Some aspects of the pyridoxine (vitamin B_6) requirement in weanling piglets

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Four trials were carried out to determine the optimal level of dietary pyridoxine (vitamin B_6) and its interaction with riboflavin (vitamin B_2) in early-weaned piglets. In Trial 1, twelve piglets were tube-fed graded supplements of B_6, 0, 10, 50 or 100 mg/kg. The level of 50 mg/kg maximized B_6 in red blood cells ($P<0.05$). In Trial 2, thirty-six piglets were tube-fed with four combinations of B_6 (0 v. 50 mg/kg) and B_2 (0 v. 25 mg/kg). The B_6 supplement increased ($P<0.01$) B_6 in red blood cells. C-peptide and insulin responses to intravenous glucose tended ($P<0.08$) to or decreased ($P<0.03$) with B_2 while no effect was observed on glucose. After gastro-enteral glucose, dietary B_2 depressed C-peptide and insulin responses in B_6-unsupplemented piglets and increased them in B_6-supplemented piglets ($P<0.03$). The glucose response tended to be higher in B_6-supplemented piglets ($P<0.06$). Trials 3 and 4 were carried out in commercial conditions using either B_6 and/or B_2 supplements given during 2 weeks after weaning (Trial 3) or a B_6 supplement alone (50 mg/kg) given between 2 (weaning) and 10 weeks of age. Despite a marked and persistent increase ($P<0.01$) of B_6 in red blood cells in B_6-supplemented piglets, the effect on growth performance was either none ($P>0.39$; Trial 3) or marginally lower ($<-2\%$; $P<0.03$; Trial 4). In conclusion, it appears that a dietary supplement of 50 mg/kg B_6 saturated the red blood cell pool in B_6 and influenced, along with B_2, the glucose homeostasis through the entero-insular axis. Nevertheless, such metabolic effects are not reflected on growth performance.

Pyridoxine: Riboflavin: Glycaemia: Growth: Piglets

The weaning period induces, in piglets, drastic challenges for homeostasis of water-soluble vitamins such as folates (Matte *et al.* 1990; Letendre *et al.* 1991), vitamin B_{12} (Bilodeau *et al.* 1989) and vitamin C (Yen & Pond, 1988). It is also the case of pyridoxine (vitamin B_6) for which the status is low at weaning (Matte *et al.* 1997, 2001). In fact, sow milk is a poor dietary source of B_6 (Benedikt *et al.* 1996), approximately $0.4\,\mu g/ml$, which is believed to cover less than half the amount required to sustain the piglet growth rate (Coburn, 1994). Moreover, as in rats (Lu & Huang, 1997) and humans (Bender, 1999), the reduced quality and increased quantity of protein in the post-weaning feed, as opposed to dam's 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, B_6-dependent. The interaction between metabolic utilization of B_6 and protein accretion or growth rate (Matte *et al.* 1997, 2001) has been also linked to its action on insulin, a key hormone for protein synthesis and deposition (Davis *et al.* 1996). Indeed, it has been reported that a deficiency in vitamin B_6 induces insulin resistance in elderly men (Ribaya-Mercado *et al.* 1991; Rogers & Mohan, 1994) whereas a supplement of pyridoxine in a diet already adequate in this vitamin decreased plasma glucose concentrations after an oral glucose load (Safaya & Bamji, 1981). However, in pigs, it is not clear if this action on insulin occurs through the glycaemic state (systemic induction) or through the neural and endocrine axis (entero-insular induction) because the response appeared to vary according to the route of glucose infusion (Matte *et al.*

1997, 2001). The dietary levels of $2-5$ mg/kg of B_6 have been suggested to maximize growth performance in weaned piglets (Adams *et al.* 1967; Kösters & Kirchgessner, 1976; Bretzinger, 1991). Recent data using growth (Woodworth *et al.* 2000) or metabolic (Matte *et al.* 2001) criteria suggest optimal responses at dietary and parenteral daily levels, two and ten times higher, respectively, than those currently recommended (NRC, 1998). Further studies are necessary to determine the precise optimal dietary level of B_6.

B_6 metabolism is closely related to other B-vitamins, in particular, riboflavin (B_2). The biologically active metabolites of B_2, FMN and FAD, are involved in the conversion of B_6 in its biologically active vitamers, pyridoxal phosphate, and its excretory form, 4-pyridoxic acid (Le Grusse & Watier, 1993). The NRC (1998) recommendations for both B_6 and B_2 in post-weaning pigs $(5-20\,kg)$ are 1.5 and $3.0-3.5$ mg/kg, respectively.

The present experiment aimed to determine the optimal dietary level of pyridoxine in interaction with riboflavin on B_6 and B_2 status, insulin response to glucose load and growth performance of piglets weaned at 2 weeks of age.

Materials and methods

Two trials (1 and 2) were carried out under intensive experimental conditions for metabolic measurements while the two others (3 and 4) were realized in commercial conditions for performance measurements.

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Trial 1

Twelve Yorkshire × Landrace × Duroc piglets (castrated males and females) weaned at 2 weeks of age and weighing (mean) 6·80 (SEM 0·10) kg were distributed in three repetitions of four animals, each repetition corresponding to a selection within the same litter. Under anaesthesia (4 % halothane–oxygen 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 which lasted 15 d. Within each repetition, the piglets were assigned to four dietary supplements of vitamin B_6 (pyridoxine.HCl) at 0 (B_60), 10 (B_610), 50 (B_650) and 100 mg/kg (B_6100) added to a commercial diet based on barley (25 %), maize (20 %), dried whey (20 %), soyabean meal (10 %), extruded soyabean (8 %) and plasma protein (4·5 %) (Table 1). The calculated total dietary level of B_6 (ingredients and synthetic addition) was 7·1 mg/kg and the analytical content was 7·6 mg/kg (expressed as pyridoxine equivalent; sum of pyridoxamine + pyridoxal + pyridoxine contents). The feeding regimen was based on a daily increment of 7·1 g feed per $kg^{0.75}$ body weight ($g/kg^{0.75}$) up to the target maximum daily value of 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 mixed with 200 g water). The mixture was infused through the gastric tube into the stomach using a syringe of 60 ml. 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 supplement of B_6 was given as adjusted amounts, according to treatments of solutions at 0 (B_60), 1·1 (B_610), 5·2 (B_650) and 10·5 (B_6100) mM-pyridoxine.HCl injected in the oesophageal tube during the morning meal. Body weight was recorded and blood samples collected by jugular venipuncture as described by Matte *et al.* (1986) before the morning meal (fasting period of 16 h) at 0 (before attribution of treatments), 4, 7, 11 and 15 d post-weaning for determination of pyridoxal-5-phosphate in serum and erythrocytes.

Table 1. Composition of the diet (Trials 1 and 2)*

Item	Calculated concentration
Digestible energy (MJ/kg)	14·6
Total protein (%)	20·7
Fat (%)	7·26
Crude fibre (%)	1·79
Lysine (%)	1·48
Methionine (%)	0·44
Tryptophan (%)	0·25
Calcium (%)	0·96
Phosphorus (%)	0·83

* The calculated total amount of trace elements per kg of diet were: Mn, 74 mg; Zn, 527 mg; Fe, 295 mg; Cu, 182 mg; I, 205 μg; Se, 300 μg. The total amount of vitamins per kg of diet were: vitamin A, 4.5 mg vitamin D_3, 37.9 μg; vitamin E, 109 mg; menadione, 2.7 mg; thiamin, 2.8 mg; riboflavin, 8.9 mg; niacin, 31.9 mg; pantothenic acid, 21.5 mg; folic acid, 0.7 mg; pyridoxine, 2.7 mg; biotin, 123 μg; vitamin B_{12}, 26 μg; choline, 1205 mg.

Trial 2

Thirty-six Yorkshire × Landrace × Duroc piglets (castrated males and females) weaned at 2 weeks of age and weighing (mean) 6·53 (SEM 0·06) kg were distributed in nine repetitions of four animals, each repetition corresponding to a selection within the same litter. Within each repetition, the piglets were assigned to four dietary supplements of B_2 and B_6, 0 and 0 (B_20–B_60), 25 and 0 (B_225–B_60), 0 and 50 (B_20–B_650) and 25 and 50 mg/kg (B_225–B_650) added to the commercial diet described in Trial 1. The calculated total dietary levels of B_6 and B_2 (ingredients and synthetic additions) were 7·1 and 15·5 mg/kg, respectively. The analytical content was 7·6 mg/kg for B_6 (expressed as pyridoxine equivalent; sum of pyridoxamine + pyridoxal + pyridoxine) and 15·8 mg/kg for B_2 (expressed as riboflavin equivalent; FMN + FAD + riboflavin). The surgical preparation of the animals and the feeding regimen were similar to those described in Trial 1. As for the supplements of B_6 (solutions at 0 (B_60) and 5·3 (B_650) mM-pyridoxine.HCl), the supplements of B_2 were given as adjusted amounts, according to treatments, of solutions at 0 (B_20) and 1·4 (B_225) mM-riboflavin (prepared with 0·01 M-NaOH and 0·06 M-HCl) injected in the oesophageal tube during the morning meal. Body weight was recorded and blood samples collected by jugular venipuncture as described by Matte *et al.* (1986) before the morning meal (fasting period of 16 h), at 0 (before attribution of treatments), 4, 7 and 11 d post-weaning for determination of riboflavin (FAD + FMN + riboflavin) in plasma and pyridoxal-5-phosphate in erythrocytes.

A jugular catheter was inserted 4 d before the end of the experiment (day 11 post-weaning) using a non-surgical technique described by Matte (1999). On the next day, after a fasting period of 18 h, a gastro-enteral or parenteral infusion of glucose (1·0 g/kg body weight) were given during 120 min to animals (*n* 16) from four out of the nine repetitions. Repeated blood samples were performed before (0), during (30, 60, 90 and 120 min) and after (150, 180, 210 and 240 min) the infusion for determination of plasma C-peptide, insulin and glucose concentrations. Two days after, the procedure was repeated on the same piglets but the route of infusion (gastro-enteral or parenteral) was alternated. Therefore, each piglet received successively the gastro-enteral and parenteral infusions of glucose and the order of infusions was balanced among repetitions.

Trials 3 and 4

The number of individuals in Trials 1 and 2 being limited for a valid interpretation of the vitamin effects on growth performance, two Trials (3 and 4) were carried out with a large number of animals (Yorkshire × Landrace × Duroc) in a commercial post-weaning unit. In these facilities, two rooms designed for controlled trials were used. In each room, sixteen pens each housing seventeen piglets were used. The piglets (*n* 544) were selected at weaning (15–18 d old) and assigned to the pens according to their sex and weight range (heavy, 5–6 kg *v.* light, 4–5 kg) at the beginning of the project. They were fed four commercial-type diets (Phases I, II, III and IV) up to 8 weeks following the initiation of the trial (Table 2). Within each sex and weight range, the piglets were allocated to one of the four factorial treatments similar to those used in Trial 2 (B_20–B_60, B_225–B_60, B_20–B_650 and B_225–B_650) given only in the feed served during the first 2 weeks following weaning (Phase I). Between

Table 2. Composition (calculated) of the diets in Trials 3 and 4*

Item	Phase I†‡	Phase II‡	Phase III‡	Phase IV‡
Digestible energy (MJ/kg)	14·7	14·3	14·2	14·2
Total protein (%)	21·7	20·2	19·5	20·3
Fat (%)	6·2	5·4	5·5	5·5
Crude fibre (%)	1·91	2·31	2·76	3·11
Lysine (%)	1·41	1·26	1·14	1·11
Methionine (%)	0·44	0·41	0·38	0·37
Tryptophan (%)	0·27	0·23	0·21	0·21
Calcium (%)	0·85	0·89	0·85	0·85
Phosphorus (%)	0·78	0·73	0·66	0·62

* The calculated total amount of trace elements per kg of diet for Phases I, II, III and IV were: Mn, 60, 67, 70, 72 mg; Zn, 3·1, 2·0, 1·9, 0·19 g; Fe, 313, 322, 345, 319 mg; Cu, 130, 182, 133, 130 mg. Iodine and selenium were added for all at 2·05 and 0·3 mg, respectively. The calculated total amount of vitamins per kg of diet for Phases I, II, III and IV were: vitamin A, 4·4, 4·4, 4·4, 3·2 mg; vitamin D_3, 37·5, 37·5, 37·5, 27·3 μg MIU; vitamin E, 103, 100, 41, 42 mg IU; menadione, 2·6, 2·6, 2·6, 1·9 mg; thiamin, 2·7, 2·7, 2·7, 2·0 mg; riboflavin, 8·8, 8·7, 8·7, 6·4 mg; niacin, 32, 32, 31, 23 mg; pantothenic acid, 21·5, 21·2, 21·2, 15·4 mg; folic acid, 0·7, 0·7, 0·7, 0·5 mg; pyridoxine, 2·7, 2·7, 2·6, 1·9 mg; biotin, 121, 75, 75, 55 μg; vitamin B_{12}, 25, 25, 25, 18 μg; choline, 900, 1380, 1500, 1560 mg.

† Analytical values of dietary B_2 (expressed as riboflavin equivalent; FMN + FAD + riboflavin) were 17·8 and 40·5 mg/kg in $B_2$0 and $B_2$25 diets. For dietary B_6 (expressed as pyridoxine equivalent; sum of pyridoxamine + pyridoxal + pyridoxine) values were 7·9 and 55·9 mg/kg, for $B_6$0 and $B_6$50 diets, respectively.

‡ Analytical values (overall means of Phases I–IV) of dietary B_6 (expressed as pyridoxine equivalent; sum of pyridoxamine + pyridoxal + pyridoxine) were 4·5 and 50·9 mg/kg, for $B_6$0 and $B_6$50 diets, respectively.

4 and 10 weeks of age, all animals received the same feed (Phases II, III and IV) without the B_2–B_6 treatments. Growth performance (body weight of overall piglets, feed intake and feed conversion) were measured every week for each pen. A first blood sample was taken before initiation of treatments from two piglets identified (average weight of the pen) in each pen. Another blood sample was collected from these selected piglets at 4, 6 and 8 weeks of age for determination of riboflavin (FAD + FMN + riboflavin) in plasma and pyridoxal-5-phosphate in erythrocytes. The fasting period preceding the blood sampling was limited to 4 h; the individual weight of the selected piglets was monitored at the time of sampling.

For Trial 4, the management of animals (n 544) was exactly the same as for Trial 3. In that case, two treatments (dietary supplements of 0 or 50 mg/kg of B_6) were distributed in each combination of gender and weight and they were incorporated in all diets (Phases I, II, III and IV) throughout the post-weaning period, from 2 to 10 weeks of age. Growth performance (body weight of overall piglets, feed intake and feed conversion) were measured every week for each pen. A first blood sample was taken before initiation of treatments from one piglet identified (average weight of the pen) in each pen. Another blood sample was collected from this selected piglet at 1, 5 and 8 weeks of age for determination of pyridoxal-5-phosphate in erythrocytes. The procedure for blood collection was the same as for Trial 3.

In all trials, animals were cared for 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

Pyridoxal-5-phosphate was determined in serum and red blood cells using a fluorometric method adapted by Matte *et al.* (1997) from Srivastava & Beutler (1973). Dietary B_6 was determined according to a HPLC method described by Matte *et al.* (2001) for body tissues. Riboflavin status was estimated by total B_2 metabolites in plasma, as described by Giguère *et al.* (2002).

Plasma glucose was measured by colorimetry (GOD/PAP # 166 391; Boehringer Mannheim, Germany). Plasma insulin

was measured by RIA kits (#KTSP 11 001, Immunocorp, Montréal, Canada) validated in our laboratory; the intra- and inter-assay CVs were 2·7 % and 3·7 %, respectively. Plasma C-peptide was assayed by a commercial porcine C-peptide RIA kit (Linco, cat. no. PCP-22K, St Louis, MO, USA); the intra and inter-assays CVs were 2·4 % and 2·8 %, respectively.

Statistical analysis

The data were analysed using the SAS procedure for Mixed models (Littell *et al.* 1996). For Trial 1, treatment effects were analysed within repetitions using orthogonal contrasts (linear, quadratic and/or cubic) to compare the dietary B_6 levels (0, 10, 50 and 100 mg/kg). For Trial 2, main effects and interaction between treatments were analysed within repetitions with factorial treatments of dietary B_2 (0 and 25 mg/kg) and B_6 (0 and 50 mg/kg). For Trial 3, main effects and interaction between treatments were analysed within combinations of sex and initial weight (heavy and light) with factorial treatments of dietary B_2 (0 and 25 mg/kg) and B_6 (0 and 50 mg/kg) given from 2 to 4 weeks of age. For Trial 4, treatment effects were analysed within combinations of sex and initial weight (heavy and light) with the two dietary levels of B_6 (0 and 50 mg/kg) given throughout the post-weaning period. For Trials 1–4, the age (body weight, average daily gain, feed conversion, and status in pyridoxine and riboflavin) along with their appropriate interactions with treatments were broken down in independent orthogonal contrasts (linear, quadratic, cubic etc.).

Results

Trial 1

The average daily gains, for the 2 weeks post-weaning, were 126 (SEM 9), 139 (SEM 10), 143 (SEM 7) and 138 (SEM 8) g/d in piglets fed 0, 10, 50 and 100 mg/kg B_6, respectively (B_6 linear, $P > 0.11$). There was no treatment effect ($P > 0.08$) on pyridoxal-5-phosphate in serum; the average values were 5·83 (SEM 0·44), 3·74 (SEM 0·21), 5·19 (SEM 0·25) and 5·02 (SEM 0·22) at 0, 4, 7 and 11 d post-weaning (age linear, $P < 0.01$). In erythrocytes, pyridoxal-5-phosphate decreased in $B_6$0 piglets to reach a minimum 4 d post-weaning, whereas in supplemented piglets the values

increased markedly up to 7 d post-weaning by 82, 365 and 437 % in $B_6$10, $B_6$50 and $B_6$100, respectively, and plateaued thereafter (B_6 quadratic × age quadratic, $P<0.05$; Fig. 1). Taking into account the quadratic effect of dietary B_6 on erythrocyte pyridoxal-5-phosphate, the dietary level of 50 mg/kg B_6 was used in the subsequent trials.

Trial 2

There was no treatment effect ($P>0.30$) on growth of piglets, the overall body weight at the end of the experimental period (2 weeks post-weaning) was 8.74 (SEM 0.07) kg. There was a main effect of the pyridoxine dietary supplement ($P<0.01$) on erythrocyte pyridoxal-5-phosphate (0.71 (SEM 0.02) *v.* 2.18 (SEM 0.14) μmol/l in $B_6$0 and $B_6$50 treatments). The profiles were similar to those presented for the corresponding treatments in Trial 1. No effect ($P>0.20$) of the dietary levels of B_2 was observed on B_2 metabolites (riboflavin, FMN and FAD) in plasma, the overall values were 190.4 (SEM 2.21) nmol/l.

The dietary supplement of B_2 tended to reduce ($P<0.08$) the C-peptide response to parenteral infusion of glucose (Table 3). The effect on insulin was similar but more marked ($P<0.03$) (Fig. 2; Table 3). No treatment effect ($P>0.13$) was observed on glucose profiles following the infusion of parenteral glucose. When the glucose infusion was gastro-enteral, there was a depressing effect of dietary B_2 on C-peptide and insulin responses in $B_6$0 piglets which was reversed in $B_6$50 (B_6 effect and interaction $B_2 × B_6$, $P<0.03$). In fact, the highest concentration of C-peptide and insulin were observed in $B_2$25–$B_6$50 piglets (Fig. 3; Table 3). The glucose profile tended to be higher ($P<0.06$) after initiation of the gastro-enteral infusion of glucose in B_6-supplemented piglets (Table 3).

Trials 3 and 4

In Trial 3, there was no treatment effect ($P>0.39$) on growth performance such as final body weight (31.08 (SEM 0.35) kg),
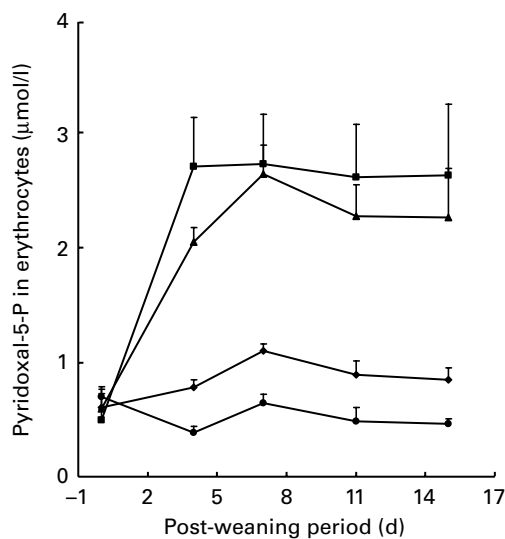

Fig. 1. Pyridoxal-5-phosphate (pyridoxal-5-P) concentrations in erythrocytes according to the dietary supplements of pyridoxine (●, 0 mg/kg ($B_6$0); ◆, 10 mg/kg ($B_6$10); ▲, 50 mg/kg ($B_6$50); ■, 100 mg/kg ($B_6$100)) given during 2 weeks following weaning (Trial 1). Values are means with standard errors of the means depicted by vertical bars.

average daily gain (0.43 (SEM 0.01) kg), feed consumption (0.67 (SEM 0.01) kg) or feed conversion (1.48 (SEM 0.01) kg/kg). There was no treatment effect ($P>0.11$) on plasma B_2 metabolites, the average overall value was 207.7 (SEM 2.1) nmol/l. For pyridoxal-5-phosphate in erythrocytes, there was a marked effect ($P<0.01$) of the dietary B_6 supplements, 2 weeks after weaning (Fig. 4), but the effect disappeared during the following 2 weeks and the values remained at their initial (weaning) level up to the end of the experimental period (interaction B_6 × post-weaning time cubic, $P<0.01$; Fig. 4).

In Trial 4, the dietary B_6 supplement increased ($P<0.03$) feed conversion (1.426 (SEM 0.008) for $B_6$0 *v.* 1.447 (SEM 0.010) for $B_6$50) by 1.4 % but decreased ($P<0.04$) average daily gain (0.423 (SEM 0.004) kg for $B_6$0 *v.* 0.410 (SEM 0.005) kg for $B_6$50) by 2.4 %. Although feed intake was also decreased by 1.4 % (0.603 (SEM 0.007) kg for $B_6$0 *v.* 0.594 (SEM 0.010) kg for $B_6$50) this was not significant ($P<0.13$). One week following weaning, the effect of dietary B_6 on pyridoxal-5-phosphate in erythrocytes was already marked ($P<0.01$; Fig. 4) and it persisted throughout the experimental period up to 10 weeks of age.

Discussion

B_6 and B_2 status

For all trials, pyridoxal-5-phosphate in erythrocytes was chosen as the indicator of the pyridoxine status of piglets, taking into account previous results (Matte *et al.* 2001) suggesting its better relevance as compared to the plasma pool. This is in agreement with the results of Trial 1 where the erythrocyte pool of pyridoxal-5-phosphate was saturated between 10 and 50 mg/kg dietary B_6 while no clear trend was observed in the plasma pool.

The values of erythrocyte pyridoxal-5-phosphate in the $B_6$50 treatments were different among trials, those in Trials 1 and 2 being approximately 50 % lower than in Trials 3 and 4. Validation tests using repeated postprandial measurements (data not shown) indicated that such a difference was related to the length of the fasting period before blood sampling (16 h in Trials 1 and 2 *v.* 4 h in Trials 3 and 4). Homeostasis of B_6 in erythrocytes appears, therefore, to be a dynamic process which responds to dietary pyridoxine intake. Pyridoxal-5-phosphate in erythrocytes also seems to respond to pyridoxine metabolic utilization, taking into account the transient increase observed during the 2 weeks of supplementation followed by the rapid decrease after the cessation of dietary supplement of pyridoxine in Trial 3. The erythrocyte metabolic pool is known to play an important role in the transport and distribution of B_6 (Coburn, 1994). It appears, therefore, that the basal level of dietary B_6 (7.6 mg/kg) which was already five times higher than the 1.5 mg/kg recommended by NRC (1998) was not sufficient to maximize pyridoxal-5-phosphate homeostasis in erythrocytes. However, for riboflavin, the absence of treatment effects suggested that the dietary levels of the $B_2$0 treatments in Trials 2 and 3, which were also five times higher than the 3.5 mg/kg recommended by NRC (1998) were sufficient to optimize the B_2 status at least in the plasma pool. Further measurements of the riboflavin status, and B_2 metabolites in erythrocytes and liver from piglets of Trial 2 (Giguère *et al.* 2002), did not reveal, either, any effect due to the dietary provision of riboflavin. Similar results were observed on erythrocyte glutathione reductase activity which is often considered as a reliable indicator of B_2 status in different species (Le Grusse & Watier, 1993) including pigs (Esch *et al.* 1981). This criterion

Table 3. C-peptide, insulin and glucose responses to parenteral or gastro-enteral infusion of glucose according to the dietary treatments of vitamins B_2 and/or B_6 (Trial 2)*

	Parenteral infusion†						Gastro-enteral infusion‡					
	C-peptide (nmol/l per min)		Insulin (nmol/l per min)		Glucose (mmol/l per min)		C-peptide (nmol/l per min)		Insulin (nmol/l per min)		Glucose (mmol/l per min)	
Treatments	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
$B_2 0 - B_6 0$	42·5	0·6	28·3	1·2	1613	134·9	37·0	3·3	25·2	2·0	1354	96·3
$B_2 0 - B_6 50$	49·6	8·3	30·6	5·0	1816	165·6	39·1	2·4	25·1	1·3	1488	19·1
$B_2 25 - B_6 0$	37·6	4·3	23·7	1·8	1544	88·6	28·3	2·2	19·6	1·4	1312	51·5
$B_2 25 - B_6 50$	36·0	1·8	21·9	0·9	1476	75·6	45·2	2·7	29·2	2·9	1432	41·8
P values												
B_6 effect	0·70		0·91		0·64		0·01		0·02		0·06	
B_2 effect	0·08		0·03		0·13		0·47		0·51		0·47	
$B_6 \times B_2$ interaction	0·53		0·56		0·32		0·02		0·03		0·90	

* Values are area under the curve from 0 to 240 min after initiation of infusions. See Materials and methods for details.
† The number of animals was three, four, four and three for treatments $B_2 0 - B_6 0$, $B_2 0 - B_6 50$, $B_2 25 - B_6 0$ and $B_2 25 - B_6 50$, respectively.
‡ The number of animals was four, four, four and three for treatments $B_2 0 - B_6 0$, $B_2 0 - B_6 50$, $B_2 25 - B_6 0$ and $B_2 25 - B_6 50$, respectively.

was not used in the present experiment because of the serious doubts raised about its validity as a biochemical indicator of vitamin B_2 status in the pig (Giguère *et al.* 2002).

Another factor supporting the validity of the present direct measurements of the B_2 and B_6 status in the blood pool was related to the experimental conditions themselves. Those animals were from similar genetic origin, they were fed the same basal diets and exactly the same amount in Trials 1 and 2. In such conditions, the fact that repeated blood samples were taken after a controlled fasting period and within the same animal should prevent any influence from the precedent meal and therefore, depending on the trials, should be a reliable estimation of the age responses of the metabolic balance of those vitamins.

Glucose tolerance

In Trial 2, the glucose tolerance tests were performed using two routes of infusion, parenteral and gastro-enteral, in order to differentiate between the glycaemic induction (parenteral) from the entero-insular or glycaemic + hormonal induction (gastro-enteral) of the insulin response to glucose. The responses of glucose and insulin to those routes of glucose infusion differed markedly from those reported by Sève *et al.* (1997) who showed that the glucose response was 50 % lower and the insulin response 50 % higher after duodenal *v.* parenteral infusions of glucose; the infusion levels were similar in both cases. In the present experiment, the values in the $B_2 0 - B_6 0$ group were lower in gastro-enteral *v.* parenteral infusions of glucose both for insulin (12 %) and glucose (20 %) responses. The present insulin response was likely to reflect the insulin secretion since C-peptide profiles were similar to those of insulin, approximately 15 % lower with gastro-enteral *v.* parenteral infusions of glucose. Peripheral C-peptide is considered a better indicator of insulin secretion than the peripheral insulin, because of its better stability to hepatic metabolism; the insulin concentration represents in fact, the balance between secretion and hepatic extraction and tissular utilization (Morgan, 1992). Some factors related to differences in experimental conditions cannot be ruled out to explain the discrepancy between the present study and Sève *et al.* (1997); those include age (4 *v.* 6 weeks, respectively) which, in rats, was sufficient to change insulin sensitivity (Issad *et al.* 1988) and genetic origin

of the piglets (Yorkshire × Landrace × Duroc *v.* Large White or Pietrain × Large White, respectively) which, in pigs, influenced insulin secretion (Gregory *et al.* 1977). Moreover, because C-peptide was not determined by Sève *et al.* (1997), the interpretation of those results on insulin synthesis is risky.

The depressing effects of B_2 were approximately of the same magnitude (20–23 %) for both insulin and C-peptide after the parenteral infusion of glucose. Such a response suggests, on the one hand, that the secretion of insulin was reduced and, on the other hand, in an apparent ($P < 0.13$) absence of any effect on glucose profiles, that the sensitivity of the systemic-induced insulin was higher in B_2-supplemented than in non-supplemented piglets. To the best of our knowledge, such an effect of B_2 has never been reported before. The relevant information is rather scarce and related mostly to the effect of deficiency and/or repletion after deficiency. In such cases, some aspects of insulin
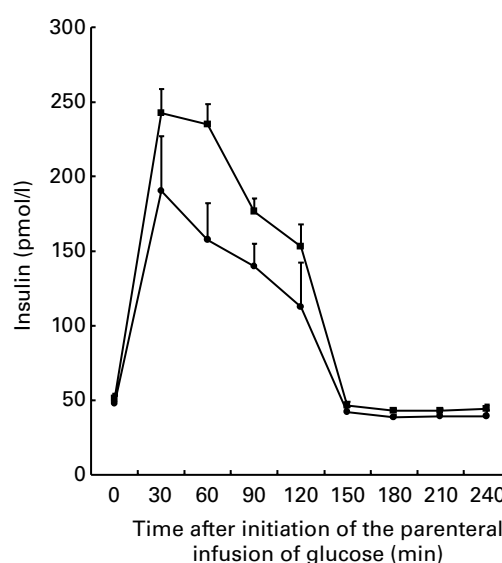

Fig. 2. Plasma insulin response to parenteral infusion of glucose (1 g/kg body weight during 120 min) according to the dietary supplements of riboflavin (■, 0 mg/kg ($B_2 0$); •, 25 mg/kg ($B_2 25$)) given during 2 weeks following weaning (Trial 2). Values are means with standard errors of the means depicted by vertical bars.
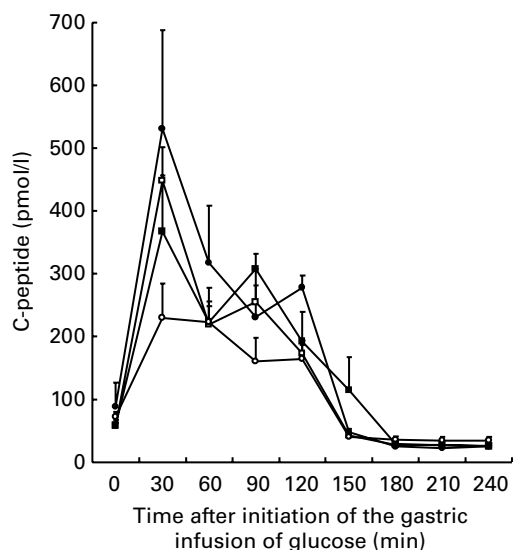
Fig. 3. Plasma C-peptide response to gastro-enteral infusion of glucose (1 g/kg of body weight during 120 min) according to the dietary supplements of riboflavin and pyridoxine (□, 0 and 0 mg/kg ($B_2$0–$B_6$0); ■, 0 and 50 mg/kg ($B_2$0–$B_6$50); ○, 25 and 0 mg/kg ($B_2$25–$B_6$0); ●, 25 and 50 mg/kg ($B_2$25–$B_6$50)) given during 2 weeks following weaning (Trial 2). Values are means with standard errors of the means depicted by vertical bars.

metabolism (e.g. the feeding response to insulin) were altered but, in agreement with the present results, not the glucose response (Matsuo & Suzuoki, 1982; Matsuo *et al.* 1983). *In vitro*, riboflavin does not stimulate or inhibit the secretion of insulin by murine pancreas in contrast to other micronutrients such as niacin (Patole & Agte, 1998).

After the gastro-enteral infusion of glucose, the glucose profile tended ($P < 0.06$) to be higher during and after the gastro-enteral infusion of glucose in B_6-supplemented piglets. Such a B_6 effect has been reported in broilers where the *in vitro* passage of enteric
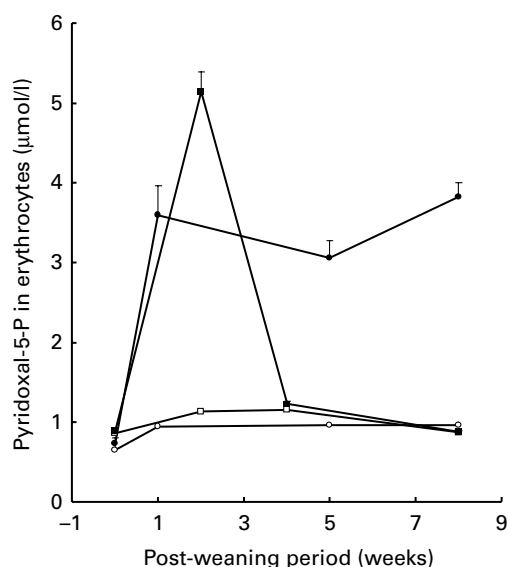

Fig. 4. Pyridoxal-5-phosphate (pyridoxal-5-P) concentrations in erythrocytes according to the dietary supplements of pyridoxine given during the first 2 weeks after weaning (□, 0 mg/kg ($B_6$0); ■, 50 mg/kg ($B_6$50); Trial 3) or from 2 to 10 weeks of age (○, 0 mg/kg ($B_6$0); ●, 50 mg/kg ($B_6$50; Trial 4). Values are means with standard errors of the means depicted by vertical bars.

glucose through duodenal and jejunal loops was twice higher when 30–60 µmol vitamin B_6 are present in the loops (Pintea & Garici, 1985). According to those last authors, the physiological mechanism involved would be related to a better efficiency of glucose transport (decreased losses) in the intestinal wall of B_6-treated tissues. Possibly, the role of B_6 in the metabolism of glucogenic amino acids such as, for example, alanine (towards pyruvate through the B_6-dependent alanine-glyoxylate transaminase (*EC* 2.6.1.44); K.E.G.G., 2004) could be involved here. This hypothesis of a vitamin-induced differential glucose absorption appeared as the most logical explanation to the concomitant high levels of insulin, C-peptide and glucose. Nevertheless, the apparent greater transfer of glucose in circulation in B_6-supplemented animals had apparently a different effect on the post-infusion insulin release depending on the level of B_2 supplement. Such a response for both insulin and C-peptide was different from what was observed after the parenteral infusion. In fact, there was a stimulating effect on insulin secretion of the combination of B_2 and B_6 supplements only when glucose was given through the gastro-enteral route. An increased insulin response to duodenal infusion of glucose was also observed in piglets receiving intramuscular injections of B_6 (Matte *et al.* 1997) although their estimated dietary provision of B_2 was rather close to the actual level in $B_2$0 groups. Nevertheless, the present results suggest that the action of B_2 and B_6 on the secretion of insulin was mediated by the entero-insular axis and, in this way, neuropeptides regulating insulin secretion appear as the most logical candidates. Indeed, some of them such as the glucose-dependent insulinotropic polypeptide, the neuropeptide YY, the peptide histidine methionine and the glucagon-like peptide GLP-1 are produced in response to oral but not to intravenous glucose and have the ability to stimulate insulin secretion (Morgan, 1992). To the best of our knowledge there is no information in the literature about involvement of both vitamins, B_2 and/or B_6 in the synthesis, secretion or release of these regulatory peptides.

Growth performance

In Trial 1, there was a tendency for an increased growth rate with levels of pyridoxine over 50 mg/kg during the first 2 weeks after weaning. No such treatment effect was found in Trial 2. Taking into account that the experimental conditions and the number of individuals in Trials 1 and 2 could be limiting factors for a valid interpretation of the vitamin effects on growth performance, two additional Trials (3 and 4) were carried out with a large number of animals in a commercial post-weaning unit. In Trial 3, as mentioned earlier, the transient increase in erythrocyte pyridoxal-5-phosphate observed during the 2 weeks of supplementation followed by the rapid decrease after the cessation of dietary supplement of pyridoxine suggest an intense utilization of this vitamin after weaning in commercial conditions. In such a case, one could argue that the transient duration of treatments could have masked an eventual effect of the dietary supplement of B_6. In order to verify this possibility, another trial was undertaken with supplements of B_6 throughout the post-weaning period but, without riboflavin supplements taking into account the previous absence of effect of this vitamin on riboflavin status and performance in Trial 3. It appears, however, that in spite of a high B_6 status brought by the B_6 supplements throughout the post-weaning period, the effects on performance, if so, were rather marginal (< 2.5 %). Therefore, the basal level of dietary B_6 (between 4.5

and 7·9 mg/kg according to the trials), which was already three to five times higher than recommended by NRC (1998); 1·5 mg/kg, was sufficient to maximize growth performance. Such dietary levels are in agreement with the range of optimal values (7·1–7·9 mg/kg) suggested by Woodworth *et al.* (2000) for post-weaning piglets. The supplement of synthetic pyridoxine (pyridoxine.HCl) needed to reach such total provision of B_6 is similar, in fact, to the average level of fortification reported to be used in the industry (BASF Corporation, 1993).

Conclusion

In conclusion, dietary concentrations between 10 (apparently inadequate) and 50 mg/kg (probably more than adequate) B_6 seemed optimal for some metabolic criteria of B_6 status. The dietary concentration of 50 mg/kg B_6 appeared beneficial in combination with vitamin B_2 for some aspects of the entero-insular control of glucose homeostasis. Nevertheless, it appears that those metabolic effects during the 2 weeks following weaning had no major impact for post-weaning growth performance, taking into account the lack of response after increasing the daily provision of B_6 beyond the B_60 level (between 4·5 and 7·9 mg/kg) in the present trials.

Acknowledgements

References

Adams CR, Richardson CE & Cunha TJ (1967) Supplemental biotin and vitamin B6 for swine. *J Anim Sci* **26**, 903.

Agriculture Canada (1993) *Recommended Code of Practice for Care and Handling of Pigs*. Publication no. 1771E. Ottawa, Ont., Canada: Agriculture Canada.

BASF Corporation (1993) *Vitamin Supplementation Rates for U.S. Commercial Poultry, Swine and Dairy Cattle*. Mount Olive, NJ: BASF Corporation.

Bender DA (1999) Non-nutritional uses of vitamin B_6. *Br J Nutr* **81**, 7–20.

Benedikt J, Roth-Maier DA & Kirchgessner M (1996) Influence of dietary vitamin B_6 supply during gravidity and lactation on total vitamin B_6 concentration (Pyridoxine, Pyridoxal and Pyridoxamine) in blood and milk. *Int J Vitam Nutr Res* **66**, 146–150.

Bilodeau R, Matte JJ, B de Passillé A-M, Girard CL & Brisson GJ (1989) Effects of floor type on serum folates, serum vitamin B_{12}, plasma biotin and on growth performances of pigs. *Can J Anim Sci* **69**, 779–788.

Bretzinger J (1991) Pyridoxine supply of early weaned piglets. Doctoral Thesis in Veterinary Medecine, Ludwig-Maximillians University of Munich.

Canadian Council on Animal Care (1993) *Guide to the Care and Use of Experimental Animals*, Vol. 1. Ottawa, Ont.: Canada Canadian Council on Animal Care.

Coburn SP (1994) A critical review of minimal B_6 requirements for growth in various species with a proposed method of calculation. *Vitam Horm* **48**, 259–300.

Cortamira NO, Sève B, Lebreton Y & Ganier P (1991) Effect of dietary tryptophan on muscle, liver and whole body protein synthesis in weaned piglets: relationship to plasma insulin. *Br J Nutr* **66**, 423–435.

Davis TA, Burrin DG, Fiorotto ML & Nguyen HV (1996) Protein synthesis in skeletal muscle and jejunum is more responsive to feeding in 7- than in 26-day-old pigs. *Am J Physiol* **270**, E802–E809.

Esch MW, Easter RA & Bahr JM (1981) Effect of riboflavin deficiency on estrous cyclicity in pigs. *Biol Reprod* **25**, 659–665.

Giguère A, Girard CL & Matte JJ (2002) Erythrocyte glutathione reductase activity and riboflavin nutritional status in early-weaned piglets. *Int J Vitam Nutr Res* **72**, 383–387.

Gregory NG, Lovell RD, Wood JD & Lister D (1977) Insulin-secreting ability in Pietrain and Large White pigs. *J Agric Sci (Cambridge)* **89**, 407–413.

Issad T, Coupé C, Pastor-Anglada M, Ferré P & Girard J (1988) Development of insulin sensitivity at weaning in the rat. Role of nutrition transition. *Biochem J* **251**, 685–690.

K.E.G.G. (2004) *Kyoto Encyclopedia of Genes and Genomes*. http://www.genome.ad.jp/kegg

Kösters WW & Kirchgessner M (1976) [Change in feed intake of early-weaned piglets in response to different vitamin B_6 supply]. *Z Tierphysio Tiern Futterm* **37**, 247–254.

Le Grusse J & Watier B (1993) *Les vitamines, données biochimiques, nutritionnelles et cliniques*. Neuilly-sur-Seine, France: Centre d'étude et d'informations sur les vitamines, produits Roche.

Letendre M, Girard CL, Matte JJ & Bernier JF (1991) Effects of intramuscular injections of folic acid on folates status and growth performance of weanling pigs. *Can J Anim Sci* **71**, 1223–1231.

Littell RC, Milliken GA, Stroup WW & Wolfinger RD (1996) *SAS System for Mixed Models*. Cary, NC: SAS Institute Inc.

Lu S-H & Huang P-C (1997) Effects of casein and soy protein isolate on vitamin B_6 nutritional status in rats. *J Biomed Sci* **4**, 120–124.

Matsuo T, Shimakawa K & Suzuoki Z (1983) Recovery from impairment in feeding response to glucoprivic stimuli and in sensitivity to diabetogenic agents in riboflavin-repleted rats. *J Nutr* **113**, 1784–1790.

Matsuo T & Suzuoki Z (1982) Feeding response of riboflavin-deficient rats to energy dilution, cold and glucoprivation. *J Nutr* **112**, 1052–1056.

Matte JJ (1999) A rapid and non-surgical procedure for jugular catheterization of pigs. *Lab Anim* **33**, 258–264.

Matte JJ, Girard CL, Bilodeau R & Robert S (1990) Effects of intramuscular injections of folic acid on serum folates, haematological status and growth performance of growing-finishing pigs. *Reprod Nutr Dev* **30**, 103–114.

Matte JJ, Girard CL & Brisson GJ (1986) Importance of folic acid administered during gestation on haematological status of piglets. *Can J Anim Sci* **66**, 523–527.

Matte JJ, Girard CL & Sève B (2001) 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. *Br J Nutr* **85**, 11–21.

Matte JJ, Ponter AA & Sève B (1997) Effects of chronic parenteral pyridoxine and acute enteric tryptophan on pyridoxine status, glycemia and insulinemia stimulated by enteric glucose in weanling piglets. *Can J Anim Sci* **77**, 663–668.

Morgan LM (1992) Insulin secretion and the entero-insular axis. In *Nutrient Regulation of Insulin Secretion* [PR Flatt, editor]. London: Portland Press.

NRC (1998) *Nutrient Requirements of Swine*, 10th ed. Washington, DC: National Academy Press.

Patole AP & Agte VV (1998) Effects of various dietary constituents on insulin secretion in vitro by pancreas culture. *J Med Arom Plant Sci* **20**, 413–416.

Pintea V & Garici I (1985) Absorption of glucose from the intestine of broilers in the presence of water soluble vitamins. In *Lucrarile simpozionului bolile de nutritie si metabolism la animale in cresterea intensir*, pp. 35–40. Romania: Societacta de medicina veterinara

Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD & Gershoff SN (1991) Vitamin B-6 requirements of elderly men and women. *J Nutr* **121**, 1062–1074.

Rogers KS & Mohan C (1994) Vitamin B-6 metabolism and diabetes. *Biochem Med Metab Biol* **52**, 10–17.

Safaya S & Bamji MS (1981) Unlikely role of tryptophan metabolites in glucose tolerance and gluconeogensis in oral contraceptive and pyridoxine treated rats. *Indian J Med Res* **74**, 236–243.

Sève B, Ronat P, Hess V, Matte JJ & Ponter AA (1997) Evidence for the entero insular axis in weanling piglets. In *Proceedings, VIIth Symposium on Digestive Physiology in Pigs*, St-Malo, France, 26–28 May, EAAP Publication 88, pp. 270–274 [JP Laplace, C Février and A Barbeau, editors]. Rome, Italy: EAAP.

Srivastava SK & Beutler E (1973) A new fluorometric method for the determination of pyridoxal 5′-phosphate. *Biochim Biophys Acta* **304**, 765–773.

Woodworth JC, Goodband RD, Nelssen JL, Tokach MD & Musser RE (2000) Added dietary pyridoxine, but not thiamin, improves weanling pig growth performance. *J Anim Sci* **78**, 88–93.

Yen JT & Pond WG (1988) Response of weanling pigs to dietary supplementation of vitamin C and/or rutin. *Nutr Rep Int* **38**, 1103–1107.