Citrulline and intestinal fatty acid-binding protein: Longitudinal markers of postweaning small intestinal function in pigs?

M. Berkeveld, P. Langendijk, J. H. M. Verheijden, M. A. M. Taverne, A. van Nes, P. van Haard and A. P. Koets

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ABSTRACT: The objective of the current study was to investigate whether plasma citrulline or intestinal fatty acid-binding protein (I-FABP) concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. Plasma citrulline and I-FABP concentrations were measured longitudinally in weaned and unweaned piglets, and related to intestinal absorption values (i.e., plasma mannitol and d-xylose concentrations in a sugar absorption test). Within each litter (n = 10), 2 piglets with a close-to-litter-average BW were selected. At 20.8 ± 0.4 d of age, the selected piglets per litter were either weaned conventionally (CW) or remained with the sow (UNW). One day before, and 0.5, 2, 4, and 7 d after weaning of the CW piglets, the selected piglets of both groups were subjected to a sugar absorption test. After a 2-h fast, piglets were administered an oral dose of 2 mL/kg of sugar solution, containing 50 mg/kg of mannitol and 100 mg/kg of d-xylose. One hour after administration, a blood sample was collected from a jugular vein for determination of plasma I-FABP, citrulline, mannitol, and d-xylose concentrations. Plasma I-FABP concentration showed great variation within treatments, and no difference was observed in plasma I-FABP concentrations between the CW and UNW treatments (P = 0.63). The absorption of d-xylose was not different between treatments (P = 0.83). Mannitol absorption, however, was less in the weaned CW piglets compared with the UNW piglets (P = 0.003), with the nadir on d 4 postweaning. Weaning also reduced plasma citrulline concentrations in the CW treatment compared with the UNW treatment (P < 0.001). On d 4 and 7 postweaning, plasma citrulline concentrations of CW piglets were less (P < 0.001 and P = 0.0013) than preweaning values. Furthermore, in the CW treatment, plasma citrulline concentrations correlated with plasma mannitol concentrations at d 4 postweaning (r = 0.89, P = 0.008) and overall (r = 0.76, P = 0.001). Based on these results, plasma citrulline concentration seems to be a possible marker for monitoring intestinal function in pigs after weaning.

Key words: biological marker, citrulline, intestinal fatty acid-binding protein, pig, sugar absorption, weaning

INTRODUCTION

Most intestinal markers used to study the effect of weaning on small intestinal structure or small intestinal function in piglets are end-point measurements (Montagne et al., 2007). A biological marker of intestinal function would enable longitudinal monitoring, and as a consequence, reduce the number of animals required. To our knowledge, such a marker has not been described for pigs.

Fatty acid-binding proteins are small cytoplasmatic proteins involved in the intracellular buffering and transport of long-chain fatty acids. Fatty acid-binding proteins are considered to be potential plasma markers for the detection of tissue injury (Pelsers et al., 2005).
Table 1. Composition of postweaning piglet diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>11</td>
</tr>
<tr>
<td>Corn</td>
<td>15</td>
</tr>
<tr>
<td>Barley</td>
<td>37</td>
</tr>
<tr>
<td>Whey powder</td>
<td>7.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>3.2</td>
</tr>
<tr>
<td>Soybeans (full-fat, toasted)</td>
<td>6.0</td>
</tr>
<tr>
<td>Extracted soybean meal</td>
<td>3.0</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>0.4</td>
</tr>
<tr>
<td>Potato protein</td>
<td>2.1</td>
</tr>
<tr>
<td>Coconunt oil</td>
<td>1.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>1.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>8.2</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>88.3</td>
</tr>
<tr>
<td>CP, %</td>
<td>17.8</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>4.2</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>2.9</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.6</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.3</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.5</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>135.0</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>160.0</td>
</tr>
<tr>
<td>ME, MJ of ME/kg</td>
<td>14.0</td>
</tr>
<tr>
<td>NE, MJ of NE/kg</td>
<td>10.3</td>
</tr>
</tbody>
</table>

1Calculated with the use of the Dutch feed tables (Everts et al., 1995).

The expression of intestinal fatty acid-binding proteins (I-FABP) is restricted to the intestinal tract (Glatz and van der Vusse, 1996). Elevated concentrations of I-FABP are detected in human patients, such as those with small bowel obstruction (Cronk et al., 2006) or necrotizing enterocolitis (Guthmann et al., 2002). Niewold et al. (2004) suggested, based on the acute increase (within 30 min) in plasma I-FABP concentrations after experimentally induced intestinal ischemia in pigs, that plasma I-FABP concentration might be used as a sensitive marker of damage to the intestinal mucosa in pigs.

Citrulline, a nonprotein AA, is the nitrogen end product of glutamine metabolism and is produced exclusively by the enterocytes of the small bowel (Windmueller and Spaeth, 1981). In patients with short bowel syndrome, the plasma citrulline concentration was found to be a simple and reliable marker of absorptive bowel length and absorptive function (Crenn et al., 2000; Jianfeng et al., 2005). It was hypothesized that plasma citrulline might be a promising marker for monitoring postweaning intestinal function in piglets. In this study, plasma citrulline and I-FABP concentrations were measured longitudinally in weaned and unweaned piglets, and were related to intestinal absorption values (i.e., plasma mannitol and D-xylose concentrations in a sugar absorption test).

MATERIALS AND METHODS

The Ethics Committee for animal experiments of the Faculty of Veterinary Medicine at Utrecht University approved the experimental design, including all procedures involving animals.

Experimental Design

Ten litters of multiparous sows were used during 4 farrowing replicates (Tolakker Research Farm, Faculty of Veterinary Medicine, Utrecht University, the Netherlands). Selection of litters was based on litter size (≥10 piglets) and selection of piglets was based on average piglet BW per litter (close to the overall average piglet BW of litters per replicate). Within each litter, 2 piglets with a close-to-litter-average BW were selected, resulting in a total of 20 piglets. During the suckling period, no solid feed was offered to the piglets. At 20.8 ± 0.4 d of age, one piglet per litter was weaned conventionally (CW) and one piglet continued lactation (UNW). The UNW piglets (n = 10) remained with their sows, whereas the CW piglets (n = 10) were weaned and housed in weaner pens together with 4 unfamiliar age-matched piglets (Pig and Poultry Research Unit, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, the Netherlands). From the day of weaning, the CW piglets onward, piglets from both treatment groups had ad libitum access to solid feed (Table 1; Romelko Geel, Sondag Voeders BV, Veghel, the Netherlands). Piglets had ad libitum access to water throughout the entire experiment.

One day before weaning the CW piglets and at 0.5, 2, 4, and 7 d after weaning, piglets from both treatment groups were subjected to a sugar absorption test. Piglets were fasted for 2 h by removal of solid feed and water in both groups and separation from the sow in the UNW group. Thereafter, an oral dose of 2 mL/kg of sugar solution, containing mannitol (50 mg/kg; Sigma, St. Louis, MO) and D-xylose (100 mg/kg; Sigma) dissolved in water, was administered. One hour after administration, a 5-mL blood sample was collected by venipuncture of a jugular vein with a 5-mL syringe (Instruvert, Cuijk, the Netherlands). Aliquots (2-mL) of each blood sample were transferred to a heparin-coated (17 IU of heparin/mL) and an EDTA-coated tube (Instruvert), and after centrifugation (10 min, 2,375 × g at 4°C), plasma samples were stored at −80°C.

Determination of Plasma Mannitol and D-Xylose Concentration

The plasma samples with EDTA obtained 1 h after administration of the sugar solution were used to determine plasma mannitol and D-xylose concentrations as markers of intestinal absorption (Miller et al., 1984; Cox et al., 1999). Because of the limited volume, some of the plasma samples could not be analyzed. The standards and reagents were from Sigma-Aldrich and the...
solvents were from Baker (Deventer, the Netherlands). Hibtane (50 mg/L) was added to all solutions containing carbohydrates to prevent bacterial growth. A 200-µL aliquot of an internal standard solution of Trehalose (300 µmol/L in 20:80 methanol:water, vol/vol) and 100 µL of salicine was added to 100 µL of each EDTA-plasma sample. The mixture was dried thoroughly under a stream of nitrogen at 40°C before adding a light-protected solution of 12.5 mg of hydroxylamin-HCl in 500 µL of aniline; the mixture was then incubated for 10 min at 60°C. After cooling to room temperature, 300 µL of N-O-bis-trimethylsilyl-trifluoroacetamide/1% trimethylchlorosilane was added, tubes were closed, and samples were incubated for 10 min at 18 to 28°C (room temperature). After centrifugation at 2,000 × g for 5 min, supernatants were transferred to autosampler vials. A 2-µL volume was injected (with a 1-µL air plug in front of the sample) into an 1177 split injector (containing a plug of glass wool) of a Varian CP-3900 type gas chromatograph equipped with an autosampler and a 50 m × 0.25 mm i.d. CPSil-5CB column (Varian, Middelburg, the Netherlands; 0.12-µm film thickness). The injector was operated at 280°C and the column gas (helium) flow rate was constantly 2 mL/min. The detector was operated at 300°C with a makeup gas (nitrogen) flow rate of 25 mL/min, flame gas (hydrogen) of 30 mL/min, and flame gas (air) of 300 mL/min. The temperature profile began initially at 184°C for 2 min, followed by an increase of 30°C/min to 200°C (7-min hold) and then an increase of 30°C/min to 240°C (17-min hold), and ending with an increase of 30°C/min to 290°C (1-min hold) to clean the column. Data handling was performed with Galaxy software (Varian) by using areas and the internal standard method.

Because of the detection limit, plasma concentrations of mannitol or d-xylose below 2 µM remained undetected. In those cases, this detection limit was the value applied for the plasma concentrations of mannitol (n = 5) or d-xylose (n = 21) in the statistical analysis.

**Determination of Plasma I-FABP Concentration**

Concentrations of I-FABP in EDTA-plasma samples were determined by using a commercial ELISA test kit (HyCult Biotechnology BV, Uden, the Netherlands). This ELISA test kit was developed to measure I-FABP concentrations in human plasma, but has been demonstrated to be suitable for measuring I-FABP concentrations in pig plasma (Niewold et al., 2004). Samples were analyzed in duplicate and concentrations are expressed as equivalents of human I-FABP. The variation between duplicates was <10% for more than 95% of the samples (variation of all duplicates was below 15%). The interassay CV was <10%. Preliminary analyses revealed that there was a strong “sow effect” on the plasma I-FABP concentration (P < 0.001). Therefore, plasma I-FABP concentrations of the sows (except for 1 slaughtered sow) were determined during the second half (approximately equal to d 70) of their subsequent pregnancy.

**Determination of Plasma Citrulline Concentration**

Plasma citrulline concentrations were analyzed by automated ion-exchange chromatography performed on a Jeol Amino-Tac (JLC-500/V, Jeol, Tokyo, Japan) with postcolumn ninhydrin derivatization. The detection range was from 3 to 1,000 µM, with a maximal inaccuracy of 14%. The plasma citrulline concentrations of 1 piglet in the UNW treatment showed distinctly different kinetics (greater values) compared with those of the other piglets. Data on plasma citrulline concentration for this piglet were considered to be outliers, and were therefore omitted from data analysis.

**Calculations and Statistics**

Postweaning growth check was defined as the reduction in ADG at d 2 postweaning compared with the ADG in the last week before weaning. The relative postweaning growth check (%) was calculated as 100[(ADGpreweaning wk – ADGd2 postweaning)/ADGpreweaning wk]. Although piglets of the UNW treatment were not weaned, the relative growth was calculated by using the same time periods as for the CW treatment.

Longitudinal measurements per animal cannot be considered as independent observations; thus, repeated measurement ANOVA (Littell et al., 1998) using PROC MIXED (SAS Inst. Inc., Cary, NC) was performed for piglet BW, piglet growth, and plasma variables. Treatment, day, and their interaction were included in the model as fixed factors; replicate and sow were included as random factors; and piglet was included as a repeated measurement, with an autoregressive covariance structure. For piglet BW, birth weight was included as a covariable, and for piglet growth and plasma variables, BW at d 21 of age (day of weaning for CW piglets) was included as a covariable. Plasma I-FABP concentrations were log-transformed to obtain homogeneity of variances. All values are presented as means ± SE. Effects were considered significant if P < 0.05, and a tendency if 0.05 ≤ P < 0.10; for post hoc testing, the Bonferroni correction was applied.

**RESULTS**

**Piglet Performance**

During the experiment, none of the piglets had to be treated for illness, and during postmortem pathologica examination of the CW piglets, none of the organs showed abnormalities. No differences in piglet BW or growth were observed between treatments at 20 d of age, before beginning the treatments (Figure 1 and Table 2). Weaning of the CW piglets resulted in markedly reduced growth compared with preweaning values,
and resulted in a slower growth rate compared with UNW piglets (overall $P < 0.001$; Figure 1). The relative postweaning growth check of CW piglets was 155 ± 9%, whereas the UNW piglets gained BW and had a relative increase in growth of 6 ± 9% in the same period. Consequently, CW piglets had less BW compared with UNW piglets from 2 d postweaning onward ($P < 0.001$; Table 2).

**Plasma Mannitol and d-Xylose Concentrations**

Plasma mannitol concentrations were different between the CW and UNW treatments (overall $P = 0.003$; Figure 2). Weaning with the CW treatment resulted in a transient decrease in plasma mannitol concentration 1 h after oral application, with decreased values observed at d 4 postweaning compared with preweaning values ($P < 0.001$; Figure 2). In contrast, no differences in mannitol absorption were observed between sampling days in the UNW treatment. The marked decrease at d 4 postweaning resulted in reduced mannitol concentrations in the CW treatment compared with the UNW treatment ($P = 0.01$). Overall, plasma mannitol concentrations on d 4 postweaning were correlated with the relative postweaning growth check ($r = -0.73$, $P = 0.005$); that is, piglets with a slower growth rate at d 2 postweaning had less mannitol absorption at d 4 postweaning. No correlations were observed overall on other sampling days, or within each separate treatment.

There was no difference in plasma d-xylose concentrations 1 h after oral application between the CW and UNW treatments (overall $P = 0.83$; Table 3). The plasma d-xylose concentration in the CW treatment was quite variable between sampling days and was greater on d 2 and 7 postweaning ($P = 0.013$ and $P = 0.05$) compared with the preweaning value. In the UNW treatment, d-xylose concentrations also varied considerably, but there was no difference between sampling days.

**Plasma I-FABP Concentration**

There was no difference in I-FABP plasma concentration between the CW and UNW treatments (overall $P = 0.63$; Figure 3). Moreover, mean I-FABP plasma concentrations were nearly similar at all sampling days in both treatments. Variation in plasma I-FABP concentration was high, resulting in large SE (Figure 3). When the data were examined in more detail, the wide variation was suspected to be caused by a sow effect; plasma I-FABP concentrations of the UNW and CW piglets from the same litter were similar (data not shown). Indeed, there was a strong correlation between the I-FABP concentration of sows and the average I-FABP concentration of their offspring on all treatment days ($r > 0.87$, $P < 0.003$).

**Plasma Citrulline Concentration**

Plasma citrulline concentrations differed between the CW and UNW treatments (overall $P = 0.0001$; Figure 4). Weaning of the CW litters reduced citrulline concentrations and resulted in reduced citrulline concentrations on d 4 and 7 after weaning ($P < 0.001$ and $P = 0.001$, respectively) compared with preweaning values. Overall, the relative postweaning growth check was correlated with plasma citrulline values on all postweaning sampling days ($r < -0.50$, $P < 0.04$), with the greatest correlation on d 4 postweaning ($r = -0.69$, $P = 0.002$).

**Table 2.** Body weight of piglets per treatment (means ± SE)

<table>
<thead>
<tr>
<th>Day after weaning</th>
<th>UNW$^1$</th>
<th>CW$^2$</th>
<th>$P$-value$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>6.9 ± 0.3$^a$</td>
<td>6.7 ± 0.4$^ab$</td>
<td>NS</td>
</tr>
<tr>
<td>0.5</td>
<td>7.4 ± 0.3$^b$</td>
<td>6.7 ± 0.4$^ab$</td>
<td>0.077</td>
</tr>
<tr>
<td>2</td>
<td>7.9 ± 0.3$^b$</td>
<td>6.5 ± 0.3$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>8.7 ± 0.4$^c$</td>
<td>6.9 ± 0.4$^ab$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>9.7 ± 0.4$^c$</td>
<td>7.3 ± 0.5$^b$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$–$^d$Different letters within a column indicate differences between means of sampling days within a treatment.
$^1$UNW = unweaned.
$^2$CW = conventionally weaned.
$^3$The $P$-value indicates differences between treatments per sampling day.
Figure 5). No correlation between relative postweaning growth check and citrulline concentration was observed within each separate treatment. Plasma citrulline concentrations correlated with plasma mannitol concentrations at d 4 postweaning in the CW treatment \( (r = 0.89, P = 0.008) \) and overall \( (r = 0.76, P = 0.001; \) Figure 6). On the other sampling days, no correlation was observed (overall or per separate treatment) between plasma citrulline and mannitol concentrations.

**DISCUSSION**

Commonly used variables of intestinal function or enterocyte mass in pigs, such as morphology (Hampson, 1986; Pluske et al., 1996b; Hedemann et al., 2003), absorption (Hampson and Smith, 1986; Nabuurs et al., 1996), or permeability (Spreeuwenberg et al., 2001; Verdonk et al., 2007) are often labor intensive, require the animals to be sacrificed, or both. The objective of the current study was to investigate whether plasma citrulline and I-FABP concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. Results of the current study indicate that plasma citrulline concentrations, but not I-FABP concentrations, seem to be a possible marker for monitoring intestinal function in postweaning pigs.

Although psychological stressors are assumed to make a contribution to weaning-associated intestinal dysfunction (Moesser et al., 2007), weaning-associated anorexia plays the major part in postweaning shortening of villi (Pluske et al., 1996a; van Beers-Schreurs et al., 1998). The severe reduction in piglet growth, together with the decreased mannitol absorption at d 4 postweaning observed in the current study seems to be indirect evidence for the occurrence of weaning-associated villous atrophy. However, in contrast with experimentally induced intestinal ischemia in weaned pigs of 20 to 25 kg (Niewold et al., 2004), no elevated concentrations of plasma I-FABP were observed after weaning of piglets in the current study. It should be noted, however, that the piglets used in the current experiment originated from only 1 farm, with no history of major weaning-associated problems. Under experimentally induced ischemic conditions, a 90% flow reduction through the superior mesenteric artery results in a rapid increase in plasma I-FABP concentrations within 30 min after onset of occlusion (Niewold et al., 2004). Villous atrophy, however, is greatest at 2 to 3 d postweaning (Spreeuwenberg et al., 2001; Hedemann et al., 2003); therefore, changes in I-FABP concentrations might have different kinetics than morphological changes. The timing of blood samples may not have captured the change in I-FABP, if any. Another possible explanation for the lack of increased I-FABP concentrations might be the type of induced tissue injury, because the mechanisms

**Table 3.** Plasma d-xylose concentrations (\( \mu M \)) 1 h after oral administration per treatment (means ± SE)

<table>
<thead>
<tr>
<th>Day postweaning</th>
<th>UNW(^1)</th>
<th>CW(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE (n)</td>
</tr>
<tr>
<td>−1</td>
<td>35.6</td>
<td>7.8 (10)</td>
</tr>
<tr>
<td>0.5</td>
<td>23.4</td>
<td>6.4 (9)</td>
</tr>
<tr>
<td>2</td>
<td>40.6</td>
<td>8.2 (10)</td>
</tr>
<tr>
<td>4</td>
<td>37.9</td>
<td>5.6 (9)</td>
</tr>
<tr>
<td>7</td>
<td>32.3</td>
<td>2.3 (9)</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Different letters within a column indicate differences between means of sampling days within a treatment.

\(^{1}\)UNW = unweaned.

\(^{2}\)CW = conventionally weaned.
causing the intestinal tissue injury during severe ischemia and after weaning might be different. Unlike with ischemia, weaning-associated villous atrophy is believed to be caused primarily by a reduced cell division at the base of the villi, while the shedding at the extrusion zone proceeds, ultimately resulting in shortening of the villi.

The observed I-FABP concentrations showed great variation between piglets. Apart from a few extreme values, preweaning I-FABP concentrations in the current study correspond to previously reported values in pigs (Niewold et al., 2004). Niewold et al. (2004) also reported considerable variation in baseline concentrations of I-FABP, and they suspected that this was caused by an unknown cross-reacting factor in the plasma not interfering with changes in I-FABP concentrations in time. Interestingly, results of the present study indicate that the variation in I-FABP concentrations is a “litter effect” because the mean I-FABP concentrations of CW and UNW littermates were highly correlated with the I-FABP concentration in the plasma of the mother during her subsequent pregnancy.

Plasma citrulline concentrations were affected by treatment in the current study, resulting in decreased citrulline concentrations after weaning. This seems to be in line with the reduced citrulline concentrations observed in patients with villous atrophy-associated small bowel diseases (Crenn et al., 2003). However, in the current study, the reduction in citrulline concentrations appeared to be induced rather quickly, because plasma citrulline concentrations already tended to be decreased at 12 h postweaning compared with preweaning concentrations. The absence of nutrients in the intestinal lumen has been demonstrated to induce villous atrophy in pigs parenterally fed for 24 h (Niinikoski et al., 2004). A 12-h fast, however, was not associated
with any detrimental effects on villous morphology in
the small intestines of pigs (Hartke et al., 2005). There-
fore, one might question whether the observed rapid
decrease in the current study is really caused by villous
atrophy, reduced intestinal function, or both. Previous
research on isolated pig enterocytes has demonstrated
that the availability of precursors (glutamine) in the
medium influences the production rate of citrulline by
the enterocytes (Wu et al., 1994b). Therefore, the rap-
id decrease in citrulline concentrations observed 12 h
postweaning in the current study might be a reflection
of the nutritional state rather than of intestinal func-
tion (or enterocyte mass).

Plasma citrulline concentrations were furthered re-
duced at d 4 and 7 postweaning, when piglet growth
was restored to preweaning concentrations, indicating
that the amount of nutrient intake was greater than
maintenance requirements. In addition, mannitol ab-
sorption was also decreased at d 4 postweaning and was
correlated with the observed plasma citrulline concen-
trations on that day (with an overall r = 0.76 and P
= 0.001). Previous studies on the correlation between
sugar absorption and citrulline concentration are con-
flicting. In a study by Jianfeng et al. (2005), citrulline
concentrations were correlated with 5-h d-xylose recov-
ery in the urine of patients with short bowel syndrome.
In contrast, no correlations were observed between
plasma citrulline concentrations and several sugar ab-
sorption or permeability tests in patients with cancer
treatment-induced gut toxicity (Lutgens et al., 2005).
The lack of correlation in the latter study was supposed-
edly due to a different time course of the markers (i.e.,
with citrulline concentrations being more sensitive and
specific for measuring small bowel epithelial cell loss).
The proposed greater sensitivity of plasma citrulline
for alterations in gut (dys)function might provide an
explanation for the lack of correlation between plasma
citrulline and mannitol on the other sampling days in
the current experiment.

An impressive amount of work has been done by Wu
and coauthors to elucidate intestinal AA metabolism
in porcine enterocytes (Wu et al., 1994a,b; Wu, 1997,
1998). An interesting finding is the fact that enter-
cytes isolated from 23-d-old weaned piglets (weaned at
d 21) showed a 14-fold increase in citrulline production
(from glutamine) compared with enterocytes isolated
from age-matched suckled piglets (Dugan et al., 1995).
This increase seemed to be independent of diet, be-
cause it was also observed in 23-d-old weaned piglets
fasted for 2 d (Dugan et al., 1995) and might have been
induced by elevated plasma cortisol concentrations
associated with weaning (Flynn and Wu, 1997b; Flynn
et al., 1999; Wu et al., 2000). The decreased plasma
citrulline concentrations after weaning observed in the
current study seem to conflict with these reports. How-
ever, it should be noted that the outcomes stem from
different studies obtained under completely different
experimental conditions. In the in vitro model, citru-
lline production is determined by using a fixed amount
of enterocytes, whereas the in vivo plasma citrulline
concentrations observed in the current study were also
determined by total enterocyte mass, which was as-
sumed to decrease after weaning. Therefore, the citru-
lline concentrations measured postweaning in an in vivo
model are a result of the increased citrulline production
potential per intestinal enterocytes, on the one hand,
and a reduced enterocyte mass, on the other hand. In
addition, in an in vivo model, plasma citrulline concen-
trations are influenced by the cellular uptake of the
citrulline produced.

Plasma citrulline concentrations of the UNW piglets
in the current study correspond rather well to the con-
centrations of 29-d-old suckling piglets (122 ± 25 µM;
\( n = 7; \text{jugular vein sample}) as reported previously by
Flynn and Wu (1997a). The somewhat reduced plasma
citricline concentrations (87 ± 6 µM) of 28-d-old UNW piglets
in the current study might be caused by the fact that
these piglets were fasted for 3 h before the blood sample
was obtained, which might also account for the smaller
variation in the current study compared with the study
by Flynn and Wu (1997a; 7 vs. 21%). Previous research
indicated that the plasma citrulline concentration in
the jejunal artery of 29-d-old weaned piglets (weaned at
d 21) decreased to 43.0 ± 4.8 µM, but was not different
from the concentration at weaning (50.6 ± 6.9 µM; Wu
et al., 1994a). However, this was a cross-sectional study

![Figure 5. Relation between relative postweaning growth check (%) and plasma citrulline concentration at d 4 postweaning for conventionally weaned (●, CW) and unweaned (○, UNW) piglets. Overall r- and P-
values are given. Piglets with a more reduced growth have a greater relative postweaning growth check. A negative postweaning growth check indicates that pig-
lets were gaining BW in the period after weaning the CW piglets.](image-url)
using cannulated piglets under complete anesthesia, which complicates a comparison with the values of the current study. To our knowledge, the present study is the first to present longitudinal data on plasma citrulline concentrations of piglets shortly after weaning.

Based on the results of the current study, plasma citrulline concentration seems to be a possible marker for the monitoring of intestinal function in pigs after weaning. It may be applied by biomedical researchers using pig models to study the processes involved in

**Figure 6.** Plasma citrulline concentration (µM) in relation to plasma mannitol concentration (µM) per sampling day for conventionally weaned (●, CW) and unweaned (○, UNW) piglets. The number of observations for plasma mannitol concentrations is similar to that for plasma d-xylose concentrations (Table 3). Overall r- and P-values are given per sampling day.
various human intestinal diseases (Burrin et al., 2003; Manzano et al., 2007; Pereira-Fantini et al., 2008). Moreover, it might offer the opportunity to investigate intestinal function in pigs under practical conditions, which was the rationale for the current study. In view of a possible practical use in the future, blood samples were obtained from a jugular vein in the current study. However, more insight is still needed in the relation between plasma citrulline concentration in jugular vein blood and a sampling location that is closer to the site of citrulline production, such as the portal vein. Further investigations should also focus on the relation between plasma citrulline concentration and macroscopic and morphologic small intestinal characteristics in pigs postweaning, which was beyond the scope of the current experiment.

LITERATURE CITED


