^b	6.59 ± 0.05^b	114 ± 4^b	56/27/17
Oligo/fiber 8%	259 ± 4^a	24.3 ± 0.8	4.54 ± 0.20^c	0.89 ± 0.04^b	6.32 ± 0.07^c	151 ± 5^c	53/32/15
Casein 14%							
Control	289 ± 3^b	23.8 ± 0.7	2.29 ± 0.12^a	0.54 ± 0.02^a	7.13 ± 0.03^a	103 ± 5^b	70/22/8
Oligo/fiber 4%	294 ± 7^b	24.7 ± 0.7	3.61 ± 0.12^b	0.81 ± 0.05^b	6.46 ± 0.06^b	151 ± 8^c	61/27/12
Oligo/fiber 8%	277 ± 3^b	24.6 ± 0.6	4.68 ± 0.22^c	1.01 ± 0.06^c	6.12 ± 0.06^d	181 ± 5^d	56/29/15
Significance of effects in ANOVA							
Casein	$P < 0.0001$	NS	NS	NS	$P < 0.004$	$P < 0.0001$	
Fiber	NS	NS	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	
Casein x Fiber	NS	NS	NS	NS	NS	NS	

Statistical evaluation of data was carried out by Super ANOVA. NS: non significant.
[1]Each value is the mean ± SEM, $n = 10$. Means not sharing a common superscript differ $P < 0.05$.

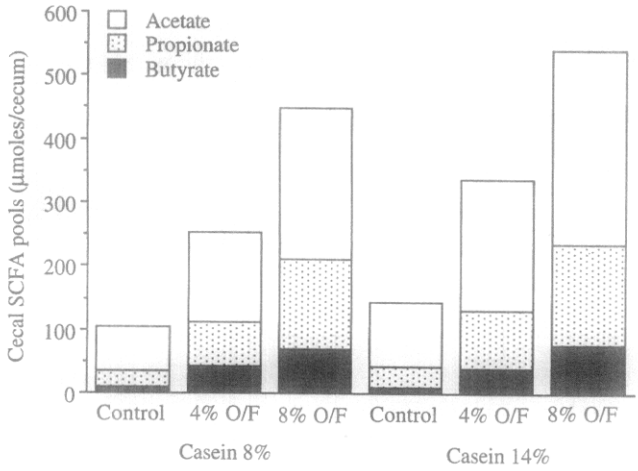

Figure 1 Effects of a complex mixture of indigestible carbohydrates (oligo/fiber: O/F) on cecal SCFA pool. The cecal SCFA pool (μmol/cecum) = SCFA concentration (mmol/L) × cecal water (mL). Each value is the mean ± SEM, n = 10.

urea was very low (as in the rats adapted to the 8% casein diet), the presence of 8% oligo/fiber in the diet still elicited a nearly 3 fold increase in cecal N flux into the cecum.

As shown in the second panel of *Figure 4*, there was also a net flux of ammonia N from the cecum to the cecal vein (the opposite direction of urea N flux). Like the flux of urea N, the magnitude of the ammonia flux was greater at the higher levels of protein and at the higher levels of oligo/fiber mix. But because urea N flux into the cecum was generally greater than opposite ammonia N flux (except in rats fed the fiber-free/8% casein), there was a positive balance in the cecum (i.e., a net flux of nitrogen into the cecum). At the higher level of oligo/fiber supplementation, overall cecal N balance was approximately 0.2 μmol/min in the rats fed 8% casein and 0.5 μmol/min in those fed the 14% casein diet.

Finally, *Figure 5* illustrates that total N excretion was much lower in rats fed the 8% casein level (≈126 mg N/day)

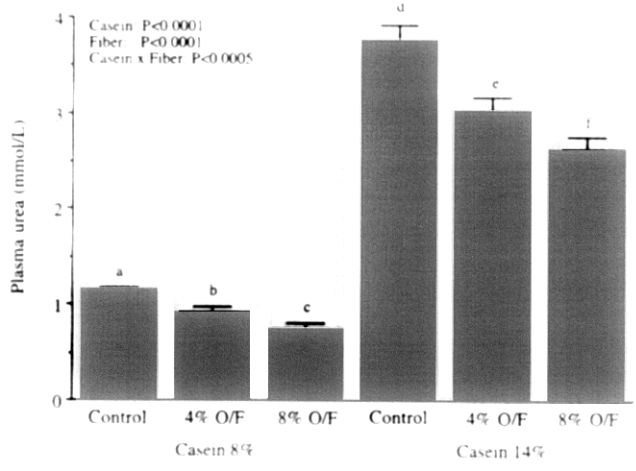

Figure 2 Effects of a complex mixture of indigestible carbohydrates (oligo/fiber: O/F) on plasma urea. Statistical evaluation of data was carried out by ANOVA. Each value is the mean ± SEM, n = 10. Means not sharing a common superscript differ $P < 0.05$.

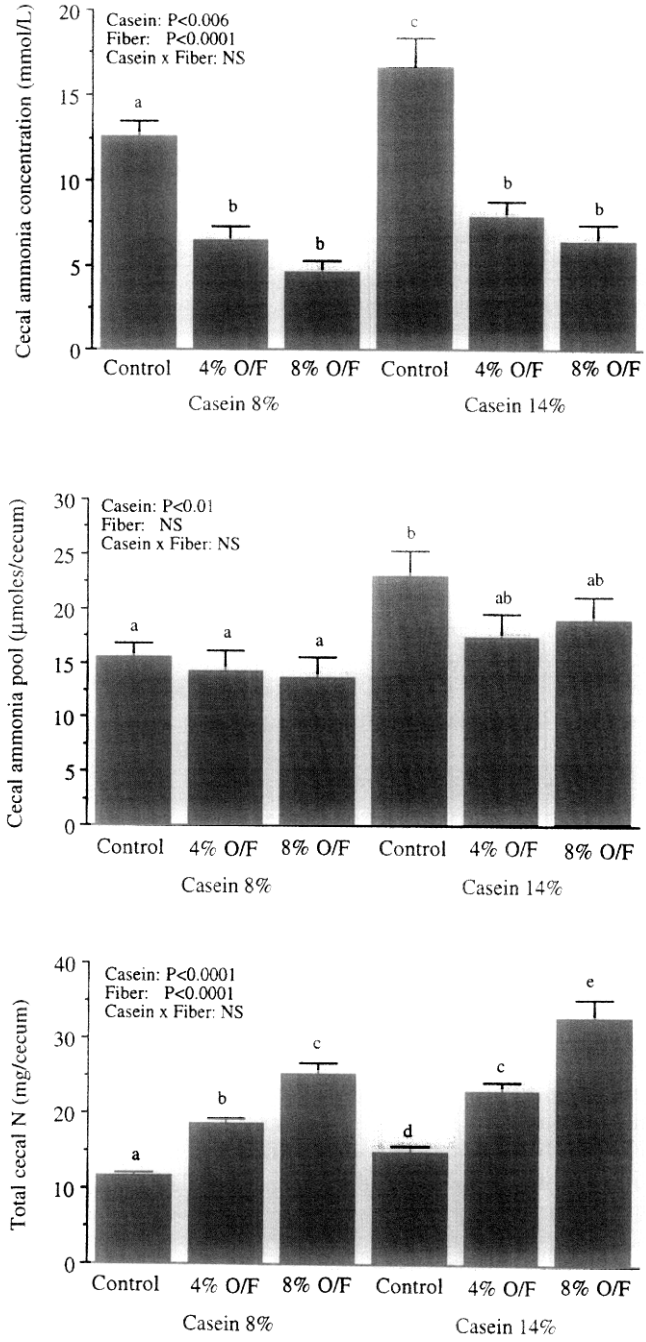

Figure 3 Effects of a complex mixture of indigestible carbohydrates (oligo/fiber: O/F) on cecal ammonia and cecal total nitrogen. The cecal ammonia pool (μmoles/cecum) = cecal ammonia concentration (mmol/L) × cecal water (mL). Statistical evaluation of data was carried out by ANOVA. Each value is the mean ± SEM, n = 10. Means not sharing a common superscript differ $P < 0.05$.

compared with those fed the 14% casein level (≈260 mg/day). The major part of N was excreted by the kidneys in rats fed fiber-free diets. With the addition of 8% oligo/fiber to the diet, fecal N excretion more than doubled in both low and high protein diet groups. It is especially noteworthy that fecal N excretion represented nearly 50% of total N excretion in rats adapted to the low protein diets containing 8% oligo/fiber. Even in rats adapted to the higher protein level, the 8% oligo/fiber elevated fecal N excretion to 26% of total

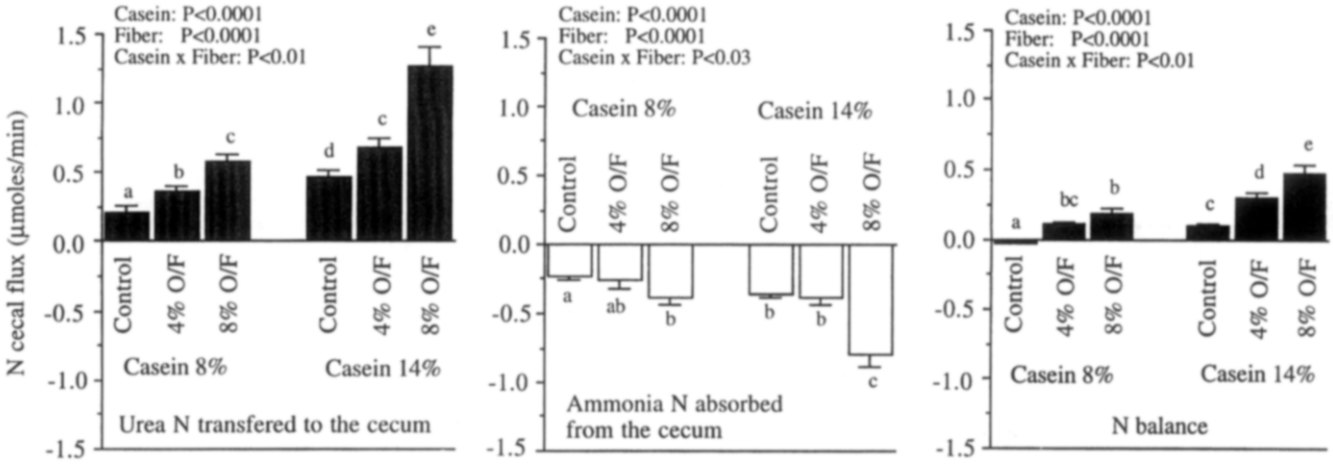

Figure 4 Effects of a complex mixture of indigestible carbohydrates (oligo/fiber: O/F) on cecal nitrogen flux. Fluxes (µmol/min) = arteriovenous differences across the cecum (mmol/L) × cecal blood flow (mL/min). Statistical evaluation of data was carried out by ANOVA. Each value is the mean ± SEM, n = 10. Means not sharing a common superscript differ $P < 0.05$.

excretion, resulting in a significant decrease in renal N excretion. As a final note interest, whole animal N balance was much more positive in the animals fed high protein levels (220 mg/day vs. 160 mg/day in the animals fed the low-protein levels), but was not significantly influenced by oligo/fiber supplementation.

Discussion

The metabolism of nitrogen in the colon is dominated in quantitative terms by the metabolic activity of the microflora, which may be modified by the availability of substrate, particularly by dietary carbohydrates.[5,10,11] Bacteria use NH_3 as a major source of nitrogen, and other forms of protein or amino acids are deaminated to NH_3 before being used metabolically.[16] With a low-fiber diet, proteins (rather than amino acids) unabsorbed from the small intestine, including those of endogenous origin (trypsin, chemotrypsin, lipase, amylase, and sloughed mucosal cells) probably sustain the microbial growth in the large bowel.[7,17] Together with indigestible carbohydrates, they provide a source of energy and nitrogen, and stimulate microbial metabolism. This can result in a substantial rise of bacterial nitrogen in the fecal matter. When the availability of indigestible carbohydrates is elevated (high-fiber diets, for example), these sources of nitrogen may be insufficient to sustain an intense bacterial growth. In such conditions, blood urea constitutes a readily available source of nitrogen for bacterial protein synthesis in the large intestine.[18,19] Various factors control the rate of urea hydrolysis: movement of urea to the site of hydrolysis, activity of microfloral urease, demand of NH_3 for bacterial synthesis, and availability of other nitrogen sources.[19] It has been shown that urea transfer is proportional to cecal size and blood urea levels, and that the breakdown of large amounts of carbohydrate increases the incorporation of urea nitrogen into bacterial proteins.[20] This type of urea disposal has been reported in various species including humans.[19,21] In herbivorous species such as rabbits, the degradation of urea in the digestive tract represents a significant pathway of urea disposal from the blood.[22]

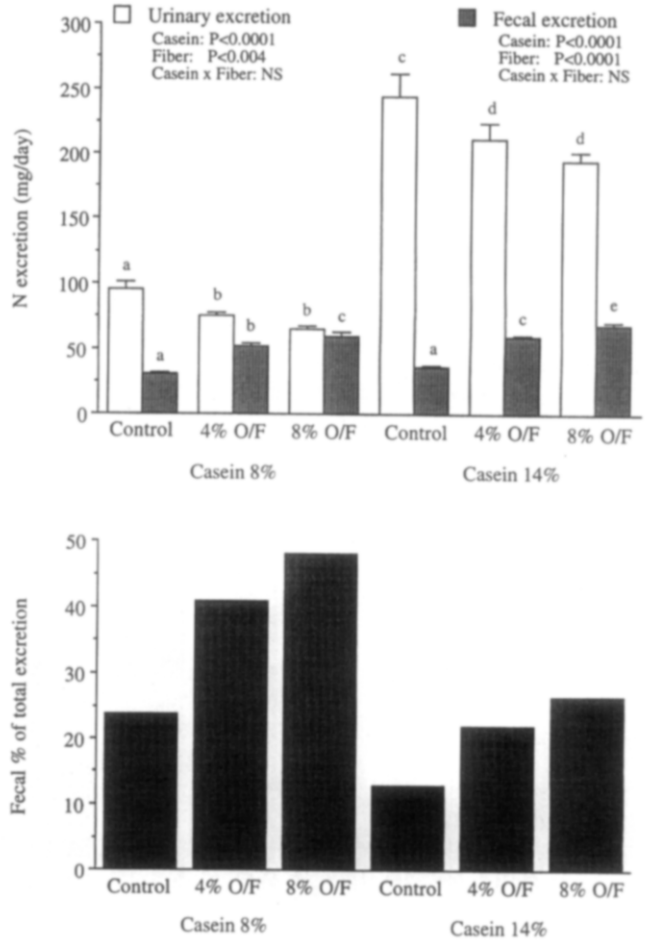

Figure 5 Effects of a complex mixture of indigestible carbohydrates (oligo/fiber: O/F) on urinary and fecal nitrogen excretions and fecal percentage of total nitrogen excretion. Statistical evaluation of data was carried out by ANOVA. Each value is the mean ± SEM, n = 10. Means not sharing a common superscript differ $P < 0.05$.

It is conceivable that indigestible carbohydrates enhance fecal nitrogen excretion by promoting bacterial proliferation and also by decreasing the digestive transit time. Stephen and Cummings[23] have demonstrated a direct relationship between the amount of fiber fermented and an increase in fecal bacterial mass. The question arises about respective effects of carbohydrates present in the fiber blend to favor urea utilization and bacterial N excretion and, in parallel, to limit the ammonia accumulation in digestive contents and the development of extensive enterohepatic cycling. In a previous work,[5] it has been shown that gum arabic and oligosaccharides have comparable effects on urea nitrogen retention but oligosaccharides led to higher concentrations of ammonia in the cecum. The efficiency of soy polysaccharides to induce an urea disposal in the large intestine was also reported by Levrat et al. (1991).[24] Oat fiber, which was poorly fermented, exerted also a significant effect on urea utilization by the cecum.

The present data support the view that there is a close relationship between the flux of urea nitrogen toward large intestine, fecal nitrogen excretion, and lowering of plasma urea. A high rate of urea transfer results from cecal hypertrophy, characterized by an enlarged surface of exchange between blood and luminal fluid and by a greater blood supply to the cecum.[11] The relative hypertrophy of the cecal wall with indigestible carbohydrates could be ascribed to high concentrations of SCFA especially butyrate, a potent trophic factor,[25] which was markedly increased. The urea lowering effect of fibers and related compounds and their effectiveness to induce urea transfer to the digestive tract are proportional to their fermentability and to their effects on cecal hypertrophy.[26] Fuctooligosaccharides, present in the fiber blend, may have specific effects, possibly via their osmotic action or by promoting the development of a flora with a high ureolytic activity.[5] In fact, most effective factor to promote urea N transfer in the large bowel was the presence of fermentable carbohydrates in sufficient amounts.

Based on the classical concepts of nitrogen utilization in the rumen,[27] fermentations in the cecum should reduce ammonia absorption, owing to a higher incorporation of nitrogen in bacterial protein and a drop of unionized NH_3 concentration. Nevertheless, in the rat cecum, whatever the amount of dietary fiber, ammonia absorption was higher than with the basal fiber-free diet. This indicates that both protein level and dietary fiber affect the ammonia availability in colonic contents. But the question arises whether nitrogen provision for bacterial metabolism is limiting for bacterial growth. With fiber diets, there was still a substantial concentration of ammonia in cecal contents of rats adapted to a 8% casein level (about 4.6 mmol/L), resulting in a noticeable ammonia reabsorption in the cecal vein. However, a higher SCFA concentration was observed in the cecum of rats fed diets containing a normal casein level, thus a minimal availability of urea or of some residual nitrogen seems required for an optimal fermentation development. By increasing uremia, dietary protein tends to enhance urea transfer towards cecal contents, which influences digestive ammonia concentration and its absorption by the cecal wall. The reabsorption of NH_3 from the large intestine observed whatever the nutritional conditions suggests that this process is not necessarily a futile cycle but also participates in the salvage of nitrogen with low protein diets.[16]

As shown in other studies[9,10] the effects of indigestible carbohydrates on fecal nitrogen excretion was dependent on the dietary protein level. Thus, in rats fed the normal protein diet containing 8% fiber, the rise of fecal excretion was not sufficient to substantially shift the route of nitrogen excretion (26% of total excretion). In contrast, with a low-protein level, the percentage of nitrogen eliminated in the feces was in the 50% range. As a result of the increase in fecal nitrogen excretion induced by fermentable carbohydrates feeding, the renal nitrogen excretion was significantly depressed, particularly in rats adapted to a low protein level (−33%). Numerous studies have demonstrated that excess protein may affect renal function,[28] and results in the development of renal lesions[29,30] in normal rats. Other studies have shown that renal lesions developing after a reduction of kidney mass are markedly influenced by the protein content of the diet.[31,32] The uremic toxicity with high-protein diets has been considered to be a prominent factor for shortening survival of uremic rats.[33] A diet rich in fiber with a low-protein level, which shifts nitrogen excretion toward the large intestine, was very effective in lowering plasma urea (down to 0.75 mmol/L). This suggests that with recommended dietary modifications, it seems possible to reduce the contribution of kidneys to nitrogen excretion.

In humans, the potential transfer of urea through the colonic wall is probably as effective as in other species,[19,21] however, the intake of dietary fiber is frequently too low to elicit a significant change in digestive urea disposal especially if the diet is rich in protein. Nevertheless, it has been demonstrated that consumption of fibers or oligosaccharides results in a high fecal excretion of nitrogen.[8,34,35] In practice, a complex mixture of fibers and oligosaccharides with different solubilities and fermentabilities could be justified to ameliorate their digestive tolerance. The effect of lowering the dietary protein level is probably reinforced by intake of sufficient amounts of fibers or related compounds.

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