Very acidic fermentations in the rat cecum during adaptation to a diet rich in amylase-resistant starch (crude potato starch)

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The effects of very acidic fermentation on the production of organic acid by the cecal microflora has been investigated in rats fed a 40% crude potato starch (CPS) diet. The rats were adapted first to a fiber-free diet for 8 days before receiving the CPS diet. The diet elicited a dramatic enlargement of the cecum, especially during the first 5 days' adaptation, along with an almost immediate drop of cecal pH down to about 5. Lactic fermentations developed during the first days' adaptations (up to 120 mmol/L D + L-lactate), then lactate concentrations progressively declined whereas volatile fatty acids (VFA) concentrations rose. VFA concentrations also exhibited an evolution, with low butyrate fermentations up to 15 days' adaptation, then a rise of butyrate to values higher than those of propionate. Even during the period of maximal lactic fermentations, lactic acid absorption was relatively limited since its efficiency was about 5–8 fold lower than that of VFA. The CPS diet led to very high concentrations of calcium (up to 80 mmol/L) and phosphate in cecal contents. The activity of ornithine decarboxylase (ODC) increased rapidly at the beginning of adaptation to the CPS diet and reached a very high value after 30 days' adaptation. Thus, it appears that diets rich in CPS may elicit various types of fermentation profile during the period of adaptation to the diet, which is particularly long. These fermentations elicit a sustained hyperactivity of ODC in the cecal mucosa.

Keywords: crude potato starch; rat cecum; lactic acid; volatile fatty acids

Introduction

The digestive and metabolic effects of fibers have been the subject of an increasing number of investigations in recent years. Classically, a distinction is established between insoluble fibers (such as cellulose and a part of hemicelluloses) and soluble fibers which include a large variety of carbohydrates (pectins, gums, etc.). Most of the soluble fibers could hardly be administered in large amounts in the diet, due to their gel-forming properties which lead to poor palatibility and to depressed digestibility of some other nutrients, especially minerals. A part of ingested starch may also escape hydrolysis by α-amylase in the small intestine and reach the large intestine where it is fermented by the digestive microflora. It has been reported that starches may have effects similar to soluble fibers on metabolic and digestive parameters, but the importance of these effects depends on the type of food processing used and on the botanical origin of starch.[1-4] Various types of starches contain a high proportion of amylase resistant starch, such as pea starch, amylomaize starch, or crude potato starch (CPS). In humans, amylase resistant starch could, under some circumstances, represent a substantial part of polysaccharides entering the colon, besides fibers *sensu stricto*.[1,5-7] A part of the chemical energy of these starches may be recovered by the host in the form of organic acids, especially volatile fatty acids (VFA). However, in recent investigations on rats fed diets rich in CPS, a relatively limited production of VFA was reported even if the cecal VFA pool was effectively enhanced.[8,9] Thus, the aim of the present work was to

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further investigate the effects of diet containing a high percentage of CPS on the digestion in the large intestine of the rat, and to study the conditions corresponding to a large production of VFA or lactic acid with such a diet. Furthermore, the consequences of very acidic fermentations elicited by a CPS diet on some parameters related to epithelial cell proliferation in the large intestine will be discussed.

Methods and materials

Animals and diets

Forty male Wistar rats (IFA-CREDO, L'Arbresle, France) weighing 180 g were fed semi-purified diets that contained the following (by dry wt.): 71% carbohydrate, namely wheat starch (Louis François, Paris), 18% casein (L. François), 5- corn oil (C.I.O., Genay), 5% mineral mixture (U.A.R., Villemoisson/Orge), and 1% vitamin mixture (U.A.R.). In the CPS diet, a part of the wheat starch supply (40%) was replaced by CPS (Roquette Frères, Lestrem, France). The animals were housed in wire-bottomed cages in a temperature-controlled room (22°C) with the dark period from 20:00 to 08:00 hours; they were first fed the 71% wheat starch diet for 8 days, and then received the 40% CPS diet for 30 days.

Sampling and analytical procedures

Groups of 8 rats were sampled at 0, 2, 5, 15, or 30 days of adaptation to the CPS diet. The procedure of blood sampling from anaesthetized animals, for measurements of arteriovenous differences across the cecum, has been described previously.[10] For blood flow measurement, bromosulfo-phthaleine in saline (4 mg/ml) was infused in one of the small veins at the surface of the cecum at a rate of 100 μl/min. Dilution of the marker in the vein draining the whole cecum allows the determination of cecal blood flow, which appeared proportional to the weight of the cecal wall (ranging from 1.0 to 1.3 ml/min/g cecal wall). After blood sampling, the cecum was removed and weighed, and about 1 g of cecal contents was transferred to microfuge tubes that were plunged immediately in liquid nitrogen for 20 seconds and then stored −20°C. The cecal wall was flushed clean with ice-cold saline, blotted on filter paper, and weighed. When the activity of ornithine decarboxylase (ODC) was measured, the cecum was opened and flushed 3 times with ice-cold phosphate buffered saline (PBS) pH 7.5. The mucosa was scraped carefully and homogenized in 2 ml PBS (Potter-Elvejeim, 10 strokes). Aliquots of the extract were centrifuged, at 4°C, for 20 minutes at 38,000*g* (ODC), and the supernatants were utilized for determinations of enzyme activities and protein concentration (BCA Pierce reagent, Interchim, France).

VFA were measured by gas-liquid chromatography, after ethanolic extraction of plasma samples[11] and on aliquots of supernatants (8,000*g*, 5 minutes) of cecal contents. Ammonia and urea were determined spectrophotometrically on neutralized perchloric extracts by enzymatic methods.[12] Magnesium and calcium were measured by atomic absorption spectrophotometry (Perkin-Elmer 400, Norwalk, CT). Phosphate was measured by a colorimetric method at 690 nm using a Biotrol kit (Paris, France). ODC activity was determined according to Craven et al.[13] from the release of $^{14}CO_2$ from 1-[^{14}C]ornithine (CEA, Gif/Yvette, France).

Values are given as the means ±SEM, and where appropriate, significance of differences between mean values were determined by analysis of variance coupled with the Student-Newman-Keuls test.[14]

Results

Changes in body weight and in parameters of cecal fermentations

In rats adapted to the 40% CPS diet, the daily food intake and the body weight gain were 23.0 and 4.6 g/day, respectively, versus 21.5 and 5.8 g/day for the animals on a 71% wheat starch diet. *Figure 1a* shows the rapid rise in cecal weight during the first 2 days' adaptation to the CPS diet. This rise of cecal weight markedly declined between 2 and 5 days, then stabilized at about 0.3 g/day up to 30 days' adaptation. The weight of the cecal wall rapidly increased during the first 5 days experiment, then more slowly (*Figure 1b*). It must be noted that the development of the cecal wall was markedly slower than the rate of cecum enlargement during the early period of adaptation.

The cecal pH was initially at 7.2 and it dropped very rapidly down to 4.9 in rats fed the CPS diet, and then it leveled off at about 5.2 (*Figure 1c*). As shown in *Figure 2a*, the cecal pool of organic acids was practically constant (≈ 130 μmoles, consisting basically in VFA) in rats fed the control diet. In contrast, the VFA pool rapidly increased in rats fed the CPS diet, up to about 1300 μmol after 30 days' adaptation. In addition, lactic fermentations also developed, resulting in the presence of large amounts of lactate (L + D) during the early stage of adaptation to the CPS diet (up to 550 μmol). The lactate pool then progressively declined down to values in the range of 250–300 μmol, due to the opposite evolution of lactate concentrations and the cecal volume. As shown in *Figure 2b*, the total concentrations of organic acids were quite constant in control animals (about 90 mmol/L) and, in rats fed the CPS diet, it also leveled off within 2 to 5 days (at about 200 mM).

Changes in cecal concentrations of organic acids and in their absorption

In spite of this stability in the concentrations of total organic acids, there were noticeable changes in the respective concentrations of the various VFA as well as in the L and D isomers of lactate. The lactic fermentations at the beginning of the experiment chiefly corresponded to the production of L-lactate (about 75%) then, when the total concentration declined, the percentage of the D-isomer was higher, in the range of

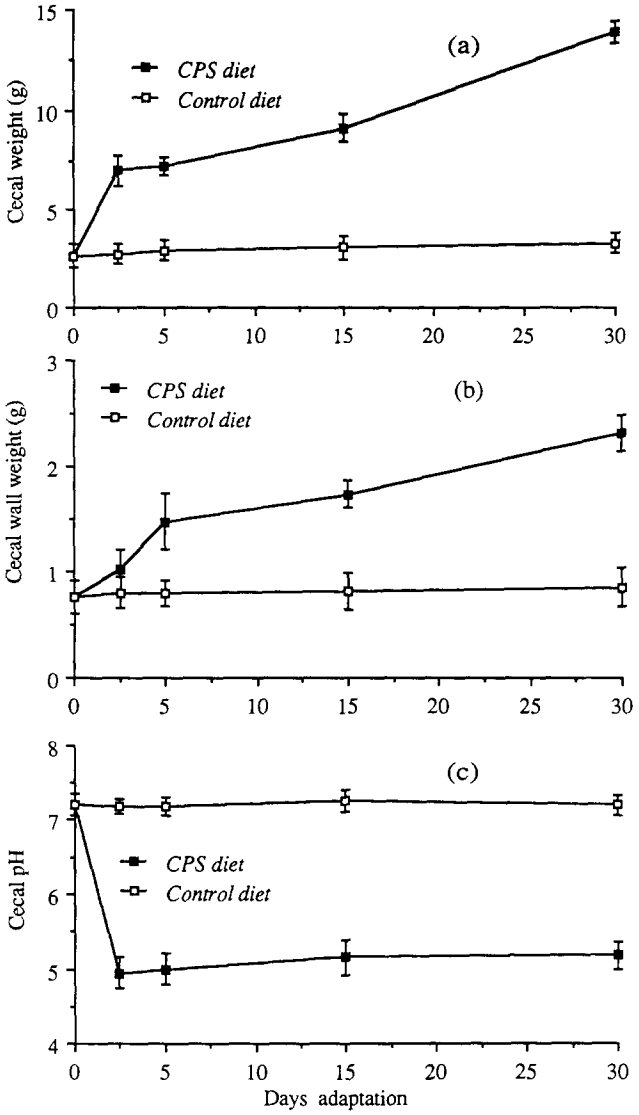

Figure 1 Changes in the weight of the cecal content (a) and the cecal wall (b), and in cecal pH (c) in rats during adaptation to a 40% CPS diet. The weight of the content was obtained by difference (total cecum − cecal wall). Results are means ± SEM for 8 rats for each experimental point.

30–35% (*Table 1*). Acetate and propionate concentrations progressively rose throughout the experiment, to about 120 mmol/L (acetate) or 20 mmol/L (propionate); thus, propionic fermentations appear rather limited with a 40% CPS diet. Butyrate presented a different evolution, since its concentration was particularly low during the first 15 days' adaptation, then markedly increased up to about 30 mM.

VFA absorption from the cecum was very low initially (about 1 μmol/min) in rats fed the control diet, as well as at the end of the experiment. In rats fed the CPS diet, the absorption of VFA increased progressively, reaching a very high value (≈ 25 μmol/min) after 30 days' adaptation. Lactate flux from the cecum was maximal (more than 1 μmol/min) after 2–5 days' adaptation. During this period, lactate and acetate concentrations in the cecum were in the same range (60–100 mmol/L) but acetate absorption was 5–7 fold higher than lactate absorption. Thereafter, lactate concentrations slowly declined and, at 30 days' adaptation, it represented no more than 5% of organic anions absorption. In rats fed the CPS diet, acetate absorption was 5 μmol/min at 2 days and close to 15 μmol/min at 30 days. Both propionate and butyrate absorption were strikingly enhanced at the end of the experiment. However, the rise of propionate absorption was progressive throughout the experiment, whereas butyrate absorption remained at a low value during the first 15 days' adaptation, then rapidly increased up to about 4 μmol/min.

Nitrogen fluxes

Ammonia concentrations in the cecum rapidly dropped from about 17 mmol/L down to 6–8 mmol/L, then leveled off (*Figure 3a*): The concentration of the NH_3 form was about 250 μmol/L in control rats against about 1 μmol/L in rats adapted to the CPS diet. The enlargement of the cecum was concomitant to a rise in the nitrogen fluxes in the cecum: transfer of urea N from blood to the cecum along with ammonia N absorption from the cecum (*Figure 3b*). These fluxes increased (more rapidly during early adaptation) in rats fed the CPS diet, up to 0.9 μmol/min for ammonia versus nearly 2 μmol/min for urea. It is noteworthy that, initially, the cycling of urea N as ammonia N was high (≈ 80%), but ammonia N absorption pla-

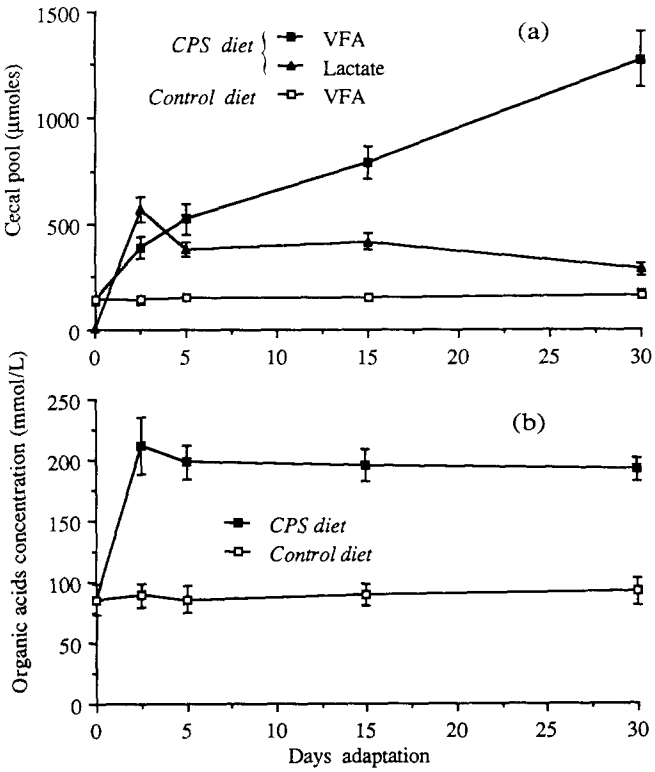

Figure 2 Changes in the cecal pool (a) and the concentrations (b) of the various organic acids. The pools were calculated as the cecal concentration (μmol ml) × cecal water (ml). Results are means ± SEM for 8 rats for each experimental point.

Table 1 Changes in the cecal concentrations and absorption of organic monocarboxylates

Days adaptation[a]	0[b]	2	5	15	30
Cecal concentrations (mmol/L)					
—Acetate	58.7 ± 6.5	78.0 ± 9.1	97.2 ± 8.6[c]	107.4 ± 7.0[c]	122.6 ± 9.4[c]
—Propionate	20.4 ± 2.2	5.3 ± 0.6[c]	11.7 ± 1.1[c]	15.1 ± 1.0	16.2 ± 1.1
—Butyrate	6.7 ± 1.2	5.2 ± 0.5	6.5 ± 0.3	11.0 ± 1.1[c]	28.1 ± 3.2[c]
—L-Lactate	5 <	93.5 ± 10.9	65.0 ± 11.2	41.7 ± 2.9	20.5 ± 1.7
—D-Lactate	5 <	28.4 ± 1.5	20.2 ± 1.9	18.9 ± 1.2	8.2 ± 1.1
Cecal flux (μmol/min)					
—Acetate	0.75 ± 0.08	4.23 ± 0.58[c]	7.88 ± 0.85[c]	10.77 ± 1.23[c]	18.46 ± 1.73[c]
—Propionate	0.20 ± 0.03	0.25 ± 0.03	1.14 ± 0.14[c]	2.07 ± 0.21[c]	3.04 ± 0.25[c]
—Butyrate	0.15 ± 0.03	0.22 ± 0.04	0.43 ± 0.05[c]	0.82 ± 0.11[c]	4.04 ± 0.43[c]
—L-Lactate	0.21 ± 0.04	0.95 ± 0.15[c]	0.74 ± 0.11[c]	0.67 ± 0.10[c]	0.50 ± 0.09[c]
—D-Lactate	≈ 0	0.27 ± 0.05[c]	0.19 ± 0.04[c]	0.17 ± 0.04[c]	0.10 ± 0.03[c]

[a] Results are means ± SEM for 8 rats for each period of sampling.
[b] The data of the concentrations and absorptions for the rats on the control fiber-free diet were not significantly modified during the 30 days period of experiment and are therefore presented only for To.
[c] Effect of the diet is significant ($P < 0.05$).

teaued after about 8 days' adaptation. As a result, the cycling of urea N decreased down to less than 50%, and large amounts of urea N were thus apparently used for bacterial protein synthesis.

Minerals

As shown in *Figure 4a*, there was an almost immediate rise in the concentrations of calcium and phosphate in the cecal supernatants of the rats fed the 40% CPS diet (Ca ≈ 80 mmol/L and phosphate ≈ 60 mmol/L vs. Ca ≈ 15 and phosphate ≈ 5–10 mmol/L, respectively, with the fiber-free diet). By contrast, the concentrations of magnesium (20 mmol/L) were moderately elevated by the CPS diet. It must be noted that the concentrations in the cecum were practically constant throughout the experimental period and the evolution of the cecal pool of calcium, magnesium, and phosphate was closely related to that of the cecum size. Calcium absorption rapidly increased during the first days of adaptation, then plateaued at about 1.2 μmol/min (*Figure 4b*). Magnesium displayed a very similar kinetic, but the magnitude of its absorption was markedly lower (about 0.5 μmol/min after 30 days).

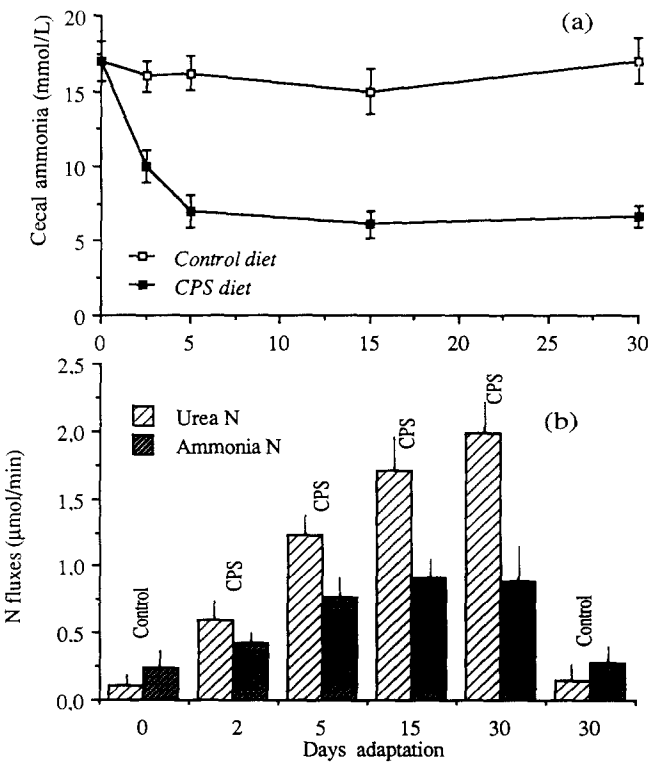

Figure 3 Changes in the cecal concentrations of ammonia (a) and in the nitrogen fluxes corresponding to urea N flux (from arterial plasma to the cecum) and ammonia N flux (from cecum to cecal vein plasma) (b). Results are means ± SEM for 8 rats for each experimental point.

Ornithine decarboxylase (ODC) activity

As shown in *Figure 5*, the activity of ODC in the cecal mucosa of rats fed a fiber-free diet is very low (≤ 0.1 nmol/min/mg prot). ODC activity readily increased in the early stage of adaptation to the CPS diet, then more slowly; after 30 days' adaptation, ODC activity remained at a very high value (1.4 nmol/min/mg prot).

Discussion

Resistant starches could be—from a functional point of view—considered as a soluble fiber;[15] however, this view has been questioned[3,4] since the characteristics of amylase resistance are not totally constant and predictable and are subject to alterations during the food processing (effects of cooking retrogradation upon cooling). The percentage of dietary starch reaching the cecum may be especially high with CPS (up to 75% with the present batch, cf[9]); lower values have been reported in the literature (from 40%[16] to 54%[8]). This discrepancy might reflect differences in the vegetal material or in the technology of starch isolation. Furthermore, it must be noted that with some resistant starches (potato, amylomaize, or pea starches), carbohydrates reaching the cecum are not completely fermented,[9,17] and, from recent data using the same type

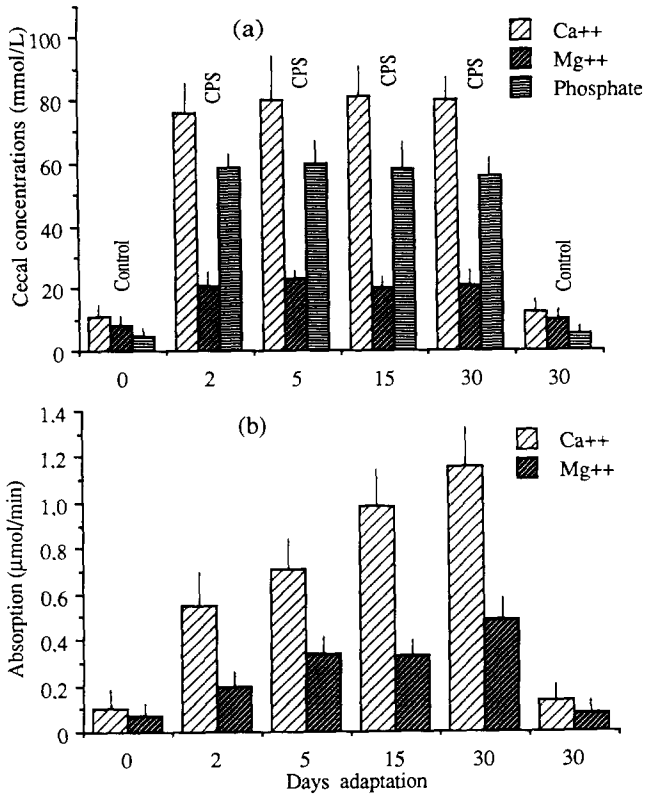

Figure 4 Changes in the cecal concentrations of Ca^{++}, Mg^{++} and phosphate in the cecum (a) and in the cecal absorption of Ca^{++} and Mg^{++} (b). There was no significant absorption of phosphate. Results are means ± SEM for 8 rats for each experimental point.

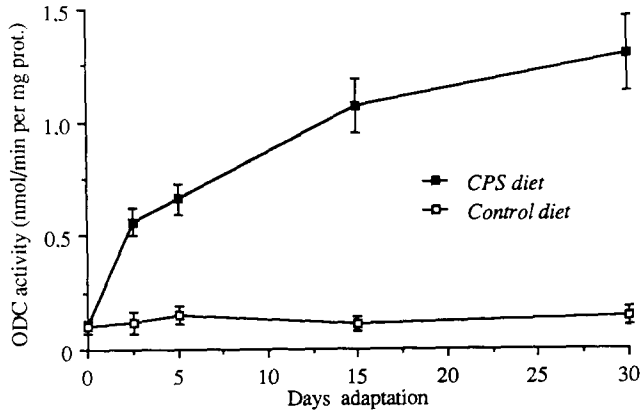

Figure 5 Changes in the activity of ornithine decarboxylase in the cecal mucosa. Results are means ± SEM for 8 rats for each experimental point.

of CPS,[9] only 75% of CPS reaching the cecum would be fermented.

In the present experiment, the rats were placed first for 8 days on a fiber-free diet before receiving the CPS diet, to study the effect of a large change in carbohydrate reaching the cecum on cecal fermentations and wall development. During the first days of adaptation, there was a considerable accumulation of lactic acid in the cecum which could be—to a large extent—accounted for by the limited capacities of absorption of the cecal wall (still poorly developed). It is noteworthy that the cecal pH readily dropped to about 5 and did not change further, in spite of further changes in cecal fermentations. Complete adaptation to the high-CPS diet was rather slow, compared to some fibers such as gums,[18] since the VFA profile characteristics of fermented starches (relatively rich in butyrate[19]) appeared after 15–20 days' adaptation. The VFA profile in rats fed CPS diets seems to depend on CPS percentage in the diet since, in previous experiments, a lower level (25%) was more favorable to propionic fermentations than to butyric fermentations.[20] In the present experiment, a noticeable production of lactic acid was still detectable after 30 days' adaptation, while VFA concentrations were about 150 mmol/L. It must be noted that, in experimental conditions close to the present (as to CPS level, duration of experiment), Mallet et al.[8] found low concentrations of VFA at pH 5.1 in adapted animals, which suggests that lactic fermentations were still predominant. Lactic acid absorption in the cecum seems much less efficient than VFA absorption; however, there is an endogenous production of L-lactic acid by the cecal wall (0.21 µmol/min at To) which precludes accurate quantification of L-lactic acid absorption. D-Lactic acid exclusively arises from bacterial metabolism, and it appears that its cecal absorption is poor, when present at 20–30 mmol/L in the cecum. This was particularly clear when VFA and lactate concentrations were very similar, at 2 or 5 days' adaptation: VFA absorption appeared 6–8 fold faster than lactic absorption. This suggests that (i) lactic fermentations are poorly efficient for the recovery of energy from fermented polysaccharides, and (ii) the rapid absorption of VFA from the cecum may lead to underestimating their importance in cecal fermentations. Even during the period of maximal lactic fermentations, there was no significant alteration of systemic L-lactate concentrations, and those of D-lactate remained very low (≤ 0.05 mmol/L).

The CPS diet appears very effective to decrease ammonia concentration in the cecum ($NH_3 + NH_4^+$ < 10 mmol/L; $NH_3 \approx 1$ µmol/L). The flux of ammonia absorption from the cecum plateaued, whereas urea transfer to the cecum was still increasing. Thus, it seems that the orientation of N flux towards bacterial synthesis is favored by a VFA-producing microflora rather than by a predominantly lactic microflora. This could be related to a lower bacterial density observed with low VFA fermentations in rats fed to CPS diet.[8]

In the present experiment, a striking rise in the cecal concentrations of calcium and phosphate was observed, up to 80 mmol/L for calcium, namely 5 to 8 fold higher than when rats were fed the 71% wheat starch diet. This could be explained by the presence of bound phosphate in potato starch,[21] but high calcium concentrations in the cecum seem to be a common feature for amylase resistant starch since it has also been observed with amylomaize starch.[15] This observation may have some nutritional bearing since calcium could counteract the stimulatory effects of bile salts or long chain fatty acids on cell proliferation in

the large intestine epithelium.[22,23] However, it must also be kept in mind that high phosphate concentration may thwart calcium effects.[24]

The activity of ODC, rate limiting enzyme for polyamines synthesis, is considered a reflection of the proliferative activity in the large intestine epithelium.[25] In the present experiment, ODC activity was actually enhanced during the early period of adaptation but it further increased (albeit more slowly) thereafter. Therefore, this observation suggests that very acidic fermentations may promote a high proliferative activity in cecal mucosa for an extended period. Yet a variety of factors should counteract this evolution: low pH conditions, low ammonia,[26] or high calcium concentrations (see above). On the other hand, it is conceivable that the rise of butyrate concentration at the end of the experiment could play a role in the maintenance of a high proliferative activity.[27] In the same view, Calvert et al.[28] have reported recently, in rats fed a 30% CPS diet, an hypertrophy of the large intestine (especially the colonic crypt column length) together with a considerable increase in the activity of the mucosal thymidine kinase. In conclusion, it appears that the cecal digestion in the rat can adapt to a high supply of CPS, with a transient period of predominantly lactic fermentations. The fact that very acidic fermentations seem to elicit a sustained hyperproliferative state in the cecal mucosa awaits further investigations and—if confirmed in humans—should be taken into consideration, besides the unfavorable effects ascribed to alkaline pH conditions in the colon.[29] Nevertheless, it must be kept in mind that, when utilized at a lower percentage, resistant starches lead to moderately acidic fermentations.[20]

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