higher proportions of propionic acid for the starchy concentrate (*ie* 28.8 *vs* 26.8%), but this difference was not significant. The proportion of acetic acid was significantly higher for the fibre concentrate (*ie* 52.4 *vs* 49.6%, $p < 0.05$). For the starchy diet, glycemia was, on average, higher (+0.02 g/l, $p < 0.05$). The β-OH butyrate content for the starchy diet was also higher from 4 h after the meal. These responses to the nature of the concentrate indicate some modifications of the energy metabolism and a reorientation of nutrient utilization. Indeed, the body weight gain was higher for the starchy diet (+11.2 kg, $p < 0.01$) and the fat content of milk was lower (33.8 *vs* 37.0 g/kg, $p < 0.05$).

Thus, the incorporation of grains into the diet of dairy cows appears to modify the milk composition in a way which conforms to the present desires of the dairy industry.

***In vivo* and *in vitro* fermentation of resistant starch and lactulose by human colonic microflora.** J Doré [1], M Durand [1], L d'Agay-Abensour [2], P Pochart [2], B Flourié [2], A Bernalier [1], M Champ [3], JC Rambaud [2] *([1] INRA, 78350 Jouy-en-Josas; [2] INSERM, hôpital Saint-Lazare, 75010 Paris; [3] INRA, 44026 Nantes, France)*

The hydrogen produced during the intracolonic fermentation of carbohydrates is excreted in breath, flatus or reutilized by microorganisms. In this study, the fermentation of retrograded high amylose corn starch by the human colonic microflora was compared with that of lactulose using *in vivo* gas production measurements, fecal microbial counts and *in vitro* comparisons of fermentation pathways.

Six healthy volunteers (3 methane- and 3 non-methane-excretors) ingested twice a day for 2 weeks during 2 periods, randomly ordered and separated by a washout period of at least 2 weeks, either 10 g lactulose, or 20 g resistant starch. On days 1, 7 and 14 of each period, H_2 and CH_4 were monitored in breath and flatus for 8 h following ingestion of the test carbohydrate, and stools were recovered and processed for microbial enumeration of total anaerobes, and H_2 utilizing methanogens, sulfate reducers and acetogens.

Three adaptation experiments (12 d) were also performed using a semi-continuous *in vitro* incubation system. Four 1 l vessels were inoculated with human fecal homogenates (100 g/l) collected either from a high, low or non-CH_4 excretor, and fed resistant starch or lactulose (5 g twice daily).

Whatever the status towards CH_4 excretion, the resistant starch tended to give lower total H_2 excretions than lactulose *in vivo*. The difference was highly significant on day 1 for CH_4 excretors and day 14 for non-CH_4 excretors. The total H_2 excretion tended to decrease further during adaptation in methane excretors and to increase in non-methane excretors, especially with lactulose.

Similarly *in vitro,* a 30% lower gas volume was observed with resistant starch, although the CH_4 production was stimulated. The latter parameters, together with the H_2 recoveries, were the only ones affected by the status of the donor towards methane excretion. Starch feeding led to stable pH (6.5) and ATP concentrations during the day, while with lactulose the pH dropped below 5.5 and the ATP concentrations increased sharply within 2 h post-feeding. The fermentation patterns of resistant starch and lactulose were different (lower butyrate and higher medium and branched chain fatty acid proportions with starch). The calculations of H_2 recoveries *in vitro* indicated that lactulose favored reductive acetogenesis as compared with resistant starch. Although the H_2 produced during *in vivo* intracolonic resistant starch fermentation appeared to be reutilized by hydrogen transfer, inter-individual variations in fecal microbial group counts did not allow the obser-

vation of significant population shifts. Due to its slower rate of hydrolysis, it is possible that resistant starch is degraded throughout the colon while lactulose hydrolysis and fermentation mainly occur in the proximal colon.

***In vitro* degradation of starch by *Bacteroides thetaiotaomicron* alone or in association with a human H_2-utilizing acetogenic bacterium.** V Rochet, A Bernalier, M Durand *(INRA, laboratoire de nutrition et sécurité alimentaire, 78350 Jouy-en-Josas, France)*

Starch that escapes digestion in the small intestine may constitute an important source of carbohydrates for colonic bacteria. Its degradation and fermentation by amylolytic bacteria produced short-chain fatty acids (SCFA) and the gases H_2 and CO_2. In methane-excreting subjects, H_2 can be reutilized *in situ* by methanogens. However it has recently been shown that when methanogenesis is low reductive acetogenesis ($4\ H_2 + 2\ CO_2 \longrightarrow 1$ acetate $+ 2\ H_2O$) may become a significant pathway of H_2 disposal (Bernalier *et al* (1994) *In: Nouvelles tendances en microbiologie anaérobie* SFM, Paris, 110-118).

The objective of the present work was to investigate *in vitro* the interactions that occur during starch hydrolysis between *Bacteroides thetaiotaomicron,* a hydrolytic bacterium of the dominant colonic flora, and an H_2-utilizing acetogenic bacterium, strain M5a3, isolated in our laboratory from human feces (Decaudin *et al* (1994) *In: Nouvelles tendances en microbiologie anaérobie* SFM, Paris, 304-307).

The kinetics of the starch degradation was studied in monoculture *(B thetaiotaomicron)* and coculture (*B thethaiotaomicron* + M5a3) after 1–8 d of incubation at 37°C. The substrate consumption was assayed enzymatically (Boehringer assay). The glucose liberated from the starch was measured by the GOP method. The fermentation products (gases and SCFA) were analysed by gas chromatography and the ethanol was determined enzymatically (Boehringer assay).

The addition of acetogenic bacterium induced an increase in the rate of starch hydrolysis by *B thetaiotaomicron.* The maximum extent of starch degradation was obtained after 3 d of coculture compared with 8 d in monoculture.

The hydrogen concentration in the coculture was always lower than in the monoculture (43 µmol/l *vs* 135 µmol/l respectively), where it accumulates, which indicates that the acetogenic bacterium was able to use the H_2 produced by the hydrolytic bacterium. Acetate, propionate and succinate were always the main products of the starch metabolism. However, a change in the fermentation pattern was observed in the presence of the acetogenic strain. The acetate concentration was higher in coculture (15.1 mmol/l *vs* 7.2 mmol/l in the monoculture). As the increase in acetate production was concomitant with a decrease in the H_2 concentration, it could be partly due to its formation by the strain M5a3 which mainly produces acetate from H_2/CO_2 metabolism. Nevertheless the existence of an interspecies H_2-transfer inducing a shift in the metabolism of *B thetaiotaomicron* to acetate is also likely.

This work demonstrates the existence of an interrelationship between *B thetaiotaomicron* and an acetogenic bacterium leading to a faster degradation of starch. It also suggests that in the colonic ecosystem, reductive acetogens have a significant effect in H_2-reutilization and acetate formation *in vivo.*

Distribution of elastase I and II messenger RNAs in calf tissue. I Le Huerou-Luron, M Gestin, R Toullec, P Guilloteau *(INRA,*