Some aspects of the pyridoxine (vitamin B₆) requirement in weanling piglets

J. J. Matte*, A. Giguère and C. L. Girard

Dairy and Swine R & D Centre, Agriculture and Agri-Food Canada, P.O. Box 90, Lennoxville, Québec, Canada, JIM 1Z3

(Received 15 June 2004 – Revised 6 January 2005 – Accepted 10 January 2005)

Four trials were carried out to determine the optimal level of dietary pyridoxine (vitamin B₆) and its interaction with riboflavin (vitamin B₂) in early-weaned piglets. In Trial 1, twelve piglets were tube-fed graded supplements of B₆, 0, 10, 50 or 100 mg/kg. The level of 50 mg/kg maximized B₆ in red blood cells (P < 0.05). In Trial 2, thirty-six piglets were tube-fed with four combinations of B₆ (0 v. 50 mg/kg) and B₂ (0 v. 25 mg/kg). The B₆ supplement increased (P < 0.01) B₆ in red blood cells. C-peptide and insulin responses to intravenous glucose tended (P < 0.08) to or decreased (P < 0.03) with B₂ while no effect was observed on glucose. After gastro-enteral glucose, dietary B₂ depressed C-peptide and insulin responses in B₆-unsupplemented piglets and increased in them in B₆-supplemented piglets (P < 0.03). The glucose response tended to be higher in B₂-supplemented piglets (P < 0.06). Trials 3 and 4 were carried out in commercial conditions using either B₆ and/or B₂ supplements given during 2 weeks after weaning. In Trial 3) or a B₆ supplement alone (50 mg/kg) given between 2 (weaning) and 10 weeks of age. Despite a marked and persistent increase (P < 0.01) of B₆ in red blood cells in B₆-supplemented piglets, the effect on growth performance was either none (P > 0.39; Trial 3) or marginally lower (P < 0.2; P < 0.03; Trial 4). In conclusion, it appears that a dietary supplement of 50 mg/kg B₆ saturated the red blood cell pool in B₆ and influenced, along with B₂, 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 B₁₂ (Bilodeau et al. 1989) and vitamin C (Yen & Pond, 1988). It is also the case of pyridoxine (vitamin B₆) for which the status is low at weaning (Matte et al. 1997, 2001). In fact, sow milk is a poor dietary source of B₆ (Benedikt et al. 1996), approximately 0.4 μg/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 importance of the protein in the post-weaning feed, as opposed to dam’s milk, would further increase the B₆ needs, because of an increased interconversion and oxidation of amino acids; those metabolic pathways are, in many cases, B₆-dependent. The interaction between metabolic utilization of B₆ 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 B₆ 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 B₆ 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 B₆.

B₆ metabolism is closely related to other B-vitamins, in particular, riboflavin (B₂). The biologically active metabolites of B₂, FMN and FAD, are involved in the conversion of B₆ in its biologically active vitamers, pyridoxal phosphate, and its excretory form, 4-pyridoxic acid (Le Grusse & Watier, 1993). The NRC (1998) recommendations for both B₆ and B₂ 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 B₆ and B₂ 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.
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 B6 (pyridoxine.HCl) at 0 (B60), 10 (B610), 50 (B650) and 100 mg/kg (B6100) added to a commercial diet based on barley (25%), maize (20%), dried whey (20%), soybean meal (10%), extruded soybean (8%) and plasma protein (4.5%) (Table 1). The calculated total dietary level of B6 (ingredients and synthetic addition) was 7.1 mg/kg and the analytical content was 7.6 mg/kg (B6100) 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 and 11 d post-weaning for determination of riboflavin (FAD + FMN + riboflavin) in plasma and pyridoxal-5-phosphate in erythrocytes.

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 B2 and B6, 0 and 0 (B20–B60), 25 and 50 mg/kg (B225–B650) added to the commercial diet described in Trial 1. The calculated total dietary levels of B6 and B2 (ingredients and synthetic additions) were 7.1 and 15.5 mg/kg, respectively. The analytical content was 7.6 mg/kg for B6 (expressed as pyridoxine equivalent; sum of pyridoxamine and pyridoxal + pyridoxine contents). The feeding regimen was based on a daily increment of 7.1 g feed per kg0.75 body weight (g/kg0.75) up to the target maximum daily value of 56 g/kg0.75. 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 20% of the daily dietary intake. The supplements of B2 were given as adjusted amounts, according to treatments of solutions at 0 (B20) and 1.4 (B225) mM-riboflavin (prepared with 0.01 M-NaOH and 0.06 mM-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)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Calculated concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy (MJ/kg)</td>
<td>14.6</td>
</tr>
<tr>
<td>Total protein (%)</td>
<td>20.7</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>7.26</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>1.79</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.48</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.96</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*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 D₃, 37.9 μg vitamin E, 109 mg; methionine, 2.7 mg; thiamin, 2.8 mg; riboflavin, 8 mg; niacin, 31.9 mg; pantothenic acid, 21.5 mg; folate, 0.7 mg; pyridoxine, 2.7 mg; biotin, 123 μg; vitamin B₁₂, 26 μg; choline, 1205 mg.

**Tiers 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 (B₂₀–B₆₀, B₂₂₅–B₆₅₀, B₂₀–B₅₅₀ and B₂₂₅–B₅₂₅) given only in the feed served during the first 2 weeks following weaning (Phase I). Between
4 and 10 weeks of age, all animals received the same feed (Phases II, III and IV) without the B2–B6 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 B6) 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 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 B6 was determined according to a HPLC method described by Matte et al. (2001) for body tissues. Riboflavin status was estimated by total B2 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 (#KTS 11001, 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. PCT-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 replications using orthogonal contrasts (linear, quadratic and/or cubic) to compare the dietary B6 levels (0, 10, 50 and 100 mg/kg). For Trial 2, main effects and interaction between treatments were analysed within replications with factorial treatments of dietary B2 (0 and 25 mg/kg) and B6 (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 B2 (0 and 25 mg/kg) and B6 (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 B6 (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 models (Littell et al. 1996). For Trial 1, treatment effects were

---

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Phase I†‡</th>
<th>Phase II†‡</th>
<th>Phase III†‡</th>
<th>Phase IV†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy (MJ/kg)</td>
<td>14.7</td>
<td>14.3</td>
<td>14.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Total protein (%)</td>
<td>21.7</td>
<td>20.2</td>
<td>19.5</td>
<td>20.3</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>6.2</td>
<td>5.4</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>1.91</td>
<td>2.31</td>
<td>2.76</td>
<td>3.11</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.41</td>
<td>1.26</td>
<td>1.14</td>
<td>1.11</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.44</td>
<td>0.41</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.27</td>
<td>0.23</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.85</td>
<td>0.89</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.78</td>
<td>0.73</td>
<td>0.66</td>
<td>0.62</td>
</tr>
</tbody>
</table>

† Analytical values (overall means of Phases I–IV) of dietary B6 (expressed as pyridoxine equivalent; sum of pyridoxamine ‡ + pyridoxal + pyridoxine) values were 7.9 and 55.9 mg/kg, for B60 and B650 diets, respectively.

‡ Analytical values were 7.9 and 55.9 mg/kg, for B60 and B650 diets, respectively.

* 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 mg; 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 D3, 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, 8.6, 8.7, 8.7, 6.4 mg; niacin, 32, 32, 31, 23 mg; pantothentic 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, 2.6 mg; biotin, 121, 75, 76, 55 µg; vitamin B12, 22, 25, 25, 18 µg; choline, 900, 1380, 1500, 1560 mg.

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 B6, respectively (B6 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 B60 piglets to reach a minimum 4 d post-weaning, whereas in supplemented piglets the values were higher.

---

**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 B6, respectively (B6 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 B60 piglets to reach a minimum 4 d post-weaning, whereas in supplemented piglets the values were higher.
increased markedly up to 7 d post-weaning by 82, 365 and 437 % in B610, B650 and B6100, respectively, and plateaued thereafter (B6 quadratic \times age quadratic, P<0.05; Fig. 1). Taking into account the quadratic effect of dietary B6 on erythrocyte pyridoxal-5-phosphate, the dietary level of 50 mg/kg B6 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 B0 and B50 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 B2 was observed on B2 metabolites (riboflavin, FMN and FAD) in plasma, the overall values were 190.4 (SEM 2.21) nmol/l.

The dietary supplement of B2 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 B2 on C-peptide and insulin responses in B0 piglets which was reversed in B50 (B6 effect and interaction B2 × B6, P<0.03). In fact, the highest concentration of C-peptide and insulin were observed in B25–B50 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 B650-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), 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 B2 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 B6 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 B6 × post-weaning time cubic, P<0.01; Fig. 4).

In Trial 4, the dietary B6 supplement increased (P<0.03) feed conversion (1.426 (SEM 0.008) for B60 v. 1.447 (SEM 0.010) for B650) by 1.4 % but decreased (P<0.04) average daily gain (0.423 (SEM 0.004) kg for B60 v. 0.410 (SEM 0.005) kg for B650) by 2.4 %. Although feed intake was also decreased by 1.4 % (0.603 (SEM 0.007) kg for B60 v. 0.594 (SEM 0.010) kg for B650) this was not significant (P<0.13). One week following weaning, the effect of dietary B6 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**

**B6 and B2 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 B6 while no clear trend was observed in the plasma pool.

The values of erythrocyte pyridoxal-5-phosphate in the B650 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 B6 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 B6 (Coburn, 1994). It appears, therefore, that the basal level of dietary B6 (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 B60 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 B2 status at least in the plasma pool. Further measurements of the riboflavin status, and B2 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 B2 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<sub>2</sub> and/or B<sub>6</sub> (Trial 2)*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parenteral infusion†</th>
<th>Gastro-enteral infusion‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-peptide (nmol/l per min)</td>
<td>Insulin (nmol/l per min)</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;0–B&lt;sub&gt;6&lt;/sub&gt;0</td>
<td>42.5 ± 0.6</td>
<td>28.3 ± 1.2</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;0–B&lt;sub&gt;6&lt;/sub&gt;50</td>
<td>49.6 ± 8.3</td>
<td>30.6 ± 5.0</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;5–B&lt;sub&gt;6&lt;/sub&gt;0</td>
<td>37.6 ± 4.3</td>
<td>23.7 ± 1.8</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;5–B&lt;sub&gt;6&lt;/sub&gt;50</td>
<td>36.0 ± 1.8</td>
<td>21.9 ± 0.9</td>
</tr>
</tbody>
</table>

* 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<sub>2</sub>0–B<sub>6</sub>0, B<sub>2</sub>0–B<sub>6</sub>50, B<sub>2</sub>5–B<sub>6</sub>0 and B<sub>2</sub>5–B<sub>6</sub>50, respectively.
‡ The number of animals was four, four, four and three for treatments B<sub>2</sub>0–B<sub>6</sub>0, B<sub>2</sub>0–B<sub>6</sub>50, B<sub>2</sub>5–B<sub>6</sub>0 and B<sub>2</sub>5–B<sub>6</sub>50, respectively.

Glucose tolerance

In Trial 2, the glucose tolerance tests were performed using two routes of infusion, parenteral and gastro-ental, in order to differentiate between the glycemic induction (parenteral) from the entero-insular or glycemic + hormonal induction (gastro-ental) 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<sub>0</sub>0–B<sub>0</sub>0 group were lower in gastro-ental 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-ental v. parenteral infusions of glucose. Peripheral C-peptide is considered a better indicator than 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<sub>2</sub> 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<sub>2</sub>-supplemented than in non-supplemented piglets.

To the best of our knowledge, such an effect of B<sub>2</sub> 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...
The infusion of glucose in B6-supplemented piglets. Such a B6 effect means with standard errors of the means depicted by vertical bars. Tended (B20–B650); (þ, 25 and 0 mg/kg (B25–B60); †, 25 and 50 mg/kg (B25–B650)) 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 (þ, 0 and 0 mg/kg (B0–B0); ■, 0 and 50 mg/kg (B0–B50); (½, 25 and 0 mg/kg (B25–B60); †, 25 and 50 mg/kg (B25–B650)) given during 2 weeks following weaning (Trial 2). Values are means with standard errors of the means depicted by vertical bars.

After the gastro-enteral infusion of glucose, the glucose profile 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 B6-supplemented piglets. Such a B6 effect has been reported in broilers where the in vitro passage of enteric glucose through duodenal and jejunal loops was twice higher when 30–60 µmol vitamin B6 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 B6-treated tissues. Possibly, the role of B6 in the metabolism of glucogenic amino acids such as, for example, alanine (towards pyruvate through the B6-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 B6-supplemented animals had apparently a different effect on the post-infusion insulin release depending on the level of B2 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 B2 and B6 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 intra-muscular injections of B6 (Matte et al. 1997) although their estimated dietary provision of B2 was rather close to the actual level in B20 groups. Nevertheless, the present results suggest that the action of B2 and B6 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 insulino-tropic 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, B2 and/or B6 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 B6. In order to verify this possibility, another trial was undertaken with supplements of B6 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 B6 status brought by the B6 supplements throughout the post-weaning period, the effects on performance, if so, were rather marginal (<2·5%). Therefore, the basal level of dietary B6 (between 4·5

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 (½, 0 mg/kg (B0); ■, 50 mg/kg (B50); Trial 3) or from 2 to 10 weeks of age (†, 0 mg/kg (B0); †, 50 mg/kg (B50); Trial 4). Values are means with standard errors of the means depicted by vertical bars.
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 B6 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) B6 seemed optimal for some metabolic criteria of B6 status. The dietary concentration of 50 mg/kg B6 appeared beneficial in combination with vitamin B2 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 B6 beyond the B60 level (between 4.5 and 7.9 mg/kg) in the present trials.

Acknowledgements

The authors would like to thank M. Guillette, F. Guay, M. Turcotte, F. Phaneuf, M.-F. Boucher (Ferme Magi-Porc), M. Vignola and D. Bussières (Shur-Gain, Maple Leaf, Brossard, Québec, Canada) for their technical support. The financial support was provided by Shur-Gain (Maple Leaf), Roche Vitamins Ltd and the Matching Investment Initiative of Agriculture and Agri-Food Canada.

References


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


Kösters WW & Kirchgessner M (1976) [Change in feed intake of early-weaned piglets in response to different vitamin B6 supply]. Z Tierphy- sio Tiern Futterm 37, 247–254.


