

Effects of three microbial probiotics on postprandial porto-arterial concentration differences of glucose, galactose and amino-nitrogen in the young pig

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Postprandial kinetics of porto-arterial concentration differences of glucose (G), galactose (Gal), L-lactic acid (LA) and amino-N (AN) were studied in the piglet after the ingestion of 10^7 colony-forming units (cfu) *Sporolactobacillus* P44 (SP), or 10^6 cfu *Bacillus cereus* IP5832 (AC), or 10^6 cfu of a combination of *Lactobacillus acidophilus*, *L. fermentum* and *L. brevis* (AB)/g feed. Sixteen fistulated piglets (portal vein and brachiocephalic trunk; mean body weight 22 (SD 2) kg) were used. The diet was based on skimmed milk (320 g/kg), barley (300 g/kg), wheat bran (110 g/kg), maize (100 g/kg) and lactose (70 g/kg). The postprandial blood kinetics, four measurements per animal at 1-week intervals, were studied for 6 h after the ingestion of test meals of 400 g basal diet (BD) or this diet supplemented with the bacteria (SP, AC and AB respectively). Areas of porto-arterial concentration differences (APACD) of G, Gal and LA were not influenced by the bacteria supplements. APACD of AN was significantly higher after the ingestion of the SP diet than that estimated for BD.

Probiotic: Absorption: Glucose: Amino-nitrogen: Piglet

Public misgivings about side-effects that often follow the use of antibiotics as growth promoters and therapeutic agents are at the origin of the probiotic concept and have strongly stimulated its development (Fuller, 1992). There is, therefore, a growing demand for an effective alternative to antibiotic growth promoters, and microbial probiotics could probably fill the gap.

Favourable changes, induced by microbial probiotics, in the activity of the digestive microflora have been described (Nguyen *et al.* 1988; Pusztai *et al.* 1990; Vanbelle *et al.* 1990). It has been demonstrated that lactobacilli exert various metabolic activities in the gut. Hill *et al.* (1970) observed that ingestion of lactobacilli significantly reduced intestinal and urinary amines in the pig. Ayebo *et al.* (1980) and Goldin *et al.* (1980) measured lower levels of β -glucuronidase (EC 3.2.1.31) and β -glucosidase (EC 3.2.1.21) activity in human faeces when lactobacilli were added to the diet. Muralidhara *et al.* (1977), Barrow *et al.* (1980), and Ratcliffe *et al.* (1986) observed modifications of the gut microbial balance in piglets after lactobacilli ingestion, in particular decreased counts of coliforms in the intestinal tissue and faeces. The beneficial effects of yoghurt, which contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, on lactose digestion has been demonstrated by Kim & Gilliland (1983), Garvie *et al.* (1984), Kolars *et al.* (1984) and Marteau *et al.* (1990). The presence of yoghurt micro-organisms in the gut resulted in a stimulating effect on the intestinal lactase (EC 3.2.1.23) activity (Besnier *et al.* 1983; Schaafsma *et al.* 1988). Recently we demonstrated that *Sporolactobacillus* P44 (Bel Industries, Vendôme, France)

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ingested by the growing–fattening pig (70 kg body weight) at 10^7 colony-forming units (cfu)/g of a feed rich in lactose, increased apparent intestinal absorption of glucose, galactose and amino-N (Rychen & Simões Nunes, 1993). The questions arising were, on the one hand, whether such effects could be observed in younger pigs having important endogenous lactase activity and, on the other hand, whether other potentially interesting bacterial strains could induce similar effects. Thus, the main aim of the present work was to study, in the weaner pig, the postprandial kinetics of porto-arterial plasma concentration differences of glucose, galactose, L-lactic acid and amino-N after ingestion of *Sporolactobacillus* P44, *Bacillus cereus* IP5832 (Paciflor®, Prodeta, 56000 Vannes, France) and a combination of *Lactobacillus acidophilus*, *L. fermentum* and *L. brevis*.

MATERIALS AND METHODS

Animals and diets

Sixteen castrated male Large White piglets (mean body weight 22 (SD 2) kg) were used. Each animal was fitted with two catheters, one placed in the portal vein and one in the brachiocephalic artery as described by Simões Nunes *et al.* (1989). Surgery was done under very strict aseptic conditions, taking into account the aim of the experimental work. The animals were not given antibiotics during or after surgery. To prevent obstruction by blood clots the cannulas were rinsed daily with a heparinized (100 IU/ml) NaCl solution (9 g/l). This was also done under strict aseptic conditions to avoid any risk of infection. The animals generally began to eat 1–2 d after the operation and rapidly recovered their normal growth rate. After surgery the animals received 400 g (twice daily) of either the mash basal diet (BD) or BD supplemented with *Sporolactobacillus* P44 at 10^7 cfu/g (SP), or *Bacillus cereus* IP5832 at 10^6 cfu/g (AC) or the combination of lactobacilli at 10^6 cfu/g (AB) according to the experimental design described below. The diet was based on maize, barley, skimmed milk and lactose, and contained 166 g crude protein/kg and 12.49 kJ digestible energy/g (Table 1). The supplemented diets were manufactured weekly at our facilities in order to achieve the requested minimal level of cfu/g feed, and stored at 4°. To avoid subsequent added-bacteria losses the supplemented feed was utilized within 1 week of preparation. Furthermore, added-bacteria counts in the feed were systematically checked by an epifluorescence technique belonging to Bel Industries for *Sporolactobacillus* P44, by numerations on a Mossel medium for *Bacillus cereus* IP5832, and by numerations on an MRS medium for the lactobacilli. The actual lowest and highest bacterial concentrations (cfu/g) in the supplemented diets were: 9×10^6 and 3×10^7 for SP; 2×10^6 and 5×10^6 for AC; 9×10^5 and 5×10^6 for AB. Lactose, glucose, galactose and amino-N contents of the four diets were determined after a storage period of 8 d (Table 2). On a weight basis the included bacteria represented 1/1000 of the supplemented diets. The experimental period began when the piglets had completely recovered from surgery (8 d). Throughout the experimental period the animals were individually housed in cages which permitted easy access to the cannulas. After surgery and under the feed restriction the piglets' mean daily weight gain was 272 (SD 60) g.

Measurements

The experimental design over a 4-week period was as follows: four piglets were given diet BD, four diet SP, four diet AC and four diet AB.

Postprandial portal and arterial plasma concentration kinetics and porto-arterial concentration differences of glucose (G), galactose (Gal), amino-N (AN), and L-lactic acid (LA) were evaluated. The sampling protocol was carried out four times for each animal at 1-week intervals. The observation period lasted for the first 6 h after ingestion of a 400 g test meal, preceded by a fasting period of 24 h. Portal vein blood and brachiocephalic trunk

Table 1. *Composition of the basal diet (g/kg)**

Skimmed milk	320
Barley	300
Wheat bran	110
Maize	100
Lactose	70
Wheat-straw meal	50
Lard	20
Vitamin and mineral mixture	30

* Calculated content (g/kg): crude protein 166, digestible energy 12.49 kJ/g, crude fibre 47.3, Ca 9.5, P 7.3, lysine 11.9, methionine 6.4.

Table 2. *Lactose, glucose, galactose and amino-nitrogen content (g/kg) of the experimental diets after a storage period of 8 d*

Diet ... Variable	BD	SP	AB	AC
Lactose	220	220	220	220
Glucose	4.7	5.0	4.9	5.0
Galactose	0.4	0.4	0.4	0.4
Amino-nitrogen	Tr	Tr	Tr	Tr

BD, based diet; SP, BD supplemented with *Sporolactobacillus* P44; AB, BD supplemented with a combination of *Lactobacillus acidophilus*, *L. fermentum* and *L. brevis*; AC, BD supplemented with *Bacillus cereus* IP5832; Tr, trace.

blood were sampled simultaneously (5 ml/site). The samples were withdrawn at intervals of 30 min during the first 3 h after the meal and hourly thereafter. Plasma samples were rapidly prepared by centrifugation (2500 g, 4°, 10 min) and stored at -20° until the assays, which were performed within 1 month after the experiment. G and LA were measured with commercial assay systems (Cobas Fara, Roche Diagnostic Systems, Neuilly-sur-Seine, France) as was Gal (Boehringer Mannheim, Meylan, France; Uvikon 930, Kontron Instruments, Birsfelden, Switzerland). AN was determined according to Palmer & Peters (1969) and Rérat *et al.* (1987). Plasma concentration values were calculated taking into account the packed cell volume measurements which were carried out throughout the 6 h observation period. The packed cell volume ranged from 27 to 34% without any apparent influence of the sampling on the relative part of plasma in whole blood.

Calculations

Postprandial kinetics of each variable in portal vein and arterial plasma were determined as well as postprandial kinetics of porto-arterial concentration differences. The area between the curve of portal vein plasma kinetic and that of the artery plasma kinetic is indicative of the apparent absorption. This area (APACD) was calculated during the first 3 and 6 h after feed ingestion for all the variables. Statistical analysis (Snedecor & Cochran, 1967) of APACD involved calculation of the mean and SD as well as a two-factor hierarchical ANOVA. The mathematical model was:

$$Y_{ijk} = \mu + A_i + B_{ij} + Z_{ijk},$$

where μ is the mean, A_i is the diet effect, B_{ij} is the combined effect of the diet and animal

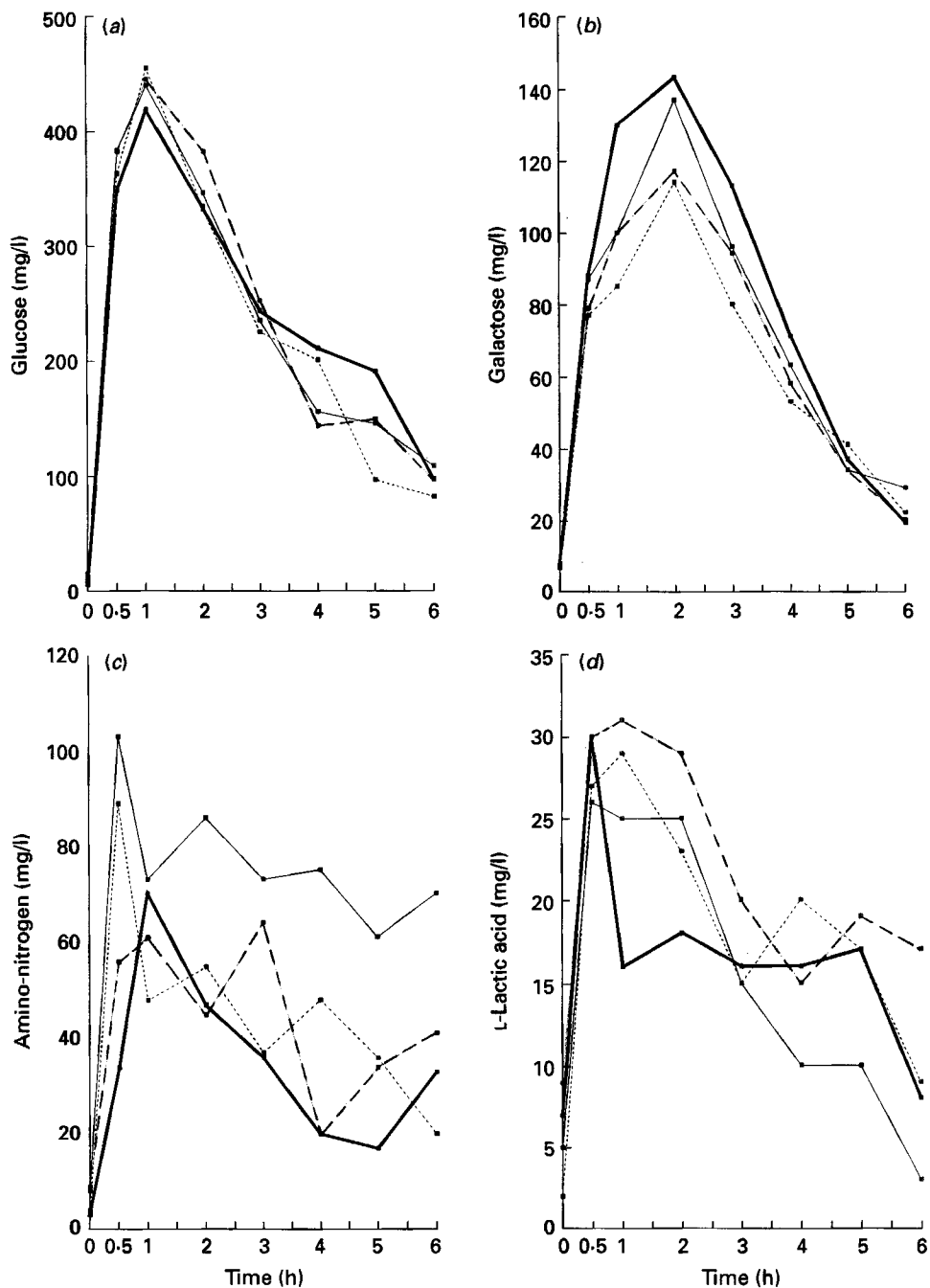


Fig. 1. Kinetics of porto-arterial differences of (a) glucose (mg/l), (b) galactose (mg/l), (c) amino-nitrogen (mg/l) and (d) L-lactic acid (mg/l) in the young pig after ingestion of a basal diet (BD) or the same diet supplemented with various probiotics. —, BD; —, BD supplemented with *Sporolactobacillus* P44; ---, BD supplemented with a combination of *Lactobacillus acidophilus*, *L. fermentum* and *L. brevis*; - - - -, BD supplemented with *Bacillus cereus* IP5832. Each point is the mean of sixteen determinations.

and Zijk is the residual term. The ANOVA was followed by Duncan's multiple range test when a significant A_i effect without B_{ij} effect was observed. These calculations were performed using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Kinetics of porto-arterial concentration differences

The kinetics of porto-arterial concentration differences of G, Gal, AN and LA are presented in Fig. 1. Before ingestion of the meal these differences were close to zero for G, Gal and AN and small for LA and very similar for all the treatments. At the end of the observation period porto-arterial differences of Gal returned to the basal level whilst those of the other variables remained higher than the preprandial levels, indicating that the appearances in the portal vein were not finished.

The porto-arterial differences of G (Fig. 1(a)), Gal (Fig. 1(b)) and LA (Fig. 1(d)) were not influenced by the bacteria supplements. Those of AN (Fig. 1(c)) were systematically higher after ingestion of the SP-supplemented diet.

Areas of porto-arterial concentration differences

Mean treatment values of APACD for each variable are presented in Table 3. It appeared that G, Gal and LA apparent absorption values were not modified by the addition of bacteria to the diet. Net appearance of AN in the portal vein after SP intake was significantly higher than that estimated after BD intake (A_i effect for AN APACD measured for the first 3 h postprandially ($P < 0.05$) and for the whole observation period ($P < 0.01$)). The 3 h and the 6 h AN APACD after the SP meal represented 169 and 198% respectively, of those measured for BD. The AN APACD after AB and AC meals were not

Table 3. Mean areas of glucose, galactose, amino-nitrogen and L-lactic acid porto-arterial concentration differences (APACD, mm^2) in the young pig, 3 h and 6 h after ingestion of a basal diet or the same diet supplemented with various probiotics*

Diet ...	BD	SP	AB	AC	SEM
Glucose					
APACD3	5745 ^a	5997 ^a	5862 ^a	5964 ^a	445.8
APACD6	9059 ^a	8733 ^a	8561 ^a	8753 ^a	706.0
Galactose					
APACD3	2113 ^a	1899 ^a	1815 ^a	1694 ^a	213.5
APACD6	3149 ^a	2849 ^a	2697 ^a	2551 ^a	260.2
Amino-nitrogen					
APACD3	832 ^c	1408 ^d	1065 ^{cd}	1131 ^{cd}	125.5
APACD6	1324 ^a	2618 ^b	1762 ^{ab}	1832 ^{ab}	254.5
L-Lactic acid					
APACD3	357 ^a	389 ^a	478 ^a	422 ^a	52.3
APACD6	631 ^a	585 ^a	782 ^a	728 ^a	87.1

BD, basal diet; SP, BD supplemented with *Sporolactobacillus* P44; AB, BD supplemented with a combination of *Lactobacillus acidophilus*, *L. fermentum* and *L. brevis*; AC, BD supplemented with *Bacillus cereus* IP5832.

^{a, b} Mean values within a row with unlike superscript letters were significantly different, $P < 0.01$.

^{c, d} Mean values within a row with unlike superscript letters were significantly different, $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 20–21.

significantly different from that measured for BD. It should be emphasized that no treatment-animal interaction effect (Bij) was observed for any of the variables studied.

DISCUSSION AND CONCLUSION

The results obtained in the present study are discussed on the assumption that portal blood flow was not significantly affected by individual variation or by addition of the bacteria. It is known that meal ingestion is followed by a small rise in portal blood flow during the first 1–2 postprandial hours (Simões Nunes *et al.* 1989). Individual variations in pig portal blood flow have been established at between 2.8 and 5.7% (Simões Nunes *et al.* 1989, 1992*a, b*). Furthermore, such variations in the portal blood flow were independent of the nature of very different experimental diets. One can easily assume that portal blood-flow variations in the present work were of the same range of amplitude as those observed previously, as well as similar for all the animals, and that they consequently interfered in the same way for all the four dietary treatments.

Microbial probiotics are thought to induce favourable changes in the activity of the digestive microflora (Nguyen *et al.* 1988; Puzstai *et al.* 1990; Vanbelle *et al.* 1990). Pig endogenous lactase activity is relatively important in weaner animals whilst it is very low in growing–fattening ones (Kidder & Manners, 1978). Thus, the similar glucose and galactose apparent absorption values observed after the ingestion of the four experimental diets suggested the absence of any apparent effect of all three probiotics on lactose digestion in individuals having enough endogenous lactase activity. Previously we demonstrated that in growing–fattening pigs *Sporolactobacillus* P44 strongly increased apparent absorption of glucose and galactose from the same diet (Rychen & Simões Nunes, 1993). Furthermore, such effects disappeared immediately after interruption of the addition of the micro-organisms to the diet, indicating absence of remnant effects of ingested bacteria after supplementation interruption. This suggests that the presence of the added bacteria in the intestinal lumen was fundamental to the observed modifications in apparent absorption. In the present study the effect on AN apparent absorption of *Sporolactobacillus* P44 ingestion was of the same magnitude for both the initial and final test meals, suggesting relatively short-term adaptation of the mechanisms involved.

Gastric, duodenal and jejuno-ileal pH values for both weaners and growing–fattening pigs were not modified by any of the bacteria or bacterial combinations studied (G. Rychen and C. Simões Nunes, unpublished results).

Sporolactobacillus is a soil bacterium and has been primarily selected because of its sporulation capacity and some likeness with lactobacilli. However, *Sporolactobacillus* P44 is a lactose-negative strain (Belville, 1990). Nothing is known about the eventual gut-colonizing capacity of the strain.

The beneficial effect of yoghurt on lactose digestion in humans (Kim & Gilliland, 1983; Garvie *et al.* 1984; Kolars *et al.* 1984; Marteau *et al.* 1990) was also demonstrated in lactase-deficient individuals.

In the present study *Sporolactobacillus* P44 (SP) supplementation significantly altered the amino-N levels in the portal vein whilst the increased apparent absorption observed after AC and AB at the doses used were very small and without statistical significance.

The effect of *Sporolactobacillus* P44 (SP) on amino-N apparent absorption confirmed the same observation previously reported in growing–fattening pigs (Rychen & Simões Nunes, 1993). Mechanisms involved in such an effect could be either a stimulation of endogenous proteolytic activity, an effect of supplemented bacterial proteolytic activity, a reduced catabolism of free amino acids in the intestinal lumen or a combination of all three. This

question calls for further experimental work. As emphasized before, strong precautions were taken to avoid, before feed ingestion, either a decrease in added bacteria or in subsequent bacterial metabolic activity. Determinations of levels of bacteria and free nutrients in the stored diets confirmed that the *in vivo* observations were a result of the added bacteria ingestion.

In conclusion, this study demonstrated that ingestion by the piglet of a diet supplemented with 10^7 cfu *Sporolactobacillus* P44/g significantly increased apparent absorption of AN, estimated by porto-arterial differences during the first six postprandial hours. Apparent absorption of G, Gal and LA were not modified by any of the added bacteria.

It would be of great interest to quantify such absorptive phenomena by the use of simultaneous determinations of portal blood flow and porto-arterial concentration differences. The study of the mechanism involved in the observed phenomena should also be undertaken.

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