Influence of inulin on urea and ammonia nitrogen fluxes in the rat cecum: consequences on nitrogen excretion

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The effect of the addition of 15% chicory inulin on the nitrogen balance and the processes of N exchanges between blood plasma and the large intestine have been investigated in rats adapted to diets having a normal (15%) or high (45%) casein level. Inulin diets elicited a marked enlargement of the cecum, together with an acidification of the cecal content and an increase of the cecal volatile fatty acids pool. The net N balance was not affected by the various dietary conditions, but N digestibility was apparently depressed in rats fed inulin. This merely reflected a shift of N excretion from the renal to the intestinal site; this last effect was more marked in rats adapted to the normal protein level. Inulin depressed cecal ammonia only in rats adapted to the normal protein level. The balance between urea N transfer (blood plasma → cecum) and ammonia N transfer (cecum → blood plasma) was positive only in rats fed inulin, reflecting a net urea N retention in the cecum. In rats fed the inulin-45% casein diet, urea N transfer was very high, but very large amounts of N were reabsorbed as ammonia, suggesting a relatively poor incorporation of this N source in bacteria. The total concentrations of free amino acids in the cecum were significantly enhanced by inulin, but the cecal reabsorption of amino acids was quantitatively marginal. It appears that inulin is very effective in increasing fecal N excretion, concomitantly to depressed renal N excretion, without altering protein bioavailability. This shift seems more evident when the dietary protein level is moderate.

Keywords: inulin; dietary protein; urea; ammonia; nitrogen balance; cecum; rat

Introduction

The interactions between fibers and the digestibility of dietary proteins have been extensively investigated.[1] It is well established that fermented polysaccharide increases the fecal nitrogen excretion, which is essentially composed of bacterial nitrogen.[2,3] The origin of nitrogen entering the large intestine is particularly complex: some dietary proteins escaping small intestine breakdown, endogenous proteins (pancreatic and small intestinal secretions and sloughed epithelial cells), digestive mucins, and blood urea diffusing in the digestive contents.[2,4] Because most of these compounds are extensively transformed by the microflora of the large intestine, it is difficult to assess the origin of fecal nitrogen and to appreciate whether dietary proteins have been incompletely digested in the small intestine.

In absolute terms, the possibility for the host to recover amino acids in the large intestine is probably limited and of minor nutritional importance, but the products of nitrogen metabolism (such as ammonia) may be very important for health. Indeed, the presence of high concentrations of ammonia in the large intestine is considered as potentially deleterious, as to (1) the risk of excessive reabsorption of ammonia that is toxic, especially in the case of hepatic failure,[5] (2) the suspected role of ammonia to disturb the mucosa cell cycle and to select for neoplasic growth.[3,6,7] Soluble polysaccharides such as inulin are readily fermented by the large intestine microflora, yielding relatively acidic fermentations,[8] but they are unlikely to affect the digestibility of dietary proteins in the upper digestive tract. The aim of the present study was thus to determine the effect of inulin on the nitrogen balance and digestibility, and on ammonia production in the large intestine in

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Table 1 Composition of the experimental diets expressed as g/100 g of diet (dry basis)

	15% casein	45% casein	15% casein/inulin	45% casein/inulin
Casein	15	45	15	45
Wheat starch	74	44	59	29
Chicory inulin[a]	0	0	15	15
Corn oil	5	5	5	5
Mineral mixture[b]	5	5	5	5
Vitamin mixture[b]	1	1	1	1

[a]Provided by COSUCRA, 7750-Pecq-Warcoing, Belgium.
[b]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-α-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, 5g; $MgCl_2$, 2.5 g; Fe_2O_3, 2.5; $MnSO_4$, 125; $CuSO_4.7H_2O$, 0.2; $ZnSO_4.7H_2O$, 100; KI, 0.4.

rats adapted to a normal (15%) or a high (45%) casein level.

Methods and materials

Animals and diets

Forty male Wistar rats (IFFA-CREDO, l'Arbresle, France), weighing 180 g, were adapted for 21 days to one of four semi-purified diets (*Table 1*). The animals were housed in stainless-steel metabolic cages with urine-feces separators in a temperature-controlled room (22° C) with the dark period from 20:00 to 08:00 hours. Daily food consumption and body weight were recorded every 3 days during the first 2 weeks, then daily. During the last 3 days of the experiment, the urine and feces were collected for nitrogen balance and digestibility studies.

Sampling and analytical procedures

The procedure of blood sampling from anesthetized animals for measurement of arteriovenous differences across the cecum and for determination of the cecal blood flow have been previously described.[9] After blood sampling, the cecum was removed and weighed, and about 1 g cecal content was transferred to microfuge tubes that were plunged immediately in liquid nitrogen and then stored at −20° C.

Volatile fatty acid (VFA) were measured by gas liquid chromatography on aliquots of supernatants (8,000g, 5 minutes) of cecal contents.[10] Ammonia and urea were determined spectrophotometrically on neutralized perchloric extracts by enzymatic methods.[11] The nitrogen content of food, urine, and feces was determined after Kjeldahl digestion in an automatic analyser (Tecator, Höganäs, Sweden). The concentrations of amino acids in plasma and cecal contents were measured after sulfosalicylic deproteinization on a Kromakon 500 Autoanalyzeur (Kontron, Zürich, Switzerland) using lithium buffers and ninhydrin detection.

Statistics

Student's t test was used to determine the statistical significance among the means. Values were considered different at a significance level of $P < 0.05$.

Results

Effects of diets on food intake, weight gain and cecal fermentations

As shown in *Table 2*, the daily food intake and weight gain of the rats were not significantly influenced by the incorporation of 15% inulin in the diet or by changes in the dietary protein level. There was no significant differences between the four groups in the final body weight of the rats (≈ 290 g).

There was a development of the cecal fermentations in rats fed the 15% inulin diets (*Table 3*): the cecum was about three-fold enlarged and the luminal pH was markedly depressed (from about 7 in controls to 5.6 in rats fed inulin). The parameters of cecal development (cecal weight, cecal VFA concentrations, and VFA pool) were not noticeably affected by changes in the dietary casein level. In rats fed inulin diets, the total concentration of VFA in the cecum was not significantly increased. However, compared with fiber-free conditions, addition of inulin in the diet resulted in a four-fold increase in the cecal pool of VFA together with a modification of the molar proportions of the various VFA (inulin enhanced propionic and butyric acid productions at the expense of acetic fermentations).

Effects of the changes in dietary casein in rats fed fiber-free or 15% inulin diets on the nitrogen balance

Table 4 shows the balance between N intake and N excretion by the fecal or urinary route. It was verified that the average N intake in rats fed the 45% casein diets was actually three-fold higher than in rats fed the basal casein level. Nevertheless, the net N balance was not significantly different in the various diet groups, in the range of 200 mg N/day. The fecal N excretion in control rats represented only 10% of total N excretion; it was only 1.5-fold enhanced in rats fed the 45% casein level, corresponding to 4% of total N excretion. In rats fed inulin diets, the fecal N excretion was markedly enhanced with both casein levels. As a result, because the total N excretion was almost unmodified, the fecal

Table 2 Body weight and food intake[a]

	Body weight (g)	Daily weight gain (g/day)	Dry matter intake (g/day)
15% casein diet	290 ± 5	6.2 ± 0.6	24.0 ± 2.1
45% casein diet	294 ± 6	5.8 ± 0.5	22.5 ± 1.8
15% casein/inulin diet	285 ± 4	5.8 ± 0.7	22.7 ± 2.0
45% casein/inulin diet	288 ± 6	6.0 ± 0.7	22.2 ± 2.1

Values are expressed as means ± SEM for 10 rats.

Table 3 Effects of changes in the dietary casein level and of the addition of 15% inulin in the diet on the parameters of cecal digestion and the cecal volatile fatty acids

	Cecum			Cecal volatile fatty acids		
	Total (g)	Content (g)	pH	Total (mmol/L)	Pool (μmoles)	Ace/Pro/But (molar ratio)
Diets						
15% casein	2.83 ± 0.12	1.70 ± 0.09	7.03 ± 0.04	87 ± 5	148 ± 9	68/25/7
45% casein	2.84 ± 0.12	1.60 ± 0.10	7.07 ± 0.04	94 ± 6	150 ± 11	70/26/4
15% casein/Inulin	8.33 ± 0.63*	5.50 ± 0.25*	5.65 ± 0.16*	111 ± 11*	610 ± 20*	43/36/21
45% casein/Inulin	8.14 ± 0.54*	5.30 ± 0.19*	5.67 ± 0.07*	109 ± 11	578 ± 18*	46/39/15

Values are means ± SEM for 10 rats.
*Significant difference ($P < 0.05$) between Inulin and fiber-free diets.

Table 4 Effects of changes in the dietary casein level and of the addition of 15% inulin in the diet on the nitrogen balance

Nitrogen:	Dietary intake (mg/d)	Urine excretion (mg/d)	Fecal excretion (mg/d)	Fecal, % of total excretion	Net[a] balance (mg/d)	Apparent[b] digestibility (% dietary intake)	Retention[c] (% dietary intake)
Diets							
15% casein	518 ± 19	290 ± 13	32 ± 2	9.9%	196 ± 23	94%	38%
45% casein	1458 ± 47*	1220 ± 57*	50 ± 2*	3.9%	188 ± 35	97%	13%
15% casein/Inulin	490 ± 20	206 ± 12†	85 ± 3†	29.2%	199 ± 26	83%	41%
45% casein/Inulin	1439 ± 55*	1117 ± 45*	117 ± 5†	9.5%	205 ± 40	92%	14%

Values are means ± SEM for 10 rats.
[a]Net balance = (Dietary intake) − (Fecal excretion + Urine excretion).
[b]Apparent digestibility = ((Dietary intake − Fecal excretion) × 100)/(Dietary intake).
[c]Retention = (Net balance) × 100/(Dietary intake).
Significant difference ($P < 0.05$): * between 15% casein diets and 45% casein diets, and † between inulin diets and fiber-free diets.

N excretion represented a higher percentage of total N excretion in rats fed the inulin diets, especially those adapted to the 15% casein level (29%).

Although the N balance and the N retention were unmodified, there was an apparent depression of the N digestibility in rats fed the inulin diets, corresponding to the rise of the fecal N excretion, which was compensated for by a concomitant reduction of the urinary N excretion.

Effect of the diets of the parameters of nitrogen digestion in the cecum

Plasma urea was strongly enhanced in rats adapted to the high-protein diets (up to about 10 mmol/L); inulin supplementation significantly decreased uremia in rats fed the 15%, but not in those fed the 45% casein diet (*Table 5*). The cecal concentration of ammonia was higher in rats fed the high-protein diets, in keeping with other investigations.[12,13] In rats fed the 15% casein diets, the cecal ammonia concentration was noticeably depressed (down to about 8 mmol/L) in the group adapted to inulin, compared with the fiber-free group. The concentration of the unionized NH_3 was in the range of 200–300 μmol/L in rats fed the fiber-free diets, and it was lower than 10 μmol/L in rats fed the inulin diets.

A substantial absorption of ammonia from the cecum was observed with all the diet conditions. In rats adapted to the fiber-free diets, ammonia absorption was doubled with the 45% casein level. Inulin in the diet drastically enhanced ammonia absorption: this flux reached a very high value (4.16 μmol/min) in rats adapted to the 45%

Table 5 Effects of changes in the dietary casein level and of the addition of 15% inulin in the diet on plasma urea and cecal ammonia, and the fluxes of urea and ammonia N between plasma and the cecum

	Plasma urea (mmol/L)	Cecal ammonia (mmol/L)	Urea N cecal flux (μmoles/min)	Ammonia N cecal flux (μmoles/min)	Balance (Urea N-Ammonia N) (μmoles/min)
Diets					
15% casein	3.50 ± 0.18	18.00 ± 0.94	0.17 ± 0.04	0.40 ± 0.07	−0.23
45% casein	9.90 ± 0.13*	23.10 ± 0.48*	0.77 ± 0.09*	0.81 ± 0.10*	−0.04
15% casein/Inulin	2.50 ± 0.39†	7.80 ± 0.56†	2.55 ± 0.36†	1.62 ± 0.20†	+0.93
45% casein/Inulin	9.60 ± 0.30*	18.05 ± 0.96*†	5.47 ± 0.54*†	4.16 ± 0.36*†	+1.31

Values are means ± SEM for 10 rats.
Significant difference ($P < 0.05$): * between 15% casein diets and 45% caseine diets, and † between inulin diets and fiber-free diets.

Table 6 Effects of changes in the dietary casein level and of the addition of 15% inulin in the diet on the concentrations of the major free amino acids in cecal content

	Fiber-free		15% inulin	
Diets	15% casein	45% casein	15% casein	45% casein
	μmles/ml cecal supernatant			
Total amino acids	7.93 ± 0.54	6.79 ± 0.41	11.38 ± 0.60†	15.25 ± 0.83*†
Taurine	0.48 ± 0.05	1.30 ± 0.12*	0.92 ± 0.10†	1.95 ± 0.18*†
Aspartate	0.77 ± 0.10	0.54 ± 0.06*	0.50 ± 0.06†	0.74 ± 0.08*
Glutamate	1.73 ± 0.12	1.85 ± 0.16	2.18 ± 0.15†	4.15 ± 0.31*†
Glutamine	0.12 ± 0.03	0.07 ± 0.02	0.10 ± 0.02	0.26 ± 0.04*†
Alanine	0.67 ± 0.08	0.68 ± 0.05	2.21 ± 0.17†	2.90 ± 0.25*†

Values are means ± SEM for 10 rats.
Significant difference ($P < 0.05$): * between 15% casein diets and 45% caseine diets, and † between inulin diets and fiber-free diets.

casein level. There was, in parallel, a net flux of urea from blood plasma to the cecum. This flux depends on both plasma uremia and cecal blood flow; as a result, compared with the basal 15% casein fiber-free diet, urea flux was 4.5-fold enhanced by increasing the casein level from 15 to 45% of the diet, and 15-fold enhanced by including 15% inulin in the diets. This accounts for the depressed uremia in rats fed the 15% inulin–low-protein diet. In rats fed inulin diets, the urea flux was doubled in rats adapted to the 45% casein level, compared with the 15% level.

The balance between urea N and ammonia N indicates that ammonia reabsorption slightly exceeded urea flux in rats fed the fiber-free diets. In rats fed the inulin diets, there was a marked N retention by the cecum (in the range of 1 μmol/min), which was only 40% greater in rats adapted to the 45% casein diet.

Effects of the diets on amino acids in the cecum and in blood plasma

As shown in *Table 6*, the total concentration of free amino acids in the cecum was not affected by the dietary protein level in rats fed the fiber-free diets. Free amino acids were elevated in rats fed the inulin diets, especially in those also adapted to the 45% casein level. Taurine was enhanced by both the high protein level and the presence of inulin in the diet. Glutamate and alanine were not affected by changes in dietary protein level in rats adapted to the fiber-free diets, but both amino acids were elevated in rats fed inulin and there was also an effect of the dietary protein level. It must be noted that the cecal concentrations of glutamine were very low, except in the group fed the high-protein–inulin diet.

The changes in the amino acid concentrations in arterial plasma are presented in *Table 7*. The most noticeable changes in plasma amino acids corresponded to the adaptation to the dietary protein level: the high protein diets led to an increase in taurine and branched-chain amino acids concentrations and to a decrease in the concentrations of glucogenic amino acids such as threonine, serine, glycine, and glutamine. Alanine, which is the major glucogenic amino acid, was significantly decreased only in rats adapted to the inulin-high protein diet which also had a particularly low glutamine concentration (0.45 mmol/L).

Discussion

Carbohydrates entering the large intestine considerably enlarge the bacterial mass, and this process requires a large supply of nitrogen. In rats fed semi-purified diets, protein such as casein is included under a purified (hence highly digestible) form; thus the amounts of dietary protein reaching the cecum should be quite limited. Increased secretion of digestive enzymes (trypsin, chymotrypsin, lipase, amylase) have been shown to occur when rats are fed diets containing pectin;[14] furthermore, it has been proposed that fiber may increase

the sloughing of mucosal cells.[15] Nevertheless, it seems unlikely that oligosaccharides would noticeably affect these digestive processes.

Blood urea may constitute a substantial source of nitrogen in the large intestine.[2,11,16,17] The present results indicate that, in rats fed fiber-free diets, urea N represented less than 50% of reabsorbed ammonia N in rats fed the 15% casein level and about 100% in those fed the 45% casein level. In rats fed the inulin diets, there was a considerable flux of urea N to the cecum; nevertheless, the N provision for bacterial metabolism was probably not limiting because substantial concentrations of ammonia were still present in the cecal contents (about 7–8 mmol/L), resulting in a noticeable reabsorption of ammonia in the cecal vein. In rats fed the normal protein level, dietary inulin significant depressed plasma uremia (−29%), probably due to urea flux in the intestinal content. Such an effect has been previously reported with fermented carbohydrates such as resistant starch.[18] The present data support the view that there is a close relationship between the flux of urea N toward the large intestine and the enhanced fecal N excretion. However, it must be kept in mind that some urea also enters the cecum by the intermediary of the ileal effluent, and that there is a substantial urea flux into the colon. Because a part of nitrogen is utilized for the synthesis of amino acids, arteriovenous differences between the cecal vein and artery have been determined: it turned out that the observed flux were negligible for most amino acids except (1) a net uptake of blood glutamine by the cecal wall and (2) a net absorption of glutamic acid, glycine, and taurine. In rats fed the inulin diet containing 15% casein, glutamine uptake represented 0.2 μmol N/min (compared with 2.55 μmol N/min for the urea flux) and the appearance of the various amino acids in the cecal vein was about 0.2 μmol N/min. Thus, the amino acid fluxes should not appreciably alter the arteriovenous N balance in the cecum.

Inulin, like other oligosaccharides, could be particularly effective in raising the transfer of urea N to the intestine: (1) inulin may have some osmotic effects in the small intestine and thus increase the amounts of urea in the distal ileum, (2) inulin may increase ureolysis either by selecting for a more actively ureolytic microflora and/or by accelerating ammonia diffusion (due to the relatively high fluidity of the cecal content). In rats fed the fiber-free diets, nitrogen excretion was poorly dependent on urea N transfer; most of this N is probably bacterial N (from endogenous sources) and casein digestibility was certainly very high. The decrease in the apparent protein digestibility in rats fed inulin thus chiefly reflects the rise of bacterial nitrogen metabolism, which has been observed with various types of fibers.[19–22] The presence of inulin in the small intestine does not affect the digestion of dietary casein, and the adequacy of the amino acid supply is illustrated by the unchanged level of most indispensable amino acids (branched-chain, threonine, methionine, lysine, etc.) in rats fed the 15% casein diet containing inulin, compared to their fiber-free controls.

In conclusion, it appears that intake of a readily fermented oligosaccharide such as inulin does not adversely affect the N balance or retention; the major difference with the fiber-free conditions is that bacterial N excretion is strongly enhanced with a proportional decrease in the renal N excretion. Thus the large intestine may represent a significant site for urea disposal. It is noteworthy that the fecal N excretion is minimally affected by a three-fold increase in dietary N intake. As previously reported,[11] oligosaccharides are very efficient in depressing ammonia in the cecum provided that plasma uremia (hence the dietary protein level) is relatively low. It has been set forth that a high bacterial proliferation in the proximal colon was at risk of providing large amounts of substrates for proteolysis and deaminations (together with alkalization of luminal pH) in the distal large bowel.[12] Nevertheless, this has been shown with fibers such as pectin, which yields moderately acidic fermentations, and it could be less critical with oligosaccharides, which could maintain relatively acidic conditions in the distal large intestine.[23] Anyway, from a nutritional point of view, it appears advisable to insure an equilibrated supply of both soluble and poorly soluble fibers together with a moderate dietary protein level. Under such conditions, it could be expected that fiber attenuate, for kidneys, the metabolic burden related to N disposal.

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