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Original article

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-artérielles de glucose, de galactose et d'azote aminé chez le porc en croissance. *Les cinétiques post-prandiales des différences de concentrations porto-artérielles 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 10^7 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-*

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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 porto-arterial plasma concentration differences of glucose, galactose, L-lactic acid, amino-nitrogen and urea after the ingestion of 10^7 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 heparinized 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 animals received daily 2 meals of 1.5 kg each of either the mash basal diet (BD) or BD supplemented with *Sporolactobacillus* P44 at 10^7 cfu/g (SD), according to the experimental design described below. The diet was based on maize, barley, skimmed milk and lactose, and contained 16.6% crude protein and 12.49 kJ/g digestible energy (table I). The diet SD was manufactured weekly at our facilities in order to have a minimal level of 10^7 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. Furthermore, *Sporolactobacillus* counts in the feed were systematically checked by an epifluorescence technique belonging to Bel Industries. The lowest and highest *Sporolactobacillus* P 44 concentrations in SD ingested feed were respectively 9 x 10^6 and 3 x 10^7 cfu/g. Lactose, glucose, galactose and amino-nitrogen content of both diets were determined after a storage period of 8 d (table II). On a weight basis the included bacteria spores represented 0.1% of the diet.

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

Ingredient	*%*
Skimmed milk	32
Barley	30
Wheat bran	11
Maize	10
Lactose	7
Wheat straw meal	5
Swine lard	2
Vitamin and mineral mixture	3

* 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.

Parameters	*BD*	*SD*
Lactose	22	22
Glucose	0.47	0.50
Galactose	0.04	0.04
Amino-nitrogen	Traces	Traces

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) received 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 concentration kinetics as well as porto-arterial concentration 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 observation period lasted for the first 3 h after ingestion of the 1 000 g test meal. The latter was preceded by a fasting period of 24 h. Portal vein and brachiocephalic trunk blood were sampled simultaneously (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 centrifugation (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 porto-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:

$$Y_{ijk} = \mu + A_i + B_j + C_{ij} + Z_{ijk}$$

In these formulae μ is the mean, A_i is the diet effect, B_j is the animal effect, B_{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

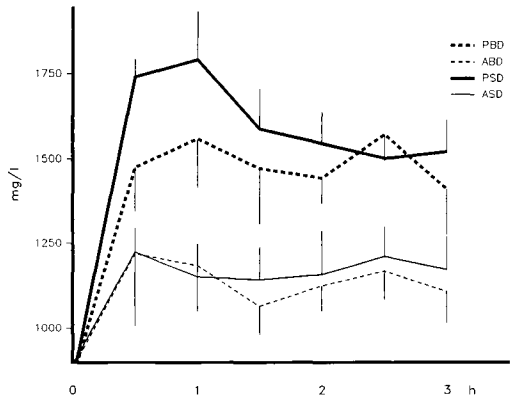


Fig 1. Means of portal (P) and arterial (A) plasma concentrations of glucose (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

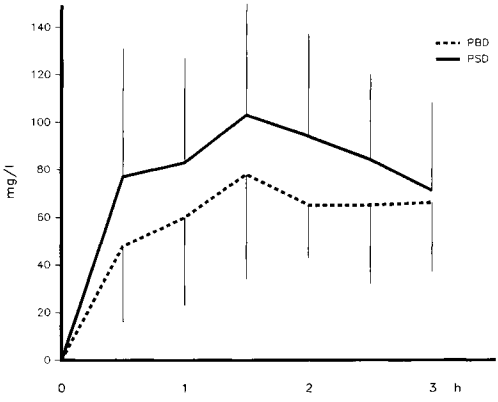


Fig 2. Means of portal (P) plasma concentrations of galactose (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

Table III. Mean areas (mm^2) of porto-arterial concentrations 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.

Pig No	*G*	*Gal*	*AN*	*LA*
BD				
3	6 386 ± 1 055 [a]	1 760 ± 162	729 ± 272	708 ± 150
6	5 212 ± 279	1 032 ± 172	499 ± 428	640 ± 350
SD				
2	8 338 ± 976	1 779 ± 113	1 294 ± 275	814 ± 163
7	6 459 ± 1 200	1 907 ± 255	981 ± 361	613 ± 220
Statistical significance	A_i $P < 0.05$	A_i $P < 0.05$ B_{ij} $P < 0.01$	A_i $P < 0.01$	NS

[a] Mean ± SD of 4 determinations; A_i: diet effect; B_{ij}: combined effect of diet and animal.

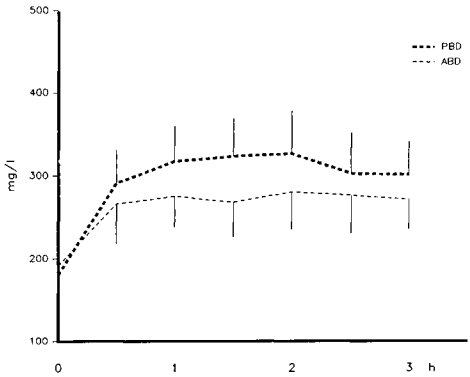
Table IV. Mean areas (mm^2) of porto-arterial concentration differences of glucose (G), galactose (Gal), amino-nitrogen (AN) and L-lactate (LA) in the pig after ingestion of diets BD and SD. Animals submitted to diet change.

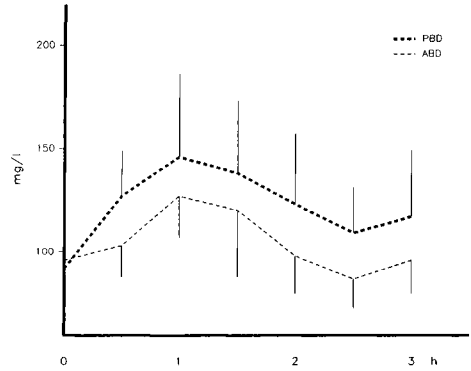
Pig No	*G*	*Gal*	*AN*	*LA*
BD				
1/8 [a]	6 580 ± 724 [b]	1 004 ± 172	827 ± 558	621 ± 470
4/5 [c]	6 881 ± 2 124	826 ± 119	649 ± 268	747 ± 373
SD				
1/8 [c]	8 576 ± 1 369	1 581 ± 435	916 ± 159	395 ± 133
4/5 [a]	6 953 ± 508	936 ± 192	799 ± 248	599 ± 119
Statistical significance				
1/8	A_i $P < 0.05$	A_i $P < 0.05$	NS	NS
4/5	NS	NS	NS	NS

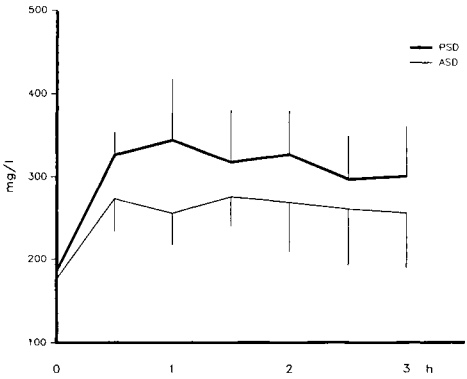
[a] First period, wk 1 and 2; [b] mean ± SD of 4 determinations; [c] second period, wk 3 and 4; A_i: diet effect.

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).







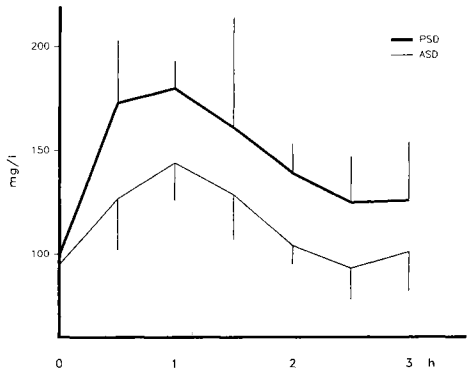


Fig 3. Means of portal (P) and arterial (P) plasma concentrations of amino-nitrogen (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

Fig 4. Means of portal (P) and arterial (P) plasma concentrations of L-lactate (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

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.

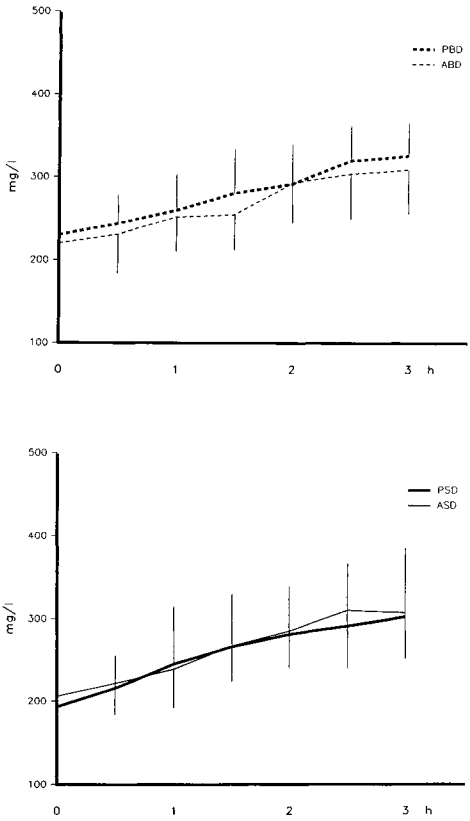


Fig 5. Means of portal (P) and arterial (P) plasma concentrations of urea (mg/l, 16 determinations/diet) in the pig after the ingestion of diets BD and SD.

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.

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 10^7 cfu/g improved the digestion of lactose. However, the mechanism of action is still unclear. Besnier *et al* (1983) and Schaafsma *et al* (1988) showed that the microorganisms in the yoghurt resulted in a stimulating effect on the intestinal lactase activ-

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 micro-organisms 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 before-feed 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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REFERENCES

Ayebo AD, Angelo IA, Shahani KM (1980) Effect of ingesting *Lactobacillus acidophilus* milk

upon fecal flora and enzyme activity in humans. *Milchwissenschaft* 35, 73-84

Barrow PA, Brooker PE, Fuller R, Newport MJ (1980) The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. *J Appl Bacteriol* 48, 147-154

Belville C (1990) Influence du bacille sporulé P 44 sur la digestibilité du lactose chez les rats gnotoxéniques. Mémoire Ingénieur Université Aix-Marseille I, 40 p

Besnier MO, Bourlioux P, Fourniat J, Ducluzeau R, Aumaître A (1983) Influence de l'ingestion de yogourt sur l'activité lactasique intestinale chez des souris axéniques ou holoxéniques. *Ann Microbiol* 134, 219-230

Chang MLV, Li BW (1984) Effect of gel-forming undigestible polysaccharides *versus* cellulose on intestinal sugar concentrations and serum glucose level in rats. *Nutr Rep Int* 30, 789-796

Fuller R (1992) History and development of probiotics. *In: Probiotics – The Scientific Basis* (R Fuller, ed) Chapman and Hall, London, 1-7

Garvie EI, Cole CB, Fuller R, Hewitt D (1984) The effect of yoghurt on some components of the gut microflora and on the metabolism of lactose in the rat. *J Appl Bacteriol* 56, 237-245

Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL (1980) Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *JNCI* 64, 255-261

Hill IR, Kenworthy R, Porter P (1970) Studies of the effect of dietary lactobacilli on intestinal and urinary amines in pigs in relation to weaning and post-weaning diarrhoea. *Res Vet Sci* 2, 320-326

Kidder DE, Manners M (1978) *Digestion in the Pig*. Scientechnica, Bristol

Kim HS, Gilliland SE (1983) *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. *J Dairy Sci* 66, 959-966

Kolars JC, Levitt MD, Aouji M, Savaiano DA (1984) Yogurt, an autodigesting source of lactose. *N Engl J Med* 5, 1-3

Marteau P, Flourie B, Pochart P, Chastang C, Desjeux JF, Rambaud JC (1990) Effect of the microbial lactase (E.C.3.2.1.23) activity in yogurt on the intestinal absorption of lactose: an *in vivo* study in lactase-deficient humans. *Br J Nutr* 64, 71-79

Muralidhara KS, Sheggeby GG, Elliker PR, England DC, Sandine WE (1977) Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissue and faeces from piglets. *J Food Protec* 40, 288-2955

Nguyen TH, Duperray J, Eckenfelder B, Lecamp B, Lefrancois S, Levesque A, Nebout JM, Ridremont B, Salle F, Sergheraert R (1988) Quelques probiotiques facteurs de croissance. *Rev Alim Anim* 3, 31-37

Palmer DW, Peters T (1969) Automated determination of free amino groups in serum and plasma using 2,4,6-trinitrobenzene sulfonate. *Clin Chem* 15, 891-901

Pusztai A, Grant G, King TP, Clarke EMW (1990) Chemical probiosis. *In: Recent Advances in Animal Nutrition* (W Haresign, DJA Cole, eds) Butterworths, London, 47-60

Ratcliffe B, Cole CB, Fuller R, Newport MJ (1986) The effect of yogurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. *Food Microbiol (Lond)* 3, 203-211

Rérat A, Simões Nunes C, Vaissade P, Roger L (1987) Comparaison de deux techniques d'estimation de l'azote des acides aminés circulants, appliquées à l'étude de l'absorption intestinale de solutions d'acides aminés libres ou de petits peptides. *Reprod Nutr Dev* 27, 955-966

Schaafsma G, Deriks P, Dekker PR, Waard H (1988) Nutritional aspects of yogurt. Microbial lactase activity and digestion of lactose. *Neth Milk Dairy J* 42, 121-134

Simões Nunes C, Rérat A, Galibois I, Vaugelade P, Vaissade P (1989) Hepatic and gut balances of glucose, amino-nitrogen, ammonia and urea in the pig after ingestion of casein or rapeseed proteins. *Nutr Rep Int* 40, 901-907

Simões Nunes C, Malmlöf K (1992) Effects of guar gum and cellulose on glucose absorption, hormonal release and hepatic metabolism in the pig. *Br J Nutr* 68, 693-700

Snedecor GW, Cochran WG (1967) *Statistical Methods*. Iowa State Univ Press, Ames, IA

Vanbelle M, Teller E, Focant M (1990) Probiotics in animal nutrition: a review. *Arch Anim Nutr* 7, 543-567