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Effects of long-term parenteral administration of vitamin B_6 on B_6 status and some aspects of the glucose and protein metabolism of early-weaned piglets

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The present experiment aimed to determine the effect of feeding level and parenteral supplements of vitamin B_6 (pyridoxine) on B_6 status as well as on glucose, C-peptide, insulin, α-amino-N and urea after a gastric bolus of glucose in weaned piglets; the plasma tryptophan, xanthurenic acid and kynurenine responses to a gastric bolus of tryptophan were also measured. Forty-two piglets weaned at 2 weeks of age were distributed in seven blocks of six animals each. Within each block, the animals were assigned to the following factorial treatments: two levels of feeding (28 (F28) and 56 (F56) g/kg$^{0·75}$ per d) administered by gastric-tube feeding and three levels of parenteral (intramuscular injections) vitamin B_6 (0 ($B_6$0), 15 ($B_6$15) and 30 ($B_6$30) mg/d). In $B_6$0 piglets, a decrease of 30 % and 20 % in erythrocyte and plasma pyridoxal-5-phosphate respectively, were observed during the 2 weeks post-weaning. In supplemented piglets, the erythrocyte pyridoxal-5-phosphate was maximised in $B_6$15 piglets at a level 3–4 times higher than in $B_6$0 piglets ($P < 0·003$). However, in plasma the maximal pyridoxal-5-phosphate concentration was reached in F28–$B_6$30 piglets ($P < 0·058$). The glucose and insulin responses to a gastric bolus of glucose were lower, and the post-bolus decrease of glucose was slower, in F28 than in F56 piglets ($P < 0·0001$). The insulin:C-peptide ratio was 25 % greater in $B_6$15 piglets ($P < 0·082$). After the bolus of glucose, the aminoacidaemia decreased differentially according to treatments ($P < 0·047$) while the uraemia was at least 2-fold higher ($P < 0·001$) in F28 piglets than in F56 piglets and tended to be maximised in $B_6$30 piglets ($P < 0·074$). The response of plasma tryptophan to the gastric bolus of tryptophan was 11 % lower in $B_6$30 piglets ($P < 0·057$). The plasma concentration of kynurenine increased continuously during the post-bolus period and this response was more marked in F56 ($P < 0·002$) and in $B_6$30 piglets ($P < 0·02$). Xanthurenic acid was undetectable in any of the treatments. The measurements on pyridoxine status suggest that the present basal dietary level of B_6 (7·7 mg/kg) was not sufficient to cover the metabolic needs. For many criteria, an optimal level was reached at 15 mg/d parenteral B_6 but the response of urea to glucose bolus suggests that 30 mg/d was detrimental. Further studies are necessary to determine the dietary level of B_6 equivalent to the present optimal parenteral supplements and its eventual effects on B_6 status and post-weaning growth performance of piglets.

Vitamin B_6: Pyridoxal: Glucose: Tryptophan: Weanling piglets

In piglets, the weaning period induces drastic changes in the amount and bioavailability of the dietary water-soluble vitamins. This seems to be the case for folic acid (Matte *et al.* 1990; Letendre *et al.* 1991), vitamin B_{12} (Bilodeau *et al.* 1989) and vitamin C (Yen & Pond, 1988), for which the serum concentration drops after 21 d of age (weaning at 28 d) to reach a minimum 2–5 weeks later. In the case of vitamin B_6, the information is limited. It is known that sows' milk is a poor source of B_6, about 0·4 μg/ml (Benedikt *et al.* 1996), which would cover, based on factorial calculation (Coburn, 1994), less than half the amount required to sustain the piglet growth rate. Therefore, the pyridoxine status is likely to be low at weaning. Moreover, as in rats (Lu & Huang, 1997) and in humans

Abbreviation: PLP, pyridoxal-5-phosphate.

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subjects (Bender, 1999), the reduced quality and increased quantity of protein in the post-weaning feed, as opposed to sows' milk, would further increase the B_6 needs, because of an increased interconversion and oxidation of amino acids; those metabolic pathways are, in many cases, pyridoxal phosphate dependent. In fact, in weaned piglets, it was suggested that the dietary levels of B_6 which maximise growth performance would be two to five times higher than for the growing–finishing pigs (Adams *et al.* 1967; Kösters & Kirchgessner, 1976; Bretzinger, 1991; National Research Council, 1998). Recent data also suggest that the metabolic utilisation of pyridoxal-5-phosphate (PLP) would be considerably reduced when growth is slowed or inhibited (Matte *et al.* 1997). Such an effect might be linked to an action of B_6 on insulin (Matte *et al.* 1997), a key hormone for protein synthesis and deposition (Davis *et al.*1996). The supply and metabolism of tryptophan is also possibly involved in the B_6 effect on insulin response to a glucose bolus (Matte *et al.* 1997).

The present experiment was therefore undertaken to determine the interaction between feeding level and parenteral supplements of pyridoxine on pyridoxine status in early-weaned piglets. The responses of glucose, C-peptide, insulin, α-amino-N and urea to an intra-gastric bolus of glucose were also assessed. In addition, the response of plasma tryptophan, kynurenine (an intermediate metabolite of tryptophan oxydation) and xanthurenic acid (an abnormal metabolite of tryptophan oxidation which accumulates during a B_6 deficiency) were measured after an intra-gastric bolus of tryptophan.

Material and methods

Animals, treatments and sampling

Forty-two Landrace piglets (castrated males and females), weaned at 2 weeks of age and weighing 5·24 (SEM 0·09) kg, were selected from seven litters. Under anaesthesia (halothane–O_2 (4:96, v/v) given by a face mask) and aseptic conditions, an oesophageal gastric tube was fitted (Cortamira *et al.* 1991) on the day of weaning. The piglets were kept at 27°C in individual adjoining metabolism cages on plastic floors (coated expanded metal) allowing free movement for the animal throughout the experimental period. Within each litter, six piglets were assigned to the following factorial treatments: two levels of feeding (low 28 and high 56 g/kg$^{0·75}$ per d) (F28 and F56, respectively) administered through a gastric tube and three levels of parenteral (intramuscular injection) pyridoxine.HCl (0, 15 and 30 mg/d ($B_6$0, $B_6$15, and $B_6$30 respectively)). The parenteral route of administration was chosen to avoid any possible bioavailability problems sometimes reported with oral pyridoxine (Gregory, 1980; Bretzinger, 1991). All piglets were fed a commercial diet based on (g/kg): barley 250, maize 200, dried whey 200, soyabean meal 100, extruded soyabean 80 and plasma protein 45 (Table 1). The calculated total dietary level of B_6 was 7·7 mg/kg (5·0 from ingredients and 2·7 mg added). The feeding regimen was based on a daily increment of 7·1 g/kg$^{0·75}$, up to the target maximum daily value of 28 or 56 g/kg$^{0·75}$. The daily intake was adjusted three times per week according to the change in body weight. The diets were mixed with water (100 g diet–200 g water). The mixture was infused into the stomach using a 60 ml syringe. One meal was given at 16.00 hours on the first day and two meals at 08.00 and 16.00 hours on the second day after weaning. Thereafter, three meals were given daily at 08.00, 11.30 and 16.00 hours; each meal represented 45, 20 and 35 % respectively of the total daily intake. The daily injections (3 ml) of pyridoxine.HCl or saline were given after the morning meal. Blood samples were taken before the morning meal by jugular venepuncture as described by Matte *et al.* (1986) immediately after weaning (before gastric cannulation) and at 2 and 3 weeks of age for determination of circulating PLP.

Table 1. Composition of the diet*†

Item	Calculated concentration
Digestible energy (MJ/kg)	349·5
Total protein (g/kg)	207
Fat (g/kg)	72·6
Crude fibre (g/kg)	17·9
Lysine (g/kg)‡	14·8
Methionine (g/kg)‡	4·4
Tryptophan (g/kg)‡	2·5
Calcium (g/kg)	9·6
Phosphorus (g/kg)	8·3

* The total amount (natural + added) of trace elements were (/kg diet): Mn 74 mg, Zn, 527 mg, Fe 295 mg, Cu 182 mg, I 205 μg, Se 300 μg.
† The total amount (natural + added) of vitamins were (/kg diet): vitamin A 45 mg, cholecalciferol 38 mg, vitamin E 73 mg, menadione 2·7 mg, thiamin 2·8 mg, riboflavin 8·9 mg, niacin, 31·9 mg, pantothenic acid 21·5 mg, pyridoxine 2·7 mg, biotin 123 μg, vitamin B_{12} 26 μg, choline 1205 mg.
‡ For lysine, it was fractionated in 11·2 (natural) + 3·6 (added) g/kg and for methionine, 3·0 (natural) + 1·4 (added) g/kg. The value for tryptophan corresponded to the total amount from natural sources.

A jugular catheter was inserted 4 d before the end of the experiment (5 weeks of age) using a non-surgical technique described by Matte (1999). After cannulation (2 d), a glucose load test was given after a fasting period of 18 h with an intragastric bolus of glucose (5·4 g/kg body weight). Repeated blood samples were performed at 0, 30, 60, 90, 120, 150, 180, 210 and 240 min post-bolus for determination of plasma insulin and glucose concentrations; plasma free amino acids (α-amino-N) and urea concentrations were measured on the samples collected at 0, 60, 120 and 180 min post-bolus. C-peptide was determined in piglets from three litters at 30, 60, 90 and 120 min post-bolus. After 2 d, a gastric bolus of tryptophan (50 mg/kg body weight) was also given after a fasting period of 18 h. Repeated blood samples were collected at 0, 90 and 180 min post-bolus for the measurement of plasma tryptophan, xanthurenic acid and kynurenine. On the day of gastric bolus of glucose or tryptophan, the intramuscular injection as well as the meals at 08.00 hours and 11.30 hours were omitted. After the last blood sample following the bolus of tryptophan, the animals were sacrificed and the overall body (intestinal content removed) was weighed and frozen for N and pyridoxine measurements. All animals were cared for and slaughtered according to the recommended code of practice of Agriculture Canada (1993) and the procedure was approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care (1993).

Laboratory analyses

PLP was determined in plasma and erythrocytes using a fluorometric method adapted by Matte *et al.* (1997) from Srivastava & Beutler (1973). In the overall body, the B_6 metabolites, pyridoxal (including PLP), pyridoxamine, pyridoxine and 4-pyridoxic acid were measured by HPLC after acid hydrolysis with HCl (0·044 M). The injected volume was 20 μl. The chromatography was done on a Beckman system (pump no. 126, autosampler no. 506A, digital analog converter no. 406, a Beckman pre-column C8 of 4·6 mm × 4·5 cm (Beckman, Fullerton, CA, USA), and a column Ultrasphere ODS 5μm of 4·6 mm × 25 cm; Ultrasphere, Fullerton, CA, USA) using, in isocratic conditions, a mobile phase with H_2SO_4 (0·04 M) at a rate of 1 ml/min during 15 min. The detection was done with a fluorometric detector (PerkinElmer LC240; PerkinElmer, Beaconsfield, Bucks., UK) adjusted at 290 and 395 nm for emission and excitation respectively. A standard curve was linear (R^2 0·99) between 0 and 1000 nM for pyridoxamine, pyridoxal and pyridoxine and between 0 and 10 μM for 4-pyridoxic acid. For tryptophan, kynurenine and xanthurenic acid, the same HPLC system was used but the precolumn and column were respectively, a Beckman C8 (4·6 mm × 3·0 cm), and a Rainin Microsorb C8 5μm (4·6 mm × 15 cm; Rainin, Walnut Creek, CA, USA); the deproteinization of the samples was done with sulfosalicylic acid (50 g/l). The mobile phase (97 ml acetate buffer, 0·17 M-acetic acid + 0·03 M-sodium acetate, 30 ml acetonitrile and 873 ml ultrapure water) was used, in isocratic conditions, at a rate of 1·6 ml/min during 20 min. The injected volume was 20 μl. The detection was done with a coulometric detector (Coulochem (Concord, Ontario, Canada), ESA, pre-column no. 5020 and analytical cell no. 5010) at 0 mV (channel 1) and 950 mV (channel 2), for tryptophan and kynurenine and with a u.v. detector set at 254 nm for xanthurenic acid.

Protein content in the overall body was determined using a micro-Kieldahl apparatus (method no 7.016; Association of Official Analytical Chemists, 1975).

Plasma glucose was measured by colorimetry (GOD/PAP no. 166 391; Boehringer Mannheim, Laval, Québec, Canada). Plasma insulin was measured by radioimmunoassay kits (no. KTSP 11001; Immunocorp, Montréal, Canada) validated in our laboratory; the intra- and inter-assay CV were 2·7 % and 3·7 % respectively. Plasma C-peptide was assayed by a commercial porcine C-peptide radioimmunoassay kit (Cat. No. PCP-22K; Linco, St Louis, MO, USA); the intra- and inter-assays CV were 2·4 % and 2·8 % respectively.

Plasma α-amino-N and urea were measured using a Technicon auto-analyser system (Technicon, Tarrytown, NY, USA) as described by Huntington (1984).

Statistical analysis

The data were analysed using the Statistical Analysis Systems procedure for mixed models (SAS Institute Inc., Cary, NC, USA; Littell *et al.* 1996) according to a 2 × 3 factorial arrangement of feeding (28 and 56 $g/kg^{0.75}$) and pyridoxine (0, 15 and 30 mg/d) treatments distributed in seven blocks. For repeated measurements such as plasma and erythrocyte PLP plasma C-peptide, insulin, glucose, α-amino-N, urea, tryptophan and kynurenine, a third factor, the age of the animal or the time following the gastric bolus of either glucose or tryptophan, was added as a sub-plot to the basal model along with the appropriate interactions. Orthogonal contrasts were used for comparisons among levels of pyridoxine and among ages or times following the bolus of either glucose or tryptophan.

Results

Growth rate, vitamin B_6 status and body composition measurements

The daily body weight gains were 22·1 (SEM 5·0), 32·1 (SEM 5·3), 15·7 (SEM 9·9), 103·2 (SEM 7·9), 95·6 (SEM 8·0) and 103·1 (SEM 18·5) (interaction feeding × B_6 quadratic, $P < 0·049$) for F28–$B_6$0, F28–$B_6$15, F28–$B_6$30, F56–$B_6$0, F56–$B_6$15 and F56–$B_6$30 respectively.

In $B_6$0 piglets, whatever the feeding level, the concentration of PLP in erythrocyte decreased by approximately 30 % (from 2·8 to 1·8 μmol/l) during the 2 weeks following weaning (Fig. 1). During the same period, the concentration tripled in $B_6$15 and $B_6$30 piglets (interaction B_6 quadratic × age quadratic, $P < 0·003$). In plasma, PLP concentration decreased by approximately 20 % in $B_6$0 piglets during the post-weaning period. The response to B_6 supplements was dependent upon the feeding level (interaction feeding × B_6 linear × age linear, $P > 0·058$) (Fig. 2). In F56 piglets, the response of plasma PLP was similar to that of erythrocytes but the difference between treatments was less pronounced (approximately 40 % higher in $B_6$15 and $B_6$30 than in $B_6$0). However, in F28 piglets, the response of PLP did not reach a plateau at 15 mg/d; the concentration was, in fact, approximately 20 % higher in $B_6$30 than in $B_6$15 piglets.

There was an effect of feeding level on body weight and protein ($P < 0·001$) and on the pyridoxine ($P < 0·08$) and 4-pyridoxic acid ($P < 0·002$) concentrations in the overall body (Table 2). There was no effect ($P > 0·21$) of the B_6 treatments on any of the B_6 metabolites in the overall body.

Glucose and amino acid responses to a gastric bolus of glucose

The maximum concentration of glucose after a gastric bolus of glucose was lower but persisted longer in F28 piglets than in F56 piglets (interaction feeding × time cubic, $P < 0·0001$) (Fig. 3). There was no feeding level effect ($P > 0·25$) on the area under the curve for glucose. The insulin profile (Fig. 4) and the area under the curve for insulin following the gastric bolus of glucose were approximately 35 % lower (interaction feeding × time cubic, $P < 0·0001$ and feeding effect, $P < 0·003$ respectively) in F28 than in F56 piglets. There was no effect ($P > 0·20$) of the B_6 treatments on either glucose or insulin responses to the gastric bolus of glucose. However, the insulin:C-peptide ratio calculated between 30 and 120 min post-bolus was approximately 25 % higher in $B_6$15 piglets than in the two other treatments (interaction B_6 quadratic ×

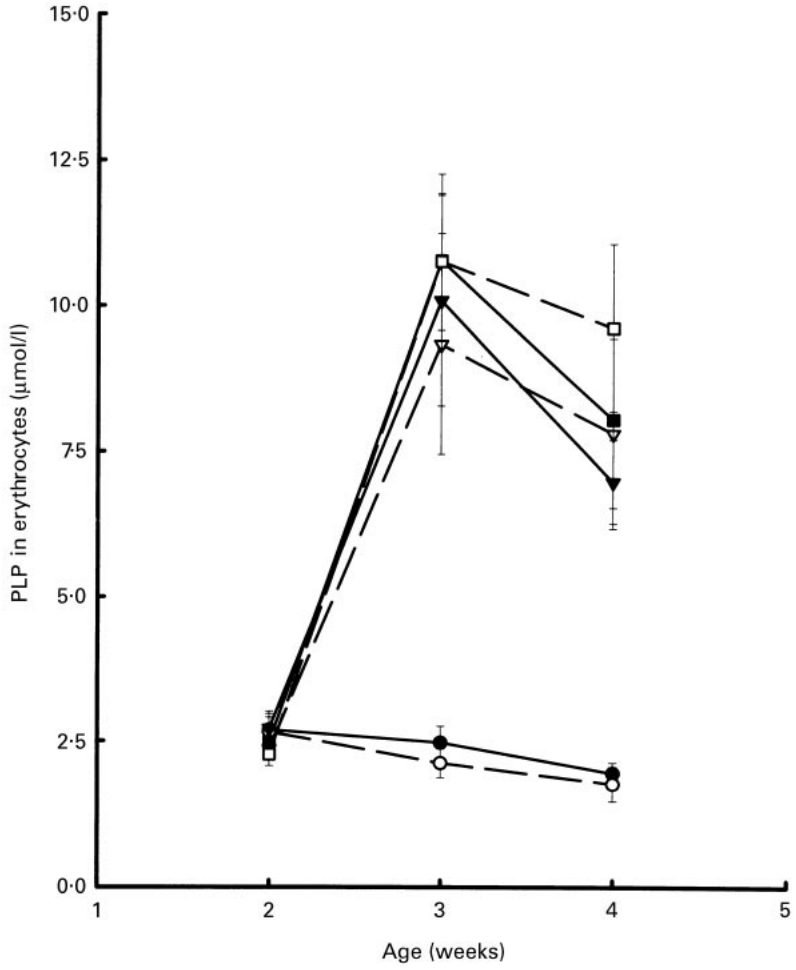

Fig. 1. Age-related changes in erythrocyte pyridoxal-5-phosphate (PLP) according to feeding level and vitamin B_6 administration. Values are means for five piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction B_6 (quadratic) × age (quadratic) $P < 0{\cdot}003$.

time linear, $P < 0{\cdot}082$). The post-bolus decrease in plasma free amino acids (α-amino-N) was different according to the combination of feeding and pyridoxine treatments. In F28 piglets, the decrease was less pronounced in the $B_6$15 piglets than in the two other B_6 treatments while no B_6 effect was apparent in F56 piglets (interaction feeding × B_6 quadratic × time quadratic, $P < 0{\cdot}047$) (Fig. 5). The post-bolus urea concentrations were twice higher in F28 than in F56 piglets ($P < 0{\cdot}001$) and tended to be higher in $B_6$30 piglets regardless of the feeding level (B_6 quadratic, $P < 0{\cdot}074$) (Fig. 6).

Tryptophan-related status after a gastric bolus of tryptophan

The concentration of plasma tryptophan reached a maximum 90 min after the gastric bolus of tryptophan and decreased thereafter up to 180 min post-bolus (Table 3); the average concentration was approximately 11 % lower in $B_6$30 piglets than for the two other treatments (B_6 quadratic, $P < 0{\cdot}057$). During the post-bolus period, the concentration of kynurenine increased continuously and the response was more marked in F56 piglets (interaction feeding × time linear, $P < 0{\cdot}002$) and in $B_6$30 piglets (interaction B_6 quadratic × time linear, $P < 0{\cdot}02$). Xanthurenic acid was undetectable ($< 0{\cdot}5$ μmol/l) in plasma after the tryptophan bolus, whatever the feeding and the B_6 treatments.

Discussion

Growth rate, vitamin B_6 status and body composition measurements

The drastic decrease in the concentration of PLP in both plasma and erythrocytes (approximately 20 % and 30 %, respectively) in $B_6$0 piglets during the 2 weeks following weaning suggests that the long-term balance between the dietary supply of pyridoxine and the metabolic utilisation was negative. The actual dietary level of 7·7 mg/kg, which

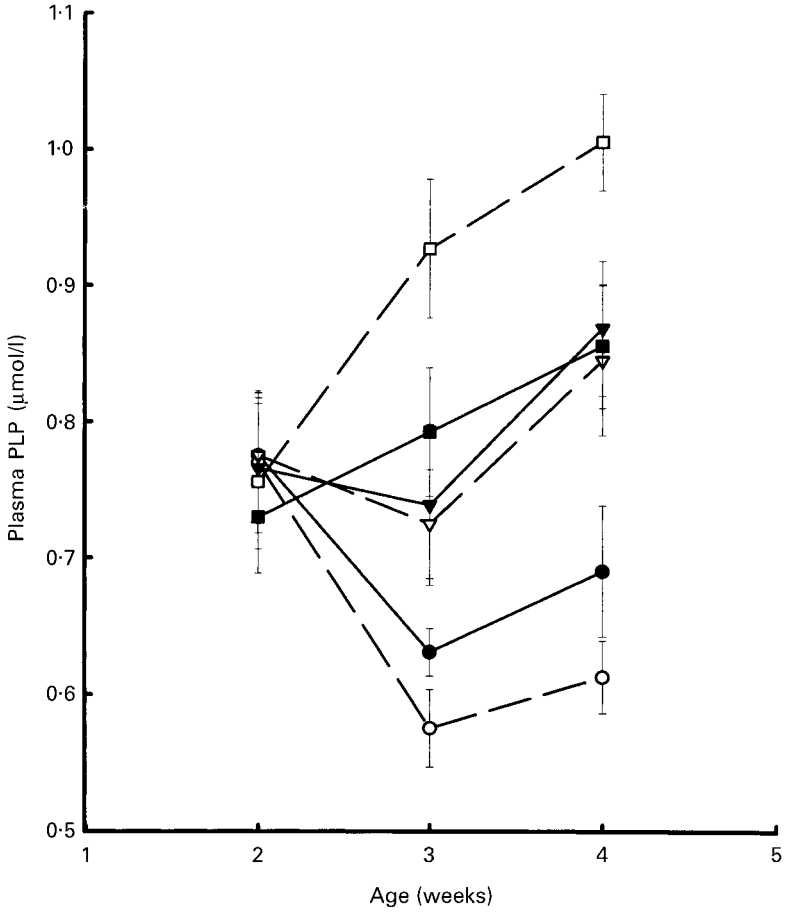

Fig. 2. Age-related changes in plasma pyridoxal-5-phosphate (PLP) according to feeding level and vitamin B_6 administration. Values are means for seven piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction feeding × B_6 (linear) × age (linear) $P < 0{\cdot}058$

was already three and five times higher than the recommended supply of B_6 (Agricultural Research Council, 1981 and National Research Council, 1998, respectively) for early-weaned piglets, was apparently not sufficient to meet their metabolic need for B_6. The response to the supplements in erythrocytes for both F28 and F56 piglets suggests that this metabolic pool, which plays an important role in the transport and distribution of B_6 (Coburn, 1994), was saturated with 15 mg B_6/d. However, in plasma, the response to supplements was in general less marked than in erythrocytes and dependent upon the feeding level. Indeed, while the maximal plasma concentration was reached at 15 mg B_6/d in F56 piglets, there was a further increase at 30 mg B_6/d in F28 piglets. As suggested previously (Matte *et al.* 1997), there seems to be a relationship between growth rate, induced in this case by the feeding treatments, and the metabolic balance of pyridoxine in the plasma pool. It seems that when the metabolic needs for growth are high, as it is probably the case in F56 piglets (average gain of approximately 100 g/d), the metabolic balance between supply and utilisation induced a saturation of the plasma pool at 15 mg B_6/d. Over that supplemented level, the PLP seems to be directed towards other metabolic pools because the concentration was similar in $B_6$15 and $B_6$30 piglets. In F28 piglets, the accumulation of PLP in plasma might be due to the lack of utilisation of that B_6 metabolite. Such an accumulation of PLP in circulation appeared detrimental in F28–$B_6$30 piglets; indeed, they had the lowest growth rate during the post-weaning period and, although not significant, the lowest body weight and protein content (data not shown). Schaeffer *et al.* (1998) reported that excesses of dietary B_6 within the range of the actual parenteral supplementation reduced growth performance in rats. They associated these effects with alterations of brain and systemic amino acid metabolism and to the binding properties of cortical serotonine receptors; this amine is synthesized from the B_6-dependent decarboxylation of 5-hydroxytryptophan (McDowell, 1989).

The concentration of B_6 vitamers in the overall body seemed to be relatively independent from the B_6 treatments. Such a lack of effect is possibly linked to the slow

Table 2. Protein content and pyridoxine vitamer concentration in piglet whole body according to the level of feeding during the experimental period*

(Mean values with standard errors of the means)

Feeding level ($g/kg^{0.75}$)	*n*	Body weight (kg)†		Body protein (g)†		Pyridoxamine (nmol/g)		Pyridoxal (nmol/g)		Pyridoxine (nmol/g)‡		4-pyridoxic acid (nmol/g)‡	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
28 (F28)	19	5·44	0·15	851·1	24·6	231·4	21·3	132·6	25·6	47·2	9·3	1109·0	164·1
56 (F56)	22	7·12	0·28	1101·4	35·1	185·0	20·2	131·0	25·3	28·5	5·9	637·3	110·0

* For details of diets and procedures see Table 1 and p. 12.
† Feeding effect $P < 0·045$
‡ Feeding effect $P < 0·08$ for pyridoxine and $P < 0·002$ for 4-pyridoxic acid.

turnover of the B_6, sequestered by the glycogen phosphorylase in the muscle mass (Russell *et al.* 1985; McDowell, 1989; Coburn, 1994). In fact, the release of the vitamin from that reservoir occurs, not necessarily in situation of deficiency, but not until the glycogen reserves are depleted (Bender, 1999). The muscle and skeleton represent a great proportion (approximately 80 %) of the overall body mass in piglets (Pond & Houpt, 1978), so it might have masked a possible accumulation of B_6 vitamers in some specific organs and induced a lack of B_6 effect for the overall body. The effect of feeding level on the body concentration of pyridoxine, the injected vitamer, and 4-pyridoxic acid, the excretory vitamer, suggests that there is a regulatory mechanism between the provision of B_6 and the excretion

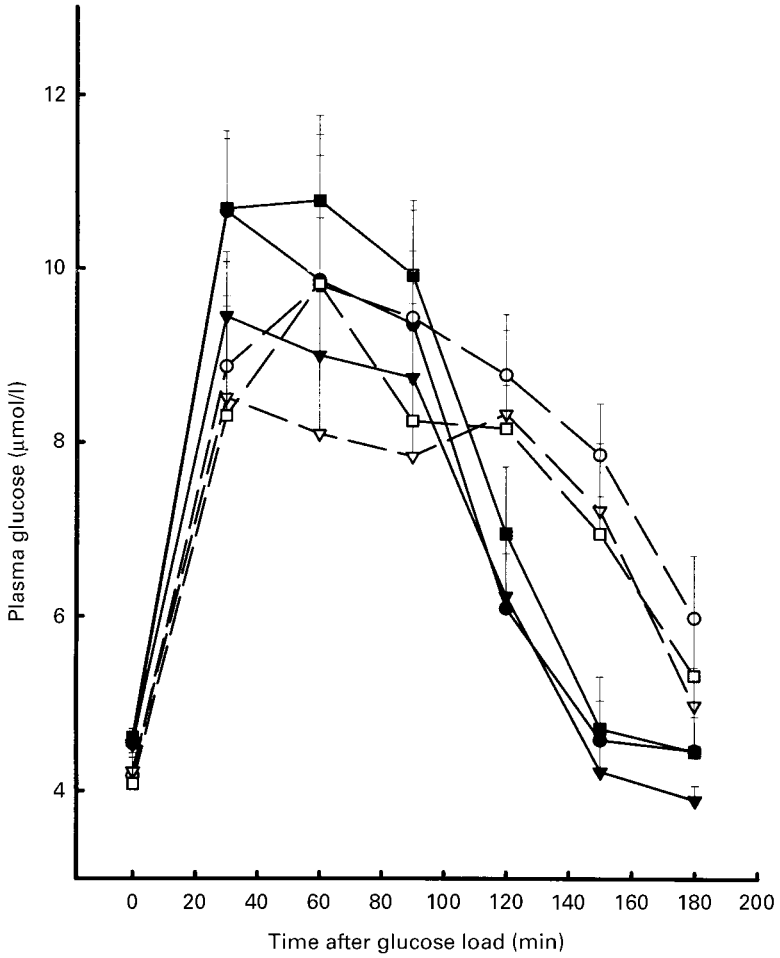

Fig. 3. Plasma glucose concentrations after a gastric bolus of glucose according to feeding level and vitamin B_6 administration. Values are means for seven piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction feeding × time (cubic) $P < 0·0001$.

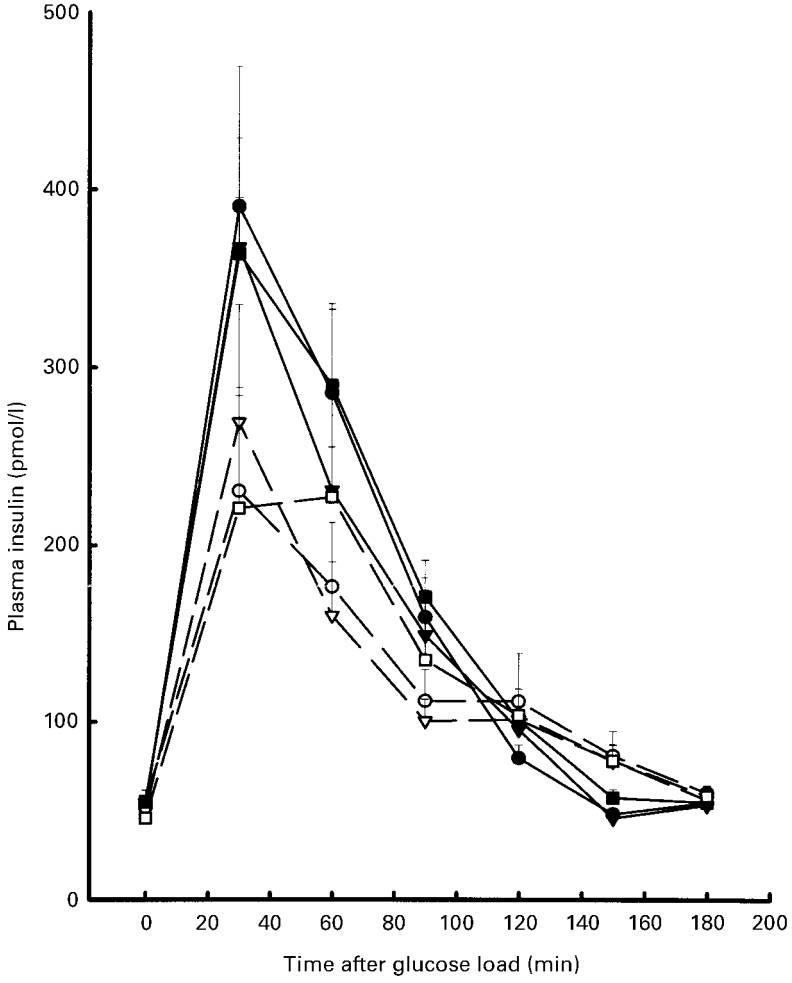

Fig. 4. Plasma insulin concentrations after a gastric bolus of glucose according to feeding level and vitamin B_6 administration. Values are means for seven piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction feeding × time (cubic) $P < 0{\cdot}0001$.

of B_6 which keeps constant the concentration of the active metabolic vitamers (pyridoxamine and pyridoxal) in the whole body of early-weaned piglets.

Glucose and protein-related status after a gastric bolus of glucose

The effects of feeding levels on glucose and insulin responses to the bolus of glucose are in agreement with the effect of feed restriction on the reduction of insulin response either to glucose, in growing–finishing pigs (van der Grift *et al.* 1985), or to a meal, in neonatal pigs (Ebner *et al.* 1994) and gilts (Prunier *et al.* 1993). In such case, the lack of specific nutrients, like arginine, has been associated with the reduced insulin response and glucose disappearance after intravenous glucose infusion (Mulloy *et al.* 1982). The present absence of B_6 effects on insulin response after the gastric bolus of glucose contrasted with the marked increase of insulin response after duodenal bolus of glucose using piglets of approximately the same age (Matte *et al.* 1997). However, the present tendency observed for a B_6 effect on the insulin:C-peptide ratio suggests a reduced hepatic degradation of insulin in $B_6$15 piglets. The increased insulin response to a duodenal bolus of glucose after parenteral B_6 supplementation (Matte *et al.* 1997) was, therefore, possibly due to a slower hepatic degradation of insulin rather than an increased pancreatic secretion. Indeed, C-peptide, a part of the proinsulin molecule secreted by the pancreas in equimolar amounts to insulin, is extracted by the liver at a much slower rate than insulin. In fact, C-peptide is considered a more reliable indication of insulin secretion than peripheral insulin concentration itself (Morgan, 1992; Uusitupa *et al.* 1992).

The basal plasma concentration of α-amino-N before the gastric bolus of glucose was not influenced by treatments but there was a marked effect of feeding on the corresponding value for urea. This last effect might be

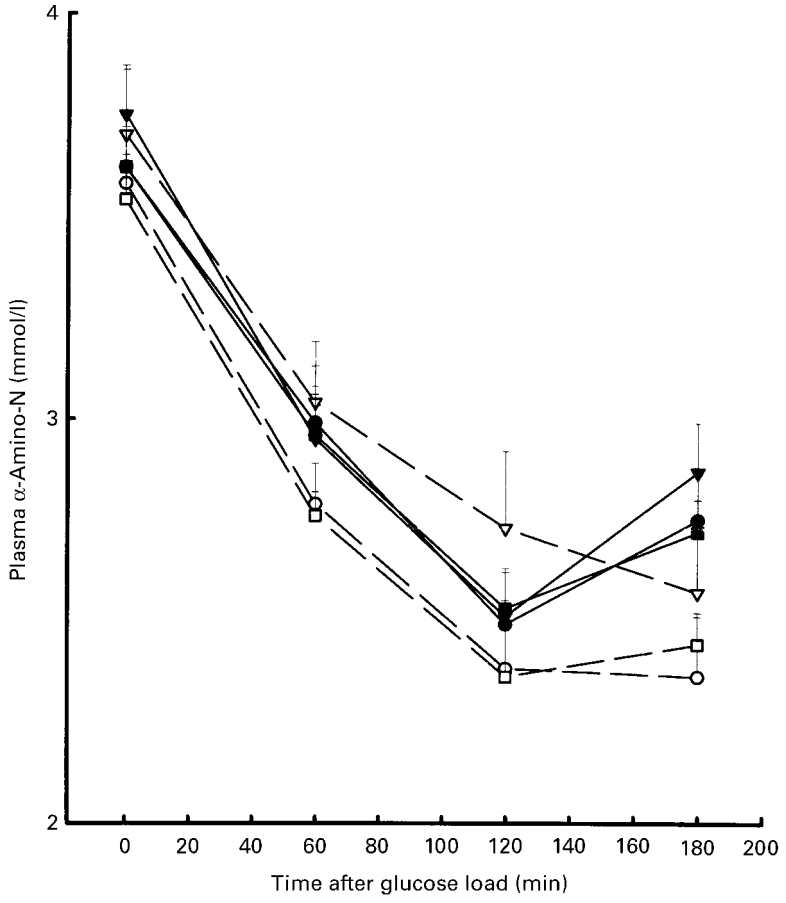

Fig. 5. Plasma α-amino-N concentrations after a gastric bolus of glucose according to feeding level and vitamin B_6 administration. Values are means for seven piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction feeding × B_6 (quadratic) time (quadratic) $P < 0{\cdot}047$.

Table 3. Plasma tryptophan and kynurenine (μmol/l) changes in response to a gastric bolus of tryptophan according to the vitamin B_6 treatments and feeding level*

(Mean values with standard errors of the means)

	Time after trytophan gastric bolus (min)											
	Tryptophan†‡						Kynurenine†§					
	0		90		180		0		90		180	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Vitamin B_6 (mg/d)												
0	43·2	3·6	395·1	38·1	203·6	17·6	0·4	0·1	2·5	0·3	4·1	0·4
15	45·2	3·0	398·3	26·9	227·0	20·1	0·3	0·1	2·5	0·3	4·2	0·5
30	44·5	3·2	360·8	28·4	164·4	13·1	0·3	0·1	2·7	0·2	4·8	0·4
Feeding level (g/kg$^{0.75}$)												
28	41·2	2·2	375·2	26·5	204·3	16·4	0·4	0·1	2·5	0·2	4·4	0·3
56	47·2	2·8	393·9	23·9	193·8	13·8	0·2	0·1	2·7	0·2	4·4	0·4

* The number of piglets were 13, 12 and 13 at 0, 90 and 180 min post-bolus in $B_6$0 treatments and 14 at all times in $B_6$15 and $B_6$30 treatments. The corresponding values were 20, 19 and 20 at 0, 90 and 180 min post-bolus in F28 treatments and 21 at all times in F56 treatments. For details of diets and procedures see Table 1 and p. 12.

† Time effect (quadratic) $P < 0{\cdot}002$.

‡ B_6 effect (quadratic) $P < 0{\cdot}057$.

§ Interaction feeding × time (linear) $P < 0{\cdot}002$ and interaction B_6 (quadratic) × time (linear) $P < 0{\cdot}02$.

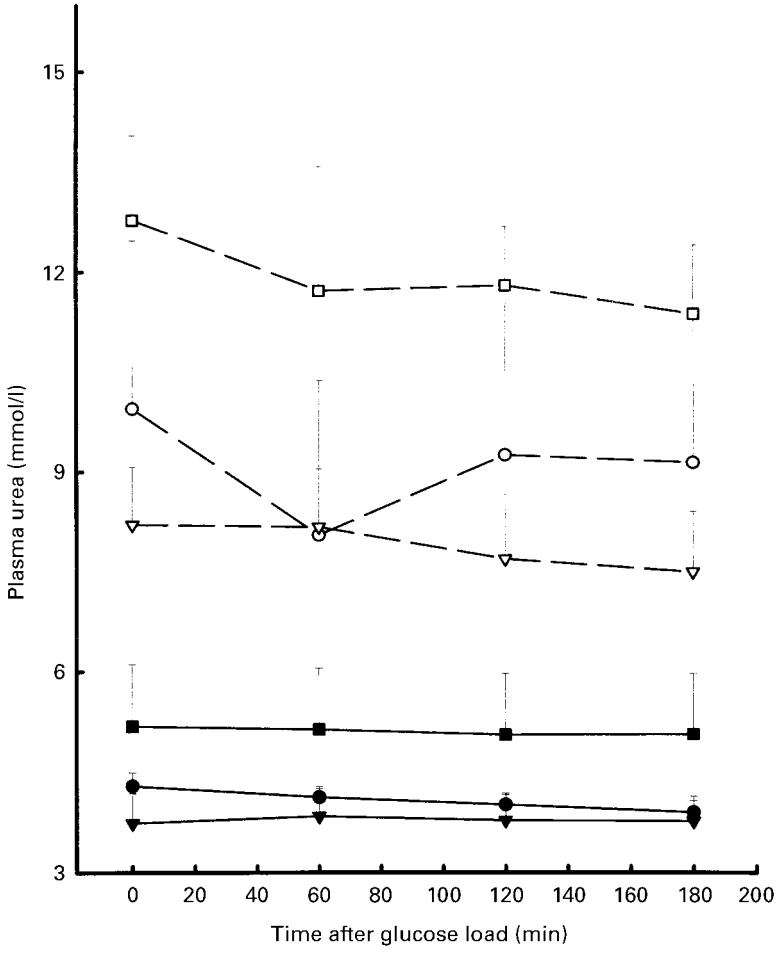

Fig. 6. Plasma urea concentrations after a gastric bolus of glucose according to feeding level and vitamin B_6 administration. Values are means for seven piglets per combination of treatments with standard errors of the means shown by vertical bars. For details of diets and procedures see Table 1 and p. 12. ●, F56–$B_6$0; ○, F28–$B_6$0; ▼, F56–$B_6$15; ▽, F28–$B_6$15; ■, F56–$B_6$30; □, F28–$B_6$30. Interaction feeding × time $P < 0{\cdot}018$.

due to the metabolic use of the glucogenic amino acids for energy. This glucose production might possibly explain the prolonged glucose response after the gastric bolus of glucose in F28 piglets. Although the main B_6 effect was only a tendency, the high urea concentrations in $B_6$30 particularly those within the F28 level of feeding might be due to an overstimulation of transamination and deamination pathways of amino acids which are, in most cases, B_6-dependent (Maynard *et al.* 1979). Such a hypothesis would be in agreement with the detrimental effect of F28–$B_6$30 for PLP homeostasis and growth rate mentioned earlier. Indeed, circulating urea is known to be positively correlated with amino acid catabolism (Reeds *et al.* 1980) and negatively correlated with weight gain, efficiency of feed utilisation and protein retention (Puchal *et al.* 1962). After the gastric load of glucose, the responses of α-amino-N and urea suggest that the short-term utilisation of free circulating amino acids, probably induced by the release of insulin, was not sufficient to induce a response on the post-bolus concentration of urea.

Tryptophan-related status after a gastric bolus of tryptophan

The lack of plasma xanthurenic acid response to any treatments after the gastric bolus of tryptophan suggests that the short-term large metabolic utilisation of PLP in $B_6$0 treatments was not sufficient to induce a B_6 deficiency in these piglets during the 2 weeks following weaning. Indeed, xanthurenic acid, an abnormal metabolite of tryptophan oxidation which accumulates in urine but also in plasma after a tryptophan load in B_6-deficient animals (Takeuchi *et al.* 1989), is a classical indicator of a deficiency of that vitamin (Bender, 1987). The results for tryptophan and kynurenine (an intermediate metabolite of tryptophan oxidation) responses suggest that the oxidation of tryptophan is stimulated by the highest level of B_6. Kynurenine is an initial step of the tryptophan oxidative pathway which releases alanine (a glucogenic amino acid) and is directed thereafter either towards acetyl-CoA (total oxidation) or to the synthesis of nicotinamide nucleotides

(NAD and NADPH) (Bender, 1987). Taking into account the preceding results showing a stimulating effect of the $B_6$30 treatments on basal plasma urea, it appears plausible that most of the plasma tryptophan disappearance after the gastric bolus of tryptophan was due to the catabolism via alanine towards acetyl-CoA.

Conclusion

In conclusion, the basal level of dietary B_6 (7·7 mg/kg), although sufficient to prevent deficiency symptoms of B_6, was not sufficient to prevent the drop in PLP in serum and erythrocytes during the 2 weeks following weaning of piglets at 2 weeks of age. Such a level was almost three and five times higher than the Agricultural Research Council (1981) and National Research Council (1998) recommendations, respectively. The present results suggest that a daily provision of 15 mg available B_6 would optimise the B_6 status in serum and erythrocytes whereas a daily level of 30 mg might have detrimental effects on some aspects of growth and protein metabolism specially in animals with restricted intake and impaired growth rate. Further studies are necessary to determine the optimal dietary concentration of B_6 which will reproduce, on blood B_6 status, the effects of a parenteral provision of that vitamin and eventually maximise the post-weaning growth performance.

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