EFFECTS OF DIETARY AMINES ON SMALL INTESTINAL VARIABLES
IN NEONATAL PIGS FED SOY PROTEIN ISOLATE¹

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ABSTRACT

Six litters of newborn crossbred piglets were utilized to examine 1) the effects of substituting 20% of the protein of an all-milk protein liquid diet with a soy protein isolate (milk-soy diet) on small intestinal variables and 2) the effects of supplementing this milk-soy diet with 25 g of either putrescine or ethylamine per kilogram diet on small intestinal variables. Small intestinal xylose absorption tended to increase from wk 1 to wk 2 of age in pigs fed the milk, putrescine and ethylamine diets, but not in pigs fed the milk, putrescine and ethylamine diets, but not in pigs fed the unsupplemented milk-soy diet. Crypt depth in pigs fed the milk-soy diet tended to be less (9.4%; P > .10) than the crypt depth in pigs fed the other diets, but mitotic index was not different (P > .10) among diets. Mucosal protein, DNA and RNA concentrations and mucosal brush border sucrase and cytosolic dipeptidase activities tended to be least in pigs fed the putrescine and ethylamine diets. Concentration of mucosal putrescine was greatest (P < .002) in the distal regions of the small intestine of pigs fed putrescine. Mucosal ornithine decarboxylase activity was inhibited by putrescine (P < .02), but it was not affected by the soybean protein isolate used in this study. Supplementing soy protein isolate diets with amines may enhance intestinal absorption and enterocyte proliferation.

Introduction

Polyamines are necessary for small intestinal mucosal growth and development, maturation, adaptation and recovery from injury (Luk et al., 1980; Luk and Baylin, 1982, 1983; Yang et al., 1984). Intraluminal infusions of the polyamine putrescine and of the amine ethylamine and feeding dimethylamine to rats have all stimulated small intestinal mucosal growth (Dembinski et al., 1984; Seidel et al., 1985). Results from our laboratory have suggested that the inhibition of polyamine biosynthesis may be responsible for reducing small intestinal mucosal growth in young calves fed soybean protein (Grant et al., 1989). Furthermore, feeding putrescine or ethylamine improved mucosal growth and intestinal nutrient absorption in calves. Supplementing piglet diets containing soybean protein with amines may enhance mucosal growth and development and thus prevent reductions in weight gain that can occur when neonatal pigs are fed unsupplemented soybean proteins (Jones et al., 1977; Wilson and Leibholz, 1981).

The objectives of the present study were to examine 1) the effects of substituting 20% of the protein of an all-milk protein liquid diet with a soy protein isolate on small intestinal nutrient absorption, mucosal morphology, cytology, digestive enzyme concentration and polyamine
biosynthesis and 2) the effects of ethylamine or putrescine supplementation of a soy protein isolate on these intestinal variables.

Materials and Methods

Animals and Diets. Six litters of crossbred (sire was Hampshire or Duroc and sow was Yorkshire-Landrace cross) piglets were obtained from the Michigan State University swine farm. Eight piglets per litter were taken from the sow at 2 days of age, randomly paired (ignoring sex) and housed as pairs in elevated, mesh wire-bottomed, stainless steel pens. Piglets were weighed when taken from the sow and daily thereafter. Pairs were assigned randomly to one of four dietary treatments. Piglets in all treatments received a liquid diet containing 15% DM. Dry diets were reconstituted with tap water just before feeding. Treatment 1 was an all-milk-protein, milk replacer diet (milk diet). Treatment 2 was Diet 1 with 20% of the protein replaced with a soy protein isolate (milk-soy diet). Treatment 3 was Diet 2 supplemented with putrescine dihydrochloride at 25 g of the salt (or .31 mol)/kg dry diet (putrescine diet). Treatment 4 was Diet 2 supplemented with ethylamine hydrochloride at 25 g of the salt (or .31 mol)/kg dry diet (ethylamine diet). Analytical composition of Diet 1 is given in Table 1. Amount of diet fed daily was equal to 30 g DM/kg BW on day 3 of the experimental period and was increased to 50 g/kg BW by day 5 for the remainder of the experimental period. Piglets were fed four times daily (at 6-h intervals) via stomach tubes and were weighed daily to adjust amount fed.

Xylose Absorption Tests. On day 5 of age, small intestinal nutrient absorption was quantitated by xylose absorptive tests (Merritt and Reed, 1980) in all piglets. A 10% xylose solution was administered via a stomach tube (.5 g xylose/kg BW) after a 12-h fast. Three milliliters of blood were collected from the vena cava in heparinized tubes at 0, 2 and 3 h after xylose administration. Plasma xylose concentration was determined by the orcinol method (Bolton et al., 1976).

Excision of Intestinal Segments. On day 7 of age, one-half of the piglets (one piglet selected randomly from each pair) were anesthetized by i.m. administration of 4.4 mg of azaperone/kg BW and 4.4 mg of ketamine/kg BW 6 h after feeding. The abdominal cavity was opened and three segments of the small intestine (each approximately 4 cm in length) were excised. The duodenal sample (Site 1) was obtained from the first 4 cm of the small intestine immediately distal to the pylorus, the jejunal sample (Site 2) was obtained 60 cm proximal to the ileocecal junction, and the ileal sample (Site 3) was obtained from the last 4 cm of the small intestine immediately proximal to the ileocecal junction. Piglets were killed by i.v. administration of T-61 Euthanasia Solution after all intestinal segments were excised.

The remaining piglets were given xylose absorption tests on day 12 of age, anesthetized on day 14 of age for collection of tissue and subsequently euthanatized.

Tissue Processing. Immediately after excising intestinal segments from piglets, segments were flushed with ice-cold physiological saline solution (9% NaCl) and blotted with a paper towel. One-third of the segment was placed in 10% phosphate buffered formalin and later used for cytological measurements. Later, a portion of this specimen was embedded in paraffin and at least four sections 5 μm thick were mounted on a slide and stained with eosin and hematoxylin. The sections were sliced along the length of the villi to form longitudinal sections of the villus-crypt.

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*Prepared by Milk Specialties Co., Dundee, IL.
1From Sigma Chemical Co., St. Louis, MO.
2From Aldrich Chemical Co., Milwaukee, WI.
3Stresnil; Pitman-Moore, Inc., Washington Crossing, NJ.
4Vetlar; Parke-Davis, Morris Plains, NJ.
5Hoechst, Somerville, NJ.

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**TABLE 1. MILK PROTEIN DIET ANALYSIS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>17.0</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>15.5</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>.2</td>
</tr>
<tr>
<td>Vitamin A, IU/kg</td>
<td>11,000</td>
</tr>
<tr>
<td>Vitamin D₃, IU/kg</td>
<td>2,750</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>22</td>
</tr>
<tr>
<td>Oxytetracycline, g/1,000 kg</td>
<td>110</td>
</tr>
<tr>
<td>Neomycin base, g/1,000 kg</td>
<td>77</td>
</tr>
</tbody>
</table>

*The milk protein diet, Chore-Time Prenursery Baby Pig Feed prepared by Milk Specialties Co., Dundee, IL, is composed of dried skim milk, animal fat, dried whey, dried milk protein, polyoxyethylene glycol (400) mono- and di-esters, dried corn syrup, minerals, vitamins and antibiotics. The milk-soy protein diet is the milk protein diet with 20% of the protein replaced by 20% soy protein isolate and also was prepared by Milk Specialties Co., Dundee, IL.*
regions. Sections were examined under a phase-contrast microscope at 250× magnification to measure the mitotic index (Hooper, 1961). Mitotic index, a measurement of intestinal epithelium proliferation, was calculated as the percentage of crypt enterocytes in mitosis. At least 500 crypt enterocytes were counted in regions where individual crypts and villi were continuous.

The remaining half of the formalin-fixed tissue was stained with New Methylene Blue and examined under a dissecting microscope equipped with an ocular micrometer at 30× magnification to measure villus height and crypt depth. Ten villi and 10 crypts were measured from each section and mean villus heights and crypt depths were calculated.

The mucosa was scraped from the remainder of the fresh intestinal segment with a glass slide and divided into three portions. One portion of fresh mucosal scrapings was homogenized in ice-cold 50 mM sodium phosphate buffer and differentially centrifuged to obtain supernatant fluids for determination of brush border sucrase activity (Dahlqvist, 1964; Messer and Dahlqvist, 1966), cytosolic dipeptidase activity (Nicholson and Kim, 1975), ornithine decarboxylase activity (ODC; Slotkin and Bartolome, 1983) and protein concentration (Bradford, 1976). Bovine serum albumin was used as a protein standard.

The second portion of fresh mucosal scrapings was placed into 10% trichloroacetic acid (TCA), homogenized and centrifuged. The TCA was removed from the supernatant fluid with diethyl ether and the aqueous supernatant was used for the determination of polyamines (putrescine, spermidine and spermine) by HPLC (N. D. Brown, personal communication).

The third portion of fresh mucosal scrapings was used for the determination of DNA and RNA concentrations (Munro and Fleck, 1966) and protein concentration (Bradford, 1976).

Statistical Analyses. Analysis of variance was conducted as outlined by Gill (1978, 1986) using least square means generated by the GLM procedure of SAS (1982). Bonferroni t-statistics (Gill, 1978) were used to test three designed contrasts among diet least square means to determine effects of soy protein and amines on small intestinal variables: milk vs milk-soy, milk vs amine diets and milk-soy vs amine diets. Bonferroni t-statistics also were used to test day and site effects within treatments. A posteriori contrasts (milk vs other three diets; putrescine vs other three diets) were performed using Scheffe's test (Gill, 1978, 1986). The error term pig (diet × day) was used to test the main effects of diets, days and the interaction of diet and day. The error term pig (diet × day × site) was used to test the main effect of sites and the interactions diet × site, day × site and diet × day × site.

Results and Discussion

General Observations. Two piglets (one fed putrescine and one fed ethylamine) had diarrhea during the experiment and, therefore, were deleted from statistical analyses. Two piglets (one fed the milk diet and one fed the ethylamine diet) died from diarrhea during the 1st wk of the experiment, but they were replaced 4 d later with two piglets from another litter and established protocol procedures were followed. All other piglets (44) were in excellent health throughout the experimental period. Daily gain for all piglets averaged 40.5 g and was not different among diets (P > .10). Schneider and Sarrett (1969) demonstrated that soy protein isolates had 83% of the growth-promoting activity of milk protein when fed to newborn pigs. Pigs in Schneider and Sarrett's study that were fed soy protein isolates as the sole source of protein gained 42% more during the first 2 wk of life than pigs in our study. Because the protein in the soy diets used in the present experiment was only 20% soy isolate, the difference in weight gain between pigs fed the soy diet and the milk diet was expected to be less than if soy protein had been the sole source of protein in the soy diet.

Absorption Measurements. Xylose absorption tests were conducted to evaluate small intestinal nutrient absorption ability. Xylose absorption quadratic regression curves are shown in Figure 1. Maximum plasma xylose concentrations, times of maximum concentrations and slopes (B1) and curvatures (B2) for the regression equations were not different (P > .10) among diets. Maximum concentrations and slope tended to increase from d 5 to d 12 in piglets fed the milk, putrescine and ethylamine diets but did not change with the milk-soy diet. In calves (Grant et al., 1987, 1989), maximum xylose concentration and slope decreased as age increased for those fed a soy protein concentrate milk replacer but tended to increase in calves fed the milk diet or the soy diet supplemented with aminos. In piglets, the maximum plasma concentration and slope tended to increase with age for those fed the milk or the soy protein isolate diet supplemented with putrescine or ethylamine. However, piglets
fed the milk soy diet had constant values for the two variables at d 5 and 12.

We interpreted the calf data to indicate that soy protein prevented the increase in xylose absorption as neonates matured and that supplementing those diets with amines allowed this increase to occur. The piglet data confirm that interpretation only partially. In piglets the increase with age occurred with amine supplementation. However, piglets fed the unsupplemented soy protein did not decrease xylose absorption as age increased. An alternate interpretation is that piglets receiving soy protein at the early age had maximum xylose flux either by absorption or other processes. This interpretation is not consistent with the data from calves.

**Mucosal Morphology.** Villus height and crypt depth, both measures of mucosal morphology, were not different among diets (Table 2). No interactions among diet, days and sites were detected for villus height or crypt depth \((P > .10)\). Villi were longer \((P < .01)\) on d 7 than on d 14 of age (430 and 242 \(\mu m\), respectively) and height was different \((P < .01)\) among sites; villi of the duodenum were shorter \((P < .01)\) than villi of the jejunum and ileum (237, 399 and 372 \(\mu m\), respectively; data not shown). Moon (1971) observed that villus height decreased during the first few weeks of life in pigs. This author also reported that crypt depth increased with age, which may

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**Table 2. Villus Height, Crypt Depth and Mitotic Index in the Small Intestine of Neonatal Pigs Fed Four Diets**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Milk</th>
<th>Milk-soy</th>
<th>Putrescine</th>
<th>Ethylamine</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus length, (\mu m)</td>
<td>341</td>
<td>359</td>
<td>320</td>
<td>324</td>
<td>50</td>
</tr>
<tr>
<td>Crypt depth, (\mu m)</td>
<td>755</td>
<td>693</td>
<td>756</td>
<td>783</td>
<td>58</td>
</tr>
<tr>
<td>Mitotic index, %</td>
<td>2.8</td>
<td>2.8</td>
<td>2.7</td>
<td>2.6</td>
<td>.53</td>
</tr>
</tbody>
</table>

*Least square means, \(n = 12, 12, 11\) and 11 pigs for milk, milk-soy, putrescine and ethylamine diets, respectively.*
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TABLE 3. MUCOSAL PROTEIN, RNA AND DNA CONCENTRATIONS IN THE SMALL INTESTINE OF NEONATAL PIGS FED FOUR DIETS TO TWO AGESa

<table>
<thead>
<tr>
<th>Variable and day</th>
<th>Diet</th>
<th>Milk</th>
<th>Milk-soy</th>
<th>Putrescine</th>
<th>Ethylamine</th>
<th>Day mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 7</td>
<td></td>
<td>51.1</td>
<td>50.1</td>
<td>54.5</td>
<td>54.9</td>
<td>52.7</td>
</tr>
<tr>
<td>D 14c</td>
<td></td>
<td>57.0</td>
<td>52.9</td>
<td>49.9</td>
<td>44.7</td>
<td>49.4</td>
</tr>
<tr>
<td>Diet mean</td>
<td></td>
<td>54.0</td>
<td>51.5</td>
<td>48.7</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 7</td>
<td></td>
<td>5.19</td>
<td>4.73</td>
<td>4.76</td>
<td>4.99</td>
<td>4.92</td>
</tr>
<tr>
<td>D 14</td>
<td></td>
<td>5.39</td>
<td>5.36</td>
<td>4.51</td>
<td>4.51</td>
<td>4.94</td>
</tr>
<tr>
<td>Diet meand</td>
<td></td>
<td>5.29</td>
<td>5.05</td>
<td>4.63</td>
<td>4.75</td>
<td></td>
</tr>
<tr>
<td>RNAe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 7</td>
<td></td>
<td>5.26</td>
<td>5.55</td>
<td>5.10</td>
<td>5.45</td>
<td>5.34</td>
</tr>
<tr>
<td>D 14</td>
<td></td>
<td>5.31</td>
<td>5.14</td>
<td>4.46</td>
<td>4.70</td>
<td>4.90</td>
</tr>
<tr>
<td>Diet mean</td>
<td></td>
<td>5.28</td>
<td>5.34</td>
<td>4.78</td>
<td>5.08</td>
<td></td>
</tr>
</tbody>
</table>

aLeast squares means of three sites; the number of pigs are 12, 12, 11 and 11 for milk, milk-soy, putrescine and ethylamine diets, respectively. Pooled SE for diet and day means are, respectively, protein 2.1, 1.4; DNA, .25, .21; DNA, .25, .21; RNA, 27, .18.
bDiet × day interaction (P < .008); contrast of d 7 vs d 14 within putrescine diet (P < .05) and within ethylamine diet (P < .10).
cMilk diet different from other diets (P < .01). Contrast of milk-soy diet vs putrescine and ethylamine diets (P < .10).
dMilk diet vs putrescine and ethylamine diets (P < .10).
eContrast of d 7 vs d 14 overall means (P < .05).

indicate that the size of the proliferative compartment for intestinal epithelial cells increased with age. Crypt depth was least in the jejunum (P < .01) and tended to be less (9.4%; P > .10) in piglets fed the milk-soy diet than in those fed the milk, putrescine or ethylamine diets (Table 2), suggesting that soybean protein might reduce intestinal proliferation in piglets.

*Mucosal Cytology.* Mitotic index, a measure of small intestinal enterocyte proliferation, was not influenced by diet (Table 2), nor were there any interactions among diets, sites and days (P > .10). However, mitotic index was greater (P < .01) on d 7 of age (3.1%) than on d 14 (2.4%) and was greater in the jejunum than in the duodenum (P < .01) or the ileum (P < .10; data not shown). Thus, the rate of proliferation of intestinal epithelium was greater on d 7 of age than on d 14 and was greater in the jejunum than in the duodenum and ileum. However, this difference in mitotic index is inconsistent with the crypt depth measurements. Measuring mitotic index only estimates the fraction of cells proliferating, whereas crypt depth estimates the relative size of the mucosal proliferative compartment. An increase in crypt cell number would not be detected with measurements of mitotic indices; thus, mitotic index can remain constant with a concomitant increase in crypt depth. These observations demonstrate the need to include measurements of crypt cell number in future studies.

*Mucosal Metabolites.* A diet × day interaction (P < .008) existed for small intestinal mucosal protein concentration (Table 3). At 14 d of age, concentration of protein in the mucosa of pigs fed the milk diet was greater than that for the mean of the other three diets (P < .01). There was a tendency for protein concentration to be greater on d 14 than on d 7 in pigs fed the milk and milk-soy diets, but there was less protein on d 14 than on d 7 in pigs fed the putrescine and ethylamine diets (P < .05 and P < .10, respectively). Protein concentration differed among sites (P < .02) and was greater in Site 1 than in Site 2 (53 > 49
mu/mg: P < .05) and intermediate in Site 3 (52
mu/mg, data not shown).

Concentration of DNA in small intestinal mu-
cosa (Table 3) tended to be greater in pigs fed the
milk diet than in pigs fed the putrescine and
ethylamine diets (P < .10). Age had no effect
(P > .10) on mucosal DNA concentration, but
DNA tended to be greater on d 14 than on d 7
(5.38 > 4.96 µg/mg) in pigs fed the milk and
milk-soy diets and to be less on d 14 than on d 7
(4.51 < 4.88 µg/mg) in pigs fed the putrescine
and ethylamine diets. Mucosal DNA concentra-
tion (µg/mg) was greater (P < .01) in Site 3 than
in Sites 1 and 2 (5.5 > 4.7 or 4.6, respectively;
data not shown). The greater DNA concentration
in the more distal regions of the small intestine
agrees with results in calves (Grant et al., 1989)
and in rats (Dembinski et al., 1984).

Mucosal RNA concentration was not different
among diets, nor were there interactions among
diets, days and sites (P > .10). However, as with
DNA, RNA concentrations tended to be greater
for pigs fed the milk and milk-soy diets than for
pigs fed the putrescine and ethylamine diets (Table 3). Mucosal RNA concentration averaged
across all diets decreased (P < .05) from d 7 to d
14 of age. The decrease was by 14% for pigs fed
putrescine and ethylamine diets, but only by 7%
for pigs fed the milk-soy diet, compared with an
increase of 1% for those fed the milk diet. Mucos-
al RNA was also greater (P < .01) in Site 1 than
in Sites 2 or 3 (5.60 > 4.91 or 4.86 µg/mg, respec-
tively; data not shown), which is consistent with
data derived from rat studies (Dembinski et al.,
1984) in which RNA concentration was greater
in the more proximal regions of the small intest-

e. The greater RNA in the proximal intestinal
region (P < .01) may be associated with greater
protein concentration (P < .05) and total sucrase
activity (P < .02), whereas the greater DNA in the
distal intestinal region (P < .01) may be associ-
ated with greater proliferative capacity. This and
further interpretations of this type data are highly
speculative.

Concentrations of polyamines in small intesti-

tinal mucosa are presented in Table 4. Dietary
putrescine increased mucosal concentration of
putrescine by a factor of 10.4 averaged across all
sites. Mucosal putrescine concentration was
greater in Site 2 and 3 of pigs fed the putrescine
diet than of pigs fed the other three diets averaged
over all sites and within each site (P < .002).
Greater concentrations of putrescine in the jeju-
nal and ileal than in the duodenal mucosa suggest
that the distal regions of the small intestine absorb
more putrescine from the luminal contents than
from the proximal regions. Kumagai and Johnson
(1987) demonstrated that uptake of putrescine by

<table>
<thead>
<tr>
<th>Variable and siteb</th>
<th>Milk</th>
<th>Milk-soy</th>
<th>Putrescine</th>
<th>Ethylamine</th>
<th>Site mean</th>
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<tbody>
<tr>
<td>Site 1</td>
<td>97</td>
<td>33</td>
<td>196</td>
<td>60</td>
<td>97</td>
</tr>
<tr>
<td>Site 2</td>
<td>71</td>
<td>32</td>
<td>760</td>
<td>34</td>
<td>224</td>
</tr>
<tr>
<td>Site 3</td>
<td>32</td>
<td>85</td>
<td>715</td>
<td>40</td>
<td>218</td>
</tr>
<tr>
<td>Diet mean</td>
<td>67</td>
<td>50</td>
<td>557</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Putrescinec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet means</td>
<td>128</td>
<td>92</td>
<td>109</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Spermidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Diet means</td>
<td>304</td>
<td>186</td>
<td>209</td>
<td>340</td>
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</table>

*Least square means of d 7 and 14; the number of pigs are 12, 12, 11 and 11 for milk, milk-soy, putrescine and ethylamine diets, respectively. Pooled SE of diet and site means for putrescine are 49 and 37, respectively. Pooled SE of diet means for spermidine and spermine are 21 and 55, respectively.

bSite 1 = duodenum, Site 2 = jejunum and Site 3 = ileum.

cDiet x site interaction (P < .0001); contrast of putrescine diet vs the other three diets within Site 2 and 3 and overall (P < .002).
### TABLE 5. SPECIFIC AND TOTAL ACTIVITIES OF MUCOSAL ORNITHINE DECARBOXYLASE (ODC), SUCRASE AND DIPEPTIDASE IN THREE SITES OF THE SMALL INTESTINE OF NEONATAL PIGS FED FOUR DIETS

<table>
<thead>
<tr>
<th>Variable and site</th>
<th>Diet</th>
<th>Site mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>34.8</td>
<td>31.1</td>
</tr>
<tr>
<td>Site 2</td>
<td>22.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Site 3</td>
<td>34.0</td>
<td>29.0</td>
</tr>
<tr>
<td>Diet mean</td>
<td>30.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Total activity of ODC</td>
<td>Site 1</td>
<td>1.33</td>
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<tr>
<td>Site 2</td>
<td>.96</td>
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</tr>
<tr>
<td>Site 3</td>
<td>1.36</td>
<td>1.29</td>
</tr>
<tr>
<td>Diet mean</td>
<td>1.22</td>
<td>1.25</td>
</tr>
<tr>
<td>Specific activity of sucrase</td>
<td>Site 1</td>
<td>541</td>
</tr>
<tr>
<td>Site 2</td>
<td>398</td>
<td>411</td>
</tr>
<tr>
<td>Site 3</td>
<td>426</td>
<td>387</td>
</tr>
<tr>
<td>Diet mean</td>
<td>455</td>
<td>412</td>
</tr>
<tr>
<td>Total activity of sucrase</td>
<td>Site 1</td>
<td>25.6</td>
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</tr>
<tr>
<td>Site 3</td>
<td>18.5</td>
<td>18.3</td>
</tr>
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<td>Diet mean</td>
<td>20.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Specific activity of dipeptidase</td>
<td>Site 1</td>
<td>557</td>
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<tr>
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<td>973</td>
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<td>Site 3</td>
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<td>695</td>
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<tr>
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<td>718</td>
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<tr>
<td>Total activity of dipeptidase</td>
<td>Site 1</td>
<td>26.3</td>
</tr>
<tr>
<td>Site 2</td>
<td>40.6</td>
<td>41.6</td>
</tr>
<tr>
<td>Site 3</td>
<td>34.2</td>
<td>32.1</td>
</tr>
<tr>
<td>Diet mean</td>
<td>33.7</td>
<td>31.9</td>
</tr>
</tbody>
</table>

*Least squares means of d 7 and 14; the number of pigs are 12, 12, 11 and 11 for milk, milk-soy, putrescine and ethylamine diets, respectively. Pooled SE of diet and site means are, respectively, specific activity of ODC, 5.5, 4.1; total activity of ODC, .19, .14; specific activity of sucrase, 57, 25; total activity of sucrase, 2.3, 1.0; specific activity of dipeptidase, 63, 27; total activity of dipeptidase, 2.7, 1.2.

Site 1 = duodenum; Site 2 = jejunum; Site 3 = ileum.

Picomoles CO₂ released h⁻¹·mg protein⁻¹; contrast of putrescine vs the other three diets within each site (P < .05) and overall (P < .01).

Picomoles CO₂ released h⁻¹·mg mucosa⁻¹; diet × site interaction (P < .02); contrast of putrescine diet vs other three diets within each site (P < .05) and overall (P < .01).

Nanomoles sucrose hydrolyzed h⁻¹·mg protein⁻¹; contrast of milk diet vs the other three diets within Site 1 (P < .05).

Nanomoles sucrose hydrolyzed h⁻¹·mg mucosa⁻¹; contrast of milk diet vs the other three diets within Site 1 (P < .05); overall Site 1 vs 3 (P < .05) and Site 1 vs 2 (P < .01).

Nanomoles phenylalanine hydrolyzed min⁻¹·mg protein⁻¹; contrast of milk diet vs the other three diets within Site 2 (P < .05) and overall (P < .05); contrast of milk diet vs putrescine and ethylamine diet (P < .10); overall site means differ (P < .01).

Nanomoles phenylalanine hydrolyzed min⁻¹·mg mucosa⁻¹; contrast of milk diet vs putrescine and ethylamine diet (P < .10); overall site means differ (P < .01).
rat small intestinal enterocytes also was more extensive in the distal than in other regions of the small intestine. Age had no effect on putrescine, spermine or spermidine concentrations ($P > .10$). Mucosal spermidine concentration was 30% greater ($P > .10$) in pigs fed the milk diet than in pigs fed the milk-soy, putrescine and ethylamine diets. Spermidine also was greater ($P > .10$) on d 14 than on d 7 (117 vs 95 pmol/mg mucosa; data not shown) for all diets, but age changes varied among diets. Spermine concentration was not different among diets or intestinal sites.

**Mucosal Enzymes**. Specific and total activities of mucosal ODC (Table 5) were least in pigs fed the putrescine diet ($P < .01$). At d 7, specific and total activity of ODC was greater ($P < .01$) in Site 1 than in Site 2 or 3, but at d 14 these differences were less ($P > .10$; data not shown). Overall, both total and specific activities of Site 1 exceeded those of Site 2 ($P < .01$). However, a diet $\times$ site interaction ($P < .02$) existed for total ODC activity. The low specific and total ODC activities in pigs fed putrescine indicate that the added dietary putrescine inhibited the activity of mucosal ODC. Dietary putrescine also reduced proximal jejunum mucosal ODC activity in young calves (Grant et al., 1989). These results contradict those that demonstrate that ileally infused putrescine and ethylamine (at a rate equal to 1 pmol/h for 66 h) stimulated ODC activity in rats (Seidel et al., 1985). These conflicting results may be due to the different routes of administration and/or different amounts of amines administered. Pegg et al. (1978) were able to inhibit rat liver ODC by i.p. administration of high concentrations of putrescine, which indicated that in some systems administration of putrescine results in feedback inhibition of ODC. This soybean protein isolate did not reduce activity of ODC, as was suggested in calves fed a soy protein concentrate (Grant et al., 1989). This inconsistency may be due to species differences or to differences in the soybean proteins used in the diets. Nevertheless, soy protein isolate apparently does not contain an inhibitor to piglet intestinal ODC when fed as in this experiment.

Specific and total activities of intestinal brush border sucrase in Site 1 (Table 5) were greater in pigs fed the milk diet than the average for pigs fed the milk-soy, putrescine or ethylamine diets ($P < .05$). This was not so evident in Sites 2 and 3. Total activity was greater in Site 1 than in Site 2 ($P < .01$) and Site 3 ($P < .02$) for all diets.

Specific activities of mucosal cytosolic dipeptidase averaged over all sites and for Site 2 (Table 5) were greater in the average of pigs fed the milk, milk-soy and ethylamine diets than in pigs fed the putrescine diet ($P < .05$). Total activity was greater in pigs fed the milk diet than in pigs fed the amine diets ($P < .10$). Activities were greatest in Site 2 and least in Site 1 ($P < .01$), regardless of diet.

If putrescine or ethylamine increased crypt cell production rate, as suggested by slightly greater crypt depth, and because villus height was unchanged, then villus cells would have less time to mature before being sloughed off. This would result in an immature villus cell population and subsequently lead to less synthesis and accumulation of sucrase and peptidase. This also might explain the decreased concentrations of protein and RNA of pigs fed the putrescine and ethylamine diets because immature cells would contain less RNA and protein.

**Implications**

Including soybean protein isolate in liquid diets for neonatal pigs may reduce small intestinal absorption and proliferation of small intestinal enterocytes. Soy protein isolate apparently contains little or no inhibitory activity toward intestinal ornithine decarboxylase. Neonatal diets formulated with soy proteins may be improved by inclusion of various amines.

**Literature Cited**


SOY PROTEIN AND AMINES IN PIGLETS


