Effects of a microbial probiotic (*Sporolactobacillus P 44*) on postprandial porto-arterial concentration differences of glucose, galactose and amino-nitrogen in the growing pig

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Summary — Postprandial kinetics of porto-arterial concentration differences of glucose (G), galactose (Gal), L-lactic acid (LA), amino-nitrogen (AN) and urea (U) were studied in the pig after the ingestion of $10^7$ colony-forming units (cfu) of *Sporolactobacillus P 44* per g of feed. Eight fistulated pigs (portal vein and brachiocephalic trunk; mean body weight 70 ± 4 kg) were used. The diet was based on skimmed milk (32%), barley (30%), maize (10%) and lactose (7%). The postprandial blood kinetics, 4 conducted per animal at 1-wk intervals, were studied during the 3 h following the ingestion of test meals of 1 000 g basal diet (BD) or the same diet supplemented by the bacteria (SD). The apparent absorption was estimated from the area between the portal and arterial concentrations. The areas of porto-arterial differences of G, Gal and AN of SD for the first 3 h after the meal were significantly higher after SD ingestion than those measured after BD intake. Plasma concentrations of U and porto-arterial differences of U and LA were not modified by the probiotic. These effects disappeared immediately after dietary supplement interruption, suggesting that added bacteria presence in the intestinal lumen was fundamental to the modifications observed in apparent absorption.

probiotic / absorption / glucose / amino-nitrogen / pig

Résumé — Effets d’un probiotique microbien (*Sporolactobacillus P 44*) sur les différences de concentrations porto-arterielles de glucose, de galactose et d’azote aminé chez le porc en croissance. Les cinétiques post-prandiales des différences de concentrations porto-arterielles de glucose (G), de galactose (Gal), d’urée (U), d’acide L-lactique (AL) et d’azote aminé (AA) ont été étudiées chez le porc après l’ingestion du probiotique *Sporolactobacillus P 44* incorporé à 10⁷ cfu/g d’aliment. Celui-ci était composé principalement de lait en poudre écrémé (32%), d’orge (30%), de maïs (10%) et de lactose (7%). L’étude a porté sur 8 porcs Large-White (70 ± 4 kg) munis de cathéters permanents dans la veine porte et dans le tronc brachiocéphalique. Les cinétiques post-
prandiales (4 pour chaque animal) ont été réalisées à intervalles d'une semaine durant les 3 premières heures suivant l'ingestion de 1 000 g d'aliment témoin (BD) ou d'aliment supplémenté (SD). L'absorption apparente a été estimée par l'aire entre les concentrations portales et artérielles. L'ingestion de SD a significativement augmenté l'aire entre les concentrations portales et artérielles du G, du Gal et de l'AA. Les concentrations plasmatiques de l'U ainsi que les différences de concentrations porto-artérielles de l'U et d'AL n'ont pas été modifiées par l'ingestion du probiotique. Ces effets ont disparu lorsque les animaux passaient du régime SD au régime BD, suggérant que la présence des micro-organismes dans la lumière intestinale a été fondamentale pour modifier l'absorption apparente des nutriments.

probiotique / absorption / glucose / azote aminé / porc

INTRODUCTION

Microbial probiotics are supposed to induce favorable changes in the activity of the digestive microflora (Nguyen et al, 1988; Pusztai et al, 1990; Vanbelle et al, 1990). The development of this concept has been partly stimulated by the public's misgivings about the side-effects that often follow the use of antibiotics as therapeutic agents and growth promoters (Fuller, 1992). There is, therefore, a growing demand for an effective alternative to antibiotic growth promoters and microbial probiotics could probably fill this gap.

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 beta-glucoronidase and beta-glucosidase activities 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 in the gut microbial balance in piglets after Lactobacilli ingestion, in particular decreased counts of coliforms in the intestinal tissue and faeces.

The beneficial effect of yoghurt, which contains Lactobacillus bulgaricus and Streptococcus thermophilus, on lactose digestion has been demonstrated by Kim and Gilliland (1983), Garvie et al (1984), Kolars et al (1984) and Marteau et al (1990). The presence of yoghurt microorganisms in the gut resulted in a stimulation effect on the intestinal lactase activity (Besnier et al, 1983; Schaafsma et al, 1988).

To our knowledge there are no experimental data on the effects of any potential interesting bacterial strain on the digestion and absorption of dietary proteins. It has been demonstrated that Sporolactobacillus P 44 improves lactose digestibility in rats (Belville, 1990) but no experimental results on the effects of this strain in pigs are available.

Thus, the aim of the present work was to study the postprandial kinetics of portal-arterial plasma concentration differences of glucose, galactose, L-lactic acid, amino-nitrogen and urea after the ingestion of 107 colony-forming units of Sporolactobacillus P44 (strain isolated by Bel Industries, France) per g of a feed based on skimmed milk, barley, maize and lactose.

MATERIALS AND METHODS

Animals and diets

Eight castrated male Large White pigs (mean body weight 70 kg ± 4) were used. Each animal was fitted with 2 catheters placed in the portal
vein and the brachiocephalic artery as described by Simões Nunes et al (1989). Surgery was
done in 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 cannulae were rinsed daily with a hepari-
nized 0.9% NaCl solution (100 IU/ml). This was
also done under aseptic conditions to avoid any
risk of infection. They generally began to eat 1–
2 d after the operation and rapidly recovered
their normal growth rate (500 g/d).

After recovering from surgery (1 wk) the ani-
mals received daily 2 meals of 1.5 kg each of ei-
ther the mash basal diet (BD) or BD supple-
mented with Sporolactobacillus P44 at 10⁷ cfu/g
(SD), according to the experimental design de-
scribed below. The diet was based on maize,
barley, skimmed milk and lactose, and con-
tained 16.6% crude protein and 12.49 kJ/g di-
gestible energy (table I). The diet SD was manu-
factured weekly at our facilities in order to have
a minimal level of 10⁶ colony-forming units per g
of feed and stored at 4°C. To avoid eventual
added bacteria losses the supplemented feed
cold storage was always inferior to 1 wk. Fur-
thermore, Sporolactobacillus counts in the feed
were systematically checked by an epifluores-
cence technique belonging to Bel Industries.
The lowest and highest Sporolactobacillus P 44
concentrations in SD ingested feed were respec-
tively 9 x 10⁶ and 3 x 10⁷ cfu/g. Lactose, glu-
cose, galactose and amino-nitrogen content of

Table I. Composition of the basal diet (%) *.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skimmed milk</td>
<td>32</td>
</tr>
<tr>
<td>Barley</td>
<td>30</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>11</td>
</tr>
<tr>
<td>Maize</td>
<td>10</td>
</tr>
<tr>
<td>Lactose</td>
<td>7</td>
</tr>
<tr>
<td>Wheat straw meal</td>
<td>5</td>
</tr>
<tr>
<td>Swine lard</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin and mineral mixture</td>
<td>3</td>
</tr>
</tbody>
</table>

* Calculated content: crude protein: 16.6%; digestible energy: 12.49 kJ/g; crude fibre: 4.73%; Ca: 0.95%; P: 0.73%; Lys: 1.19%; Met: 0.64%.

Table II. Lactose, glucose, galactose and amino-nitrogen content (%) of BD and SD diets after a storage period of 8 d.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BD</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.47</td>
<td>0.50</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>Traces</td>
<td>Traces</td>
</tr>
</tbody>
</table>

both diets were determined after a storage peri-
od of 8 d (table II). On a weight basis the includ-
ed bacteria spores represented 0.1% of the diet.

The experimental period began when the pigs
had completely recovered from surgery (8–
10 d). Throughout the experimental period the
animals were kept individually in cages which
permitted easy access to the cannulae.

Measurements

The experimental design over a 4-wk period
was the following: 2 pigs (numbers 3 and 6)
were allowed BD; 2 pigs (numbers 2 and 7) re-
ceived SD; 2 pigs (numbers 1 and 8) ingested
successively BD during a 15-d period and then
SD during the next 15-d period; and the last 2
animals (numbers 4 and 5) received first SD and
then BD for 2 periods of 15 days.

Postprandial portal and arterial plasma con-
centration kinetics as well as porto-arterial con-
centration differences of glucose (G), galactose
(Gal), amino-nitrogen (AN), urea (U) and L-lactic
acid (LA) were evaluated.

The sampling protocols, 4 for each animal,
were conducted at 1-wk intervals. The observa-
tion period lasted for the first 3 h after ingestion
of the 1 000 g test meal. The latter was preced-
ed by a fasting period of 24 h. Portal vein and
brachiocephalic trunk blood were sampled si-
multaneously (10 ml/site). The samples were
withdrawn at 30-min intervals during the first 3 h
after the meal. Withdrawn blood was replaced
by a heparinized 0.9 NaCl solution (100 IU/ml).
Plasma samples were rapidly prepared by cen-
trifugation (2 500 g, 4°C, 10 min) and stored at
–20°C until the assays, which were performed
within 1 month of the experiment. G, U and LA were measured with commercial assay systems (Cobas Fara, Roche Diagnostic Systems, Neuilly-sur-Seine, France) as was Gal (Boehringer Mannheim France, Meylan, France;UVikon, Kontron Instruments, Birsfelden, Switzerland). AN was determined according to Palmer and Peters (1969) and Rérat et al (1987). Hemato-crit measurements were carried out throughout the 3-h observation period. The packed cell volume ranged from 27 to 34% without any apparent influence of the sampling on the relative part of the plasma in whole blood.

Calculations

Postprandial kinetics of each parameter in the portal vein and the arterial plasma were determined as well as postprandial kinetics of portal-arterial concentration differences. The area between the curve of portal vein plasma kinetics and that of the artery plasma kinetics is indicative of the apparent absorption. This area (APACD) was calculated during the 3 h immediately following ingestion for G, Gal, AN and LA.

Statistical analysis (Snedecor and Cochran, 1967) involved calculation of the mean and standard error as well a 2-factor analysis of variance. For APACD of pigs 2, 3, 6 and 7 the hierarchical mathematical model was:

\[ Y_{ijk} = \mu + A_i + B_{ij} + Z_{ijk} \]

For APACD of pigs 1, 4, 5 and 8 the mathematical model was:

\[ Y_{ijk} = \mu + A_i + B_{ij} + C_{ij} + Z_{ijk} \]

In these formulae \( \mu \) is the mean, \( A_i \) is the diet effect, \( B_{ij} \) is the animal effect, \( C_{ij} \) is the combined effect of diet and animal, \( C_{ij} \) is the interaction between the animal and the diet and \( Z_{ijk} \) the residual difference.

The calculations were performed with the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

The addition of *Sporolactobacillus* P 44 to the diet produced a larger rise in portal plasma concentration of G than that observed after the basal diet ingestion (fig 1). Arterial plasma G concentrations were not affected by the diets. Thus, the mean APACD for G (tables III, IV) was significantly higher during the observation period after ingestion of SD.

After both meals an increase in portal concentration of Gal was observed (fig 2). Nevertheless, the rise in portal Gal was

![Fig 1. Means of portal (P) and arterial (A) plasma concentrations of glucose (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.](image1)

![Fig 2. Means of portal (P) plasma concentrations of galactose (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.](image2)
higher after SD ingestion. Gal was undetectable in arterial plasma. Mean APACD for Gal (tables III, IV) was significantly higher for SD than for BD.

The increase in portal plasma AN concentration was higher during the first 90 min after the beginning of the meal for SD (fig 3) whilst arterial plasma concentrations were very similar for both diets. Mean APACD for AN appeared to be significantly higher for SD than that calculated for the basal diet (tables III, IV).

Table III. Mean areas (mm²) of porto-arterial concentration differences of glucose (G), galactose (Gal), amino-nitrogen (AN) and L-lactate (LA) in the pig after ingestion of diets BD and SD. Animals ingesting the same diet for all the experimental period.

<table>
<thead>
<tr>
<th>Pig No</th>
<th>G</th>
<th>Gal</th>
<th>AN</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6 386 ± 1 055 a</td>
<td>1 760 ± 162</td>
<td>729 ± 272</td>
<td>708 ± 150</td>
</tr>
<tr>
<td>6</td>
<td>5 212 ± 279</td>
<td>1 032 ± 172</td>
<td>499 ± 428</td>
<td>640 ± 350</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8 338 ± 976</td>
<td>1 779 ± 113</td>
<td>1 294 ± 275</td>
<td>814 ± 163</td>
</tr>
<tr>
<td>7</td>
<td>6 459 ± 1 200</td>
<td>1 907 ± 255</td>
<td>981 ± 361</td>
<td>613 ± 220</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>Aᵢ P &lt; 0.05</td>
<td>Aᵢ P &lt; 0.05</td>
<td>Aᵢ P &lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Mean ± SD of 4 determinations; Aᵢ: diet effect; Bᵢ: combined effect of diet and animal.

Table IV. Mean areas (mm²) of porto-arterial concentration differences of glucose (G), galactose (Gal), amino-nitrogen (AN) and L-lactate (LA) in the pig after ingestion of diets BD and SD. Animals submitted to diet change.

<table>
<thead>
<tr>
<th>Pig No</th>
<th>G</th>
<th>Gal</th>
<th>AN</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8 a</td>
<td>6 580 ± 724 b</td>
<td>1 004 ± 172</td>
<td>827 ± 558</td>
<td>621 ± 470</td>
</tr>
<tr>
<td>4/5 c</td>
<td>6 881 ± 2 124</td>
<td>826 ± 119</td>
<td>649 ± 288</td>
<td>747 ± 373</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8 c</td>
<td>8 576 ± 1 369</td>
<td>1 581 ± 435</td>
<td>916 ± 159</td>
<td>395 ± 133</td>
</tr>
<tr>
<td>4/5 a</td>
<td>6 953 ± 508</td>
<td>936 ± 192</td>
<td>799 ± 248</td>
<td>599 ± 119</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>Aᵢ P &lt; 0.05</td>
<td>Aᵢ P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a First period, wk 1 and 2; b mean ± SD of 4 determinations; c second period, wk 3 and 4; Aᵢ: diet effect.
The meal intake was followed by a rise in LA plasma concentrations (fig 4). The higher LA rise in portal plasma after SD ingestion was associated with a parallel higher arterial LA concentrations. Any difference was noted in the APACD for LA whatever the ingested diet (tables III and IV).

Detailed results of G, Gal and AN APACD of pigs receiving successively the 2 diets (1, 4, 5 and 8) are presented in table IV. The G, Gal and AN APACD of pigs 1 and 8 were higher during the last 2 experimental weeks when the animals were fed SD. In pigs 4 and 5, G, Gal and AN APACD appeared smaller during the second part of the experimental period when the animals received the diet BD. This strongly suggests the absence of a remnant effect of the ingested bacteria after supplementation interruption.
Ingestion of both diets resulted in a systematic increase in both portal and arterial plasma U levels throughout the time period studied. The plasma U concentrations in portal and arterial blood withdrawn at the same time were found to be very similar. This was found with both diets and resulted in porto-arterial differences of plasma U which, at all the times studied, were close to zero.

**DISCUSSION AND CONCLUSION**

The results obtained in the present study are discussed on the assumption that portal blood flow was not significantly affected by individuals or by addition of *Sporolactobacillus* P 44. 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). In pigs with a body weight close to that of those used in the present work such a blood flow increase represented about 8% of the basal flow (Simões Nunes et al, 1992). Individual variations in portal blood flow have been established in 2.8 and 5.7% of the mean flow determined for pigs weighing 60–70 kg (Simões Nunes et al, 1989, 1992). Furthermore, such variations in the portal blood flow were independent of the nature of very different tested diets. One can easily assume that portal blood flow variations in the present work were of the same range of amplitude as those observed before and consequently interfering in a similar way for both dietary treatments.

Microbial probiotics are supposed to induce favorable changes in the activity of the digestive microflora (Nguyen et al, 1988; Pusztai et al, 1990; Vanbelle et al, 1990). Beneficial effects of the microorganisms in yoghurt on lactose digestion have been demonstrated by several authors (Kim and Gilliland, 1983; Garvie et al, 1984; Kolars et al, 1984 and Marteau et al, 1990). In agreement with their findings, we observed that in the growing pig galactose and glucose apparent absorptions were significantly higher with the diet SD, suggesting that *Sporolactobacillus* P44 at 10⁷ cfu/g improved the digestion of lactose. However, the mechanism of action is still unclear. Besnier et al (1983) and Schaaftsmma et al (1988) showed that the microorganisms in the yoghurt resulted in a stimulating effect on the intestinal lactase activ-

![Graph](https://via.placeholder.com/150)

**Fig 5.** Means of portal (P) and arterial (A) plasma concentrations of urea (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

Ingestion of both diets resulted in a systematic increase in both portal and arterial plasma U levels (fig 5) throughout the time period studied. The plasma U concentrations in portal and arterial blood withdrawn at the same time were found to be very similar. This was found with both diets and resulted in porto-arterial differences of plasma U which, at all the times studied, were close to zero.
ity. Sporolactobacillus P44 could exert similar effects in the gut of growing pigs.

Pig endogenous lactase activity decreases quickly after weaning and is very low in the growing animal (Kidder and Manners, 1978). Thus, the higher galactose and glucose absorptions observed after the probiotic supplementation was probably either the result of a stimulated intestinal lactase activity or that of the hydrolytic capacity of the added bacteria. The question arising is whether such an effect is also evident in younger animals.

One interesting finding was the improvement of apparent intestinal absorption of amino-nitrogen by addition of microorganisms to feed. To our knowledge this is the first time that a probiotic effect on amino-acid apparent absorption was studied and observed. The involved mechanism could be a stimulation of endogenous proteolytic activity, an effect of the proteolytic activity of the bacteria, a reduction of amino-acid catabolism by the endogenous microflora or the result of all of these effects. This aspect request also further experimental work.

This study has demonstrated that ingestion by the growing pig of a diet supplemented with Sporolactobacillus P44 significantly increased apparent intestinal absorption, estimated by porto-arterial differences of G, Gal and AN during the first 3 h postprandial.

As emphasized before, extensive precautions were taken to prevent beforefeed ingestion and a decrease in either added bacteria spores or eventual bacteria metabolic activity. Determinations of Sporolactobacillus P 44 spore levels and free nutrients in the diets after storage confirmed that the in vivo observations resulted from the added bacteria ingestion.

It is known that several dietary components influence gastric emptying (Chang et al, 1984) and conversely the rate of intestinal absorption. The question arising was whether the observed differences in apparent absorption after ingestion of the bacteria supplemented diet were, at least in part, a consequence of induced changes in gastric emptying. It appeared highly improbable that the addition of Sporolactobacillus P 44 spores could strongly influence gastric emptying. This is for 2 main reasons, the added spores represented 1.5 g per meal, and there is no evidence that such or closely related bacteria can induce changes in gastric emptying.

Porto-arterial differences of LA and plasma concentrations of U were not modified by the added bacteria. These effects disappeared immediately after interruption of the addition of micro-organisms to the diet. This suggests that the presence of the added bacteria in the intestinal lumen was fundamental to the modifications observed in apparent absorption.

Our results obtained on growing pigs underline some interesting effects of the probiotic, particularly those related to amino-nitrogen appearance in the portal vein. It would be of great interest to evaluate such effects in younger animals and 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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