Effects of cecal oxytetracycline infusion, and dietary avidin and biotin supplementation on the biotin status of nongravid gilts

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ABSTRACT: The objective of this 49-d experiment was to test effects of cecal oxytetracycline (OTC) infusion, and dietary avidin and biotin supplementation on the biotin status of nongravid gilts. Twenty-eight cross-bred gilts with an initial age of 160 d and BW of 120 kg were surgically fitted with a T-cannula in the terminal ileum, a cecal fistula, and an indwelling catheter in the anterior vena cava, and allotted to 7 dietary treatments. Treatments with the basal semipurified (SP) diet fed at 1.86 kg/d were: SP-1, negative control; SP-2, positive control with 270 μg of biotin/kg; SP-3, with spray-dried egg albumen (EA, 100 g/d) and OTC (2.56 g/d by cecal infusion); and SP-4, with EA, OTC, and 700 μg of biotin/kg. Treatments with the basal corn-soybean meal (CS) diet fed at 1.80 kg/d were: CS-1, negative control; CS-2, with EA and OTC; and CS-3, with EA, OTC, and 700 μg of biotin/kg. Response criteria were: fecal bacteria counts; plasma concentrations of biotin, glucose, and urea N (PUN); liver pyruvate carboxylase (PC) activity; kidney and epithelial tissue histology; ileal and fecal biotin concentrations; ileal and total tract N and energy utilization; daily gilt observation; and BW gain. Blood samples were collected every 7 d with serial samples collected on d 49. Total urine collections and fecal grab samples were made twice daily from d 44 to 49. Gilts were killed on d 50 and liver, kidney, and skin samples were collected. No gilts had symptoms of biotin deficiency. There were no treatment differences in BW gain, plasma glucose concentrations, liver PC activity, kidney and epithelial tissue histology, or fecal bacteria counts. Ileal and total tract N and energy digestibilities (%) did not differ among treatments within the same protein source, with greater (P ≤ 0.05) values for gilts on the SP treatments than the CS treatments. However, N retained/N absorbed and N retained/N intake (%) were less (P ≤ 0.05) and PUN concentrations were greater (P ≤ 0.05) for SP treatments with cecal OTC infusion. The overall fecal biotin concentration mean was 2.6-fold greater than the overall ileal biotin concentration mean. In conclusion, no gilts in the current experiment became biotin deficient because the biotin requirements were met primarily by microbial synthesis and absorption of biotin from the distal small intestine and large intestine, with corn and soybean meal contributing endogenous biotin. Therefore, supplementation of diets for gilts entering the breeding herd with 100% of the current NRC biotin requirement for sows is adequate.

Key words: biotin status, cecal oxytetracycline, dietary avidin, ileal digestibility, swine, total tract digestibility

INTRODUCTION

Biotin functions in mammals as a coenzyme for the carboxylase enzymes (acetyl-CoA, 3-methylcrotonyl-CoA, propionyl-CoA, and pyruvate), which are essential cellular enzymes for gluconeogenesis, lipogenesis, cholesterol, and AA metabolism, and insulin secretion (McMahon, 2002; Chou et al., 2009; Zempleni et al., 2009). Also, biotin functions in the regulation of gene expression and the systemic processes of growth, development, and immunity (Balamurugan et al., 2003, 2005; Fernandez-Mejia, 2005). Mammals cannot synthesize biotin in vivo and must depend on dietary sources or on microbes in the intestine to syn-
thesize D(-)-biotin, the active form (Livianiu et al., 2000; McMahon, 2002; Zempleni et al., 2009).

Protein feeds contain slightly more available biotin than cereal grains, which are inconsistent sources of biotin (Misir and Blair, 1988; Sauer et al., 1988; Kopinski et al., 1989b). There is considerable microbial synthesis of biotin in the hind-gut of the pig (Barth et al., 1986; Sauer et al., 1988; Scholtissek et al., 1990), although absorption of microbial biotin from the large intestine may be low (Kopinski et al., 1989a; Mosenthin et al., 1990).

However, biotin supplementation of a corn-based starter diet (Washam et al., 1975) and corn-soybean meal diets for growing-finishing pigs (Hamilton and Veum, 1986) and sows (Hamilton and Veum, 1984; Lewis et al., 1991; Watkins et al., 1991) had little or no effect on pig or sow performance, indicating that biotin supplementation of practical swine diets may be unnecessary.

The objective of this experiment was to evaluate the effects of cecal oxytetracycline (OTC) infusion, and dietary avidin and biotin supplementation on the biotin status of nongravid gilts. Response criteria were: fecal bacteria counts; plasma concentrations of biotin, glucose, and urea N; liver pyruvate carboxylase (PC) activity; tissue histology; apparent ileal N and energy digestibility; apparent ileal N and energy digestibility; apparent total tract N and energy retention; daily gilt observation; and BW gain.

**MATERIALS AND METHODS**

The procedures and use of animals in this experiment were approved by the University of Missouri Animal Care and Use Committee.

**Animals, Ileal Cannulation, Cecostomy, Vena Cava Catheterization, and Housing**

A total of 28 crossbred gilts (Yorkshire-Landrace × Duroc) at an average of 120.2 ± 1.0 kg BW and 160 ± 1 d of age from 4 surgery groups arranged in time (7 gilts/group) were surgically fitted with a flexible T-cannula in the terminal ileum and a cecal fistula as described by Hamilton et al. (1985). During the surgical procedure, an indwelling catheter made from plastic tubing (Tygon S-50-HL medical tubing, 101 cm length, 1.3 mm i.d. and 0.5 mm wall thickness; Saint-Gobain Performance Plastics, Akron, OH) was inserted into the anterior vena cava. The catheter tubing was exteriorized on the back at a point medial to the right and left scapula for blood collection, and held in place with surgical tape. Catheters were checked for patency and flushed daily with 8 mL of heparinized (250 units of Na heparin/mL; Sigma-Aldrich, St. Louis, MO) sterile saline. Gilts were housed in a temperature controlled (21 ± 1°C) and ventilated room with a slotted floor (12.7-cm concrete slats with 2.2-cm slots). During

the 14-d surgery recovery period and from d 0 to 39 of the experiment, gilts were tethered about 2.5 m from each other with an adjustable neck collar and twist chain (QC Supply, Schuyler, NE) that allowed the gilts to move in a complete circle, and water was provided ad libitum from 0800 to about 2000 h. The surgery recovery period also served as a tether acclimation period to minimize animal stress before starting the experiment (Becker et al., 1984, 1985). The floor was cleaned after each feeding to minimize coprophagy. On d 39, gilts were placed in individual stainless-steel metabolism cages (1.3 m²/pig) equipped with stainless-steel nozzle drinkers (water available ad libitum), feeders, and slotted flooring. Apparent fecal biotin status and total tract N and energy retention were determined from d 44 to 49. Apparent ileal biotin status and ileal N and energy digestibility were determined on d 49. Gilts were weighed on d 0, 14, 35, 42, and 49. Light was provided daily from 0700 to 2000 h.

**Experimental Design and Treatments**

During the 14-d surgery recovery period, all gilts were fed 1.80 kg/d of the basal corn-soybean meal (CS) diet that contained 100 μg of endogenous biotin/kg (analyzed basis; Table 1). After the surgery recovery period, gilts in each surgery group were allotted to 1 of 7 treatments in a randomized complete block design for the 49-d experiment. The 2 basal diets were the semipurified (SP) basal diet containing lactic acid casein (New Zealand Milk Products; Harlan Teklad, Madison, WI) and the CS basal diet (Table 1). Three other treatment variables were supplemental crystalline D(-)-biotin (DSM Nutritional Products, Basel, Switzerland), spray-dried egg albumen (EA; M. G. Waldbaum Co., Wakefield, NE) fed at 100 g/d as a source of avidin, and the cecal infusion of 2.56 g of OTC/d (HCl salt and soluble powder, Terramycin; Pfizer Animal Health, New York, NY). The purpose of the cecal infusion of OTC was to evaluate the effects of OTC on the total fecal bacteria count and the microbial synthesis of biotin in the distal small intestine and the large intestine. Oxytetracycline has been widely used as a treatment for intestinal and respiratory disease in swine, and the cecal infusion of 2.56 g of OTC/d (about 21 mg/kg of BW) in specific treatments in the current experiment was within the range of the oral OTC doses tested on growing swine (Mevius et al., 1986; Pijpers et al., 1991; Nielsen and Gyrd-Hansen, 1996), and the maximum oral dose of soluble OTC powder recommended for use in swine (22 mg/kg).

Four treatments were made with the basal SP diet (Table 2) that was fed at 1.86 kg/d. Treatment SP-1 was the SP negative control without biotin, EA, or cecal OTC. Treatment SP-2 was the positive control with 270 μg of biotin/kg of diet. Treatment SP-3 included EA and cecal
Table 1. Ingredient and chemical composition of air-dried basal corn-soybean meal (CS) and semi-purified (SP) diets, as-fed basis.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal SP diet</th>
<th>Basal CS diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn</td>
<td>-</td>
<td>80.30</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>-</td>
<td>11.50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.90</td>
<td>5.00</td>
</tr>
<tr>
<td>Macromineral premix</td>
<td>3.75</td>
<td>2.45</td>
</tr>
<tr>
<td>Trace mineral premix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Corn starch</td>
<td>35.50</td>
<td>-</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>39.50</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.60</td>
<td>-</td>
</tr>
<tr>
<td>Casein</td>
<td>13.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Chemical composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal SP diet</th>
<th>Basal CS diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>90.50</td>
<td>90.00</td>
</tr>
<tr>
<td>Biotin, μg/kg</td>
<td>5.00</td>
<td>100.00</td>
</tr>
<tr>
<td>CP, %</td>
<td>11.00</td>
<td>12.00</td>
</tr>
<tr>
<td>Lys, %</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td>Met + Cys, %</td>
<td>0.42</td>
<td>0.45</td>
</tr>
<tr>
<td>Thr, %</td>
<td>0.51</td>
<td>0.46</td>
</tr>
<tr>
<td>Trp, %</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Val, %</td>
<td>0.85</td>
<td>0.58</td>
</tr>
<tr>
<td>Ile, %</td>
<td>0.74</td>
<td>0.48</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>Total P, %</td>
<td>0.37</td>
<td>0.63</td>
</tr>
<tr>
<td>Available P, %</td>
<td>0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>K, %</td>
<td>0.27</td>
<td>0.51</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Cl, %</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>3.45</td>
<td>3.56</td>
</tr>
</tbody>
</table>

1The basal SP and CS diets were fed at 1.86 and 1.80 kg/d, respectively, for all treatments with the same protein source.
2Macromineral premix for the basal SP diet provided per kilogram of diet: 2.00 g of Na and 3.09 g of Cl from NaCl; 3.33 g of Ca and 2.80 g of P from CaHPO4; 2.57 g of Ca from CaCO3; 0.60 g of Mg from MgSO4; and 2.70 g of K from K2CO3.
3Macromineral premix for the basal CS diet provided per kilogram of diet: 2.10 g of Na and 3.24 g of Cl from NaCl; 3.74 g of Ca and 3.16 g of P from CaHPO4; and 3.15 g of Ca from CaCO3.
4Trace mineral premix provided per kilogram of diet: 100 mg of Fe from FeSO4; 100 mg of Zn from ZnCO3; 50 mg of Mn from MnSO4; 15 mg of Cu from CuSO4; 1.0 mg of I from Ca(IO3)2; and 0.2 mg of Se from NaSeO3.
5The vitamin-free premix provided per kilogram of diet: 5,500 IU of vitamin A acetate; 550 IU of vitamin D3; 22 IU of vitamin E from DL-a-tocopheryl acetate; 4.0 mg of vitamin K from menadione sodium dimethylprimidinol bisulfite; 2.0 g of choline as choline chloride; 38.5 mg of pantothenic acid from D-calcium pantothenate; 33.0 mg of niacin; 5.5 mg of riboflavin; 2.2 mg of pyridoxine from pyridoxine·HCl; 2.2 mg of thiamin from thiamin·HCl; 1.1 mg of folic acid; 53.0 μg vitamin B12; and 55 mg of ethoxyquin as a preservative.
6SolkaFloc, James River Corp., Berlin, NH.
7Lactic acid casein, New Zealand Milk Products, Harlan Teklad, Madison, WI.
8Analyzed diet samples for biotin [triplicate samples; microbiological method; Wright and Skeggs (1944) as modified by Frigg and Brubacher (1976)], N (triplicate samples; AOAC, 1984), and total amino acids [duplicate samples; Spies and Chambers (1949) and Benson and Patterson (1971)].
9Calculated mineral and ME composition of the diets with the values for ground corn, soybean meal, and casein from the NRC (1982).
and plates were incubated for 7 d at 37°C before the bacterial colonies were counted (Salanitro et al., 1977; Iannotti et al., 1978, 1982). Aerobic bacteria plates (5 replicates) were aseptically prepared with sterile media, inoculated (0.2 mL) with a serially diluted sample (10⁻⁵, 10⁻⁶, and 10⁻⁷), and incubated at 37°C for 24 h before the aerobic bacterial colonies were counted.

**Plasma Concentrations of Biotin, Urea N, and Glucose**

Blood samples (10 mL) were collected from the indwelling jugular catheter 4 h after feeding (1200 h) on experimental d 0, 7, 14, 21, 28, 35, 42, and 49. Five milliliters of blood was placed in a heparinized tube (32 units of heparin/mL of blood; Sigma-Aldrich, St. Louis, MO) for plasma urea N (PUN) analysis (Sigma, 1980b), and 5 mL of blood was placed in another heparinized tube (32 units of heparin/mL of blood) that were infused after feeding at 0800 and 1700 h daily. At each feeding 1.28 g of OTC and 3.0 g of sucrose were mixed in 25.0 mL of distilled water immediately before infusion into the cecum. Gilts not infused with OTC received a sham infusion of 3.0 g of sucrose with 25.0 mL of distilled water after each feeding.

Chromic oxide was included in all diets (0.07%) from d 0 to 49, and was added ad libitum to diets SP-3, SP-4, and CS-2 that provided 50 mg of avidin/gilt daily (Osgua and Feeney, 1977; Li-Chan et al., 1995). Crystalline D-(+)-biotin premix (DSM Nutritional Products, Basal, Switzerland) was added to diets SP-2, SP-4, and CS-3. EA was added to the daily ration of each gilt fed diets SP-3, SP-4, CS-2, or CS-3 that provided 50 mg of avidin/gilt daily (Osuga and Feeney, 1977; Li-Chan et al., 1995). The total daily OTC dose of 2.56 g was divided into 2 doses of 1.28 g that were infused after feeding at 0800 and 1700 h daily. At each feeding 1.28 g of OTC and 3.0 g of sucrose were mixed in 25.0 mL of distilled water immediately before infusion into the cecum. Gilts not infused with OTC received a sham infusion of 3.0 g of sucrose with 25.0 mL of distilled water after each feeding.

Blood samples (10 mL) were collected from the indwelling jugular catheter in each gilt 4 h after the 0800 h feeding. Plasma was harvested by centrifugation (1,600 × g for 10 min at 4°C), deproteinized (0.2 M trichloroacetic acid), and analyzed for urea N (Sigma, 1980b). Plasma biotin concentrations were also determined on heparinized blood samples (5 mL) collected on d 0 and 49 using *Lactobacillus plantarum* (ATCC 8014) in a microbiological assay (Wright and Skeggs, 1944; as modified by Frigg and Brubacher, 1976). In addition, serial blood samples (10 mL) were collected from all gilts on d 49, sampling every 30 min from 0800 to 1300 h and hourly from 1400 to 1700 h (before the 1700 h feeding, 15 total samples/gilt) for plasma glucose and PUN analysis.

**Ileal and Fecal Biotin Status and N and Energy Digestibility**

Chromic oxide was included in all diets (0.07%) from d 39 to 49 to allow fecal grab-samples (about 100 g) to be collected from each gilt twice daily (about 0900 and 1800 h) from d 44 to 49 and frozen (−20°C) in plastic bags. Bladder catheters (Foley No. 18 French; Midwest Medical Supply, Earth City, MO) were inserted into all gilts on d 44 for total urine collections from d 44 to 49. At each collection, total urine volume was recorded and 5% was saved and frozen (−20°C) in screw-capped plastic bottles.

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**Table 2. Effect of dietary avidin and biotin, and the cecal infusion of oxytetracycline (OTC) on plasma concentrations of biotin and urea N in nongravid gilts**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>SP² dietary treatments</th>
<th>CS³ dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP-1</td>
<td>SP-2</td>
<td>SP-3</td>
<td>SP-4</td>
</tr>
<tr>
<td>Added avidin, mg/d:</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Added avidin, μg/kg:</td>
<td>0</td>
<td>270</td>
<td>0</td>
<td>700</td>
</tr>
<tr>
<td>Cecal infusion of OTC, g/d:</td>
<td>0</td>
<td>0</td>
<td>2.56</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Plasma biotin, ng/100 mL

| d 0 | 89.5 | 101.8 | 44.8 | 68.8 | 100.0 | 78.3 | 79.8 | 27.0 | 0.680 |
| d 49 | 35.0ᵃ | 78.1ᵇ | 65.5ᵇ | 89.8ᵇ | 51.6ᵃ | 37.2ᵃ | 147.2ᶜ | 17.0 | 0.002 |

Plasma urea N, mg/dL

| d 0 | 4.50 | 3.97 | 5.54 | 5.61 | 5.02 | 5.53 | 5.26 | 0.70 | 0.620 |
| d 7 | 3.26ᵇ | 3.34ᵃ | 6.16ᵇ | 5.81ᵇ | 5.19ᵇ | 5.46ᵇ | 5.68ᵇ | 0.65 | 0.005 |
| d 14 | 3.17ᵃ | 3.42ᵃ | 6.46ᵇ | 6.48ᵇ | 4.58ᵇ | 5.44ᵇ | 4.66ᵇ | 0.72 | 0.031 |
| d 21 | 3.98ᵇ | 3.7ᵃ | 7.18ᶜ | 7.31ᶜ | 4.40ᵇ | 5.91ᵇ | 4.75ᵇ | 0.70 | 0.008 |
| d 28 | 4.11ᵃ | 3.27ᵃ | 8.65ᶜ | 8.56ᶜ | 5.28ᵇ | 6.64ᵇ | 5.15ᵇ | 0.68 | < 0.001 |
| d 35 | 3.21ᵃ | 3.7¹ᵃ | 8.20ᶜ | 8.44ᶜ | 4.89ᵇ | 6.14ᵇ | 6.03ᵇ | 0.60 | < 0.001 |
| d 42 | 4.64ᵇ | 4.24ᵃ | 8.16ᶜ | 8.00ᶜ | 4.70ᵇ | 6.62ᵇ | 6.00ᵇ | 0.70 | 0.005 |
| d 49 | 4.43ᵇ | 4.5¹ᵇ | 8.21ᵇ | 8.87ᵇ | 4.61ᵇ | 7.74ᵇ | 5.97ᵃ | 0.68 | < 0.001 |
| d 49 (overall serial bleeding mean)⁶ | 5.00ᵇ | 4.5¹ᵇ | 7.80ᵈ | 8.65ᵈ | 4.69ᵇ | 7.6³ᵇ | 6.0¹ᵇ | 0.35 | < 0.001 |

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ᵃWithin a row, means lacking a common superscript letter differ (P ≤ 0.05).

ᵇThe basal SP and CS diets (without avidin) were fed at 1.86 and 1.80 kg/d, respectively. Spray-dried egg albumen (EA) was the avidin source, with 100 g of EA added to the daily ration of each gilt fed diets SP-3, SP-4, CS-2, or CS-3 that provided 50 mg of avidin/gilt daily (Osgua and Feeney, 1977; Li-Chan et al., 1995). Crystalline D-(+)-biotin premix (DSM Nutritional Products, Basal, Switzerland) was added to diets SP-2, SP-4, and CS-3.

ᶜSP = semipurified; CS = corn-soybean meal.

³The OTC was infused into the cecum through a cecal fistula (Hamilton et al., 1985). The total daily OTC dose of 2.56 g was divided into 2 doses of 1.28 g that were infused after feeding at 0800 and 1700 h daily. At each feeding 1.28 g of OTC and 3.0 g of sucrose were mixed in 25.0 mL of distilled water immediately before infusion into the cecum. Gilts not infused with OTC received a sham infusion of 3.0 g of sucrose with 25.0 mL of distilled water after each feeding.

⁴A blood sample (5 mL) was collected from the indwelling jugular catheter in each gilt 4 h after the 0800 h feeding. Plasma was harvested by centrifugation (1,600 × g for 10 min at 4°C) and analyzed for biotin (Wright and Skeggs, 1944; as modified by Frigg and Brubacher, 1976).

⁵A blood sample (5 mL) was collected from the indwelling jugular catheter in each gilt 4 h after the 0800 h feeding. Plasma was harvested after centrifugation (1,600 × g for 10 min at 4°C), deproteinized (0.2 M trichloroacetic acid), and analyzed for urea N (Sigma, 1980b).

⁶Overall serial bleeding means for blood samples collected from each gilt every 30 min from 0800 to 1300 h, and hourly from 1400 to 1700 h (15 samples/gilt) on d 49.
Immediately after each individual collection, the metabolism cage and the plastic urine container were washed and 60 mL of toluene and 60 mL of 6N HCl (Fisher Scientific, St. Louis, MO) were added to the urine container to prevent evaporation and N loss. Pooled fecal samples from individual gilts were dried at 55°C in a forced-air oven and ground to pass a 2-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Triplicate samples of diet and duplicate samples of feces were analyzed for N and DM (Methods 7.014 and 7.007; AOAC, 1984) and for biotin as described for plasma. Duplicate samples of the basal SP and CS diets were also analyzed for total AA (Spies and Chambers, 1949; Benson and Patterson, 1971). Duplicate samples of urine were analyzed for N. The analyzed values for dietary N were used to determine N digestibility. Total Cr concentrations of diet and fecal samples were determined (Jackson et al., 1980) with an atomic absorption spectrophotometer (Model 2380; Perkin Elmer Corp., Norwalk, CT). The GE content of the diet, fecal, and urine samples was determined with an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). To determine the GE content of urine, 50.0 mL of urine was mixed with 2.0 g of finely ground cellulose. The mixture was freeze-dried, reground with a mortar and pestle, and analyzed for GE. The GE value for cellulose was subtracted from the total to obtain the GE value for urine.

On d 49, ileal contents were collected continuously from each gilt for 12 h (0800 to 2000 h). Plastic tubing (Tygon R-3603 flexible tubing, 22 mm i.d., 3 mm wall; Saint-Gobain Performance Plastics, Akron, OH) was attached to the ileal cannula with the free end inserted in a plastic collection jug containing 20 mL of 10 N H2SO4. Collection jugs were placed in ice baths with collection by gravity flow. Ileal contents were frozen (−20°C), freeze dried for 72 h, ground with a mortar and pestle, and analyzed for biotin, DM, N, Cr, and GE as described for the diets and feces. Biotin concentrations in ileal digesta and feces were expressed as microgram of biotin per kilogram of diet intake as described by Sauer et al. (1988).

**Daily Observation of Gilts and Tissue Samples**

All gilts were observed daily after the 0800 h feeding by the same person during the entire experiment for any symptoms of biotin deficiency (Cunha et al., 1946; Glaetli, 1975; Kopinski et al., 1989c). On d 50 of the experiment, all gilts were stunned by electric shock and killed by exsanguination. Livers were removed and weighed (without the gall bladder), and a 2.0 g sample of liver tissue was collected from the left lobe to determine PC activity (EC 6.4.1.1) in vitro (Deodhar and Mistry, 1969; as modified by Hamilton et al., 1983). A biotin-containing enzyme, PC catalyzes the carboxylation of pyruvate to oxaloacetate in gluconeogenesis and to replenish oxaloacetate in the tricarboxylic acid cycle, and functions in the generation of NADPH for lipogenesis (Whitehead, 1981; Hamilton et al., 1983; Jitrapakdee et al., 2008). For histology, tissue samples were collected from skin (4 samples about 1 cm2 each dorsal to the last lumbar vertebrae), the apex of the tongue, and a cross-section of kidney. Samples were fixed in a solution of 10% formalin, embedded in paraffin, sliced in cross-section to make glass slides, stained (H&E solution; Sigma Chemical Co., St. Louis, MO), and examined histologically (College of Veterinary Medicine, University of Missouri) for abnormalities associated with a biotin deficiency.

**Statistical Analysis**

Data were analyzed by ANOVA as a randomized complete block design with 7 treatments (4 treatments for fecal bacteria counts) and 4 blocks (surgical groups) using the Mixed model procedure, with serial bleeding data analyzed as repeated measurements in time (Snedecor and Cochran, 1989; SAS Inst. Inc., Cary, NC). Individual pigs were the experimental units. Treatment means were separated with the F-protected LSD test, with significance at $P \leq 0.05$.

**RESULTS**

Gilts readily consumed the experimental diets. The average increase in BW was 20.1 kg/gilt, and it was not different among the treatment groups (data not shown), with an overall average ending BW of 140.3 ± 2.2 kg on d 49 of the experiment.

**Plasma Concentrations of Biotin, Urea N, and Glucose**

Plasma biotin concentrations for the treatments were not different on d 0 (Table 2). However, on d 49, gilts on treatment SP-4 had greater ($P \leq 0.05$) plasma biotin concentrations than gilts on treatment SP-1. The plasma biotin concentrations of gilts on treatments SP-1 and SP-2 were not different from gilts on treatments CS-1 or CS-2. However, on d 49, gilts on treatment CS-3 had greater ($P \leq 0.05$) plasma biotin concentrations than gilts on any other treatment.

For PUN concentrations, gilts on treatments SP-3 and SP-4 had greater ($P \leq 0.05$) PUN concentrations on d 7, 14, 21, 28, 35, 42, and 49 than gilts on treatments SP-1 and SP-2 (Table 2). Gilts on treatments SP-3 and SP-4 also had greater ($P \leq 0.05$) PUN concentrations than gilts on treatments CS-1 and CS-3 on d 21, 42, and 49, and treatments CS-1, CS-2, and CS-3 on d 28 and 35. For gilts on the CS treatments, there were no treatment differences in PUN concentrations on d 7, 14, 21, 28 35, or 42. How-
ever, on d 49, gilts on treatment CS-2 had greater ($P \leq 0.05$) PUN concentrations than gilts on treatments CS-1 or CS-3. For serial bleeding over time on d 49, there were no differences in PUN concentrations within treatment serial bleeding time. Therefore, the overall serial bleeding means for each treatment are presented in Table 2. For plasma glucose concentrations, there were no treatment differences for weekly bleedings or the serial bleedings on d 49 (data not shown), with overall experimental means of 74.2 ± 1.8 and 74.6 ± 1.6 mg/100 mL, respectively.

**Ileal and Fecal Biotin Status and N and Energy Digestibility**

Apparent ileal digesta biotin concentrations ($\mu$g/kg of diet intake; Table 3) were greater ($P \leq 0.05$) for treatments supplemented with 700 $\mu$g of biotin/kg of diet (SP-4 and CS-3) than for any other treatment. Also, ileal digesta biotin concentrations were greater ($P \leq 0.05$) for gilts on treatment CS-2 than for gilts on treatments SP-1, SP-2, or SP-3, with gilts on treatment CS-1 being intermediate in ileal digesta biotin concentration. Apparent fecal biotin concentrations ($\mu$g/kg of diet intake) were greater ($P \leq 0.05$) for gilts on treatments CS-3, CS-2, and SP-4 than gilts on the other treatments. Also, fecal biotin concentrations were greater ($P \leq 0.05$) for gilts on treatment CS-1 than for gilts on treatment SP-2, with intermediate values for gilts on treatments SP-1 or SP-3.

The fecal biotin concentration minus the ileal digesta biotin concentration value was greater ($P \leq 0.05$) for gilts on treatment CS-2 than for gilts on treatments SP-1, SP-2, SP-3, SP-4, and CS-1, but was not different from gilts on treatment CS-3. However, the fecal minus ileal digesta biotin concentration was greater ($P \leq 0.05$) for gilts on treatment SP-4 than for gilts on treatments SP-2 or SP-3, and was not different from gilts on treatments SP-1, CS-1, or CS-3.

For apparent ileal and total tract N digestibility, gilts on the SP treatments had greater ($P \leq 0.05$) ileal and total tract N digestibility values (%) than gilts on the CS treatments, with no treatment differences within the same protein source. For apparent biological value ($%\text{BV} = \frac{N \text{ retained}}{N \text{ absorbed}} \times 100$) and apparent net protein utilization ($%\text{NPU} = \frac{N \text{ retained}}{N \text{ intake}} \times 100$), gilts on treatments SP-3 and SP-4 had lower ($P \leq 0.05$) BV and NPU values than gilts on the other treatments. Gilts on treatment SP-2 had a greater ($P \leq 0.05$) NPU than the gilts on treatments CS-1 or CS-2.

For apparent ileal DE and apparent total tract DE and ME (%), gilts on the SP treatments had greater ($P \leq 0.05$) values than gilts on the CS treatments, with no treatment differences within the same protein source. There were no treatment differences for apparent ileal or total tract DM digestibilities (data not shown), with overall experimental means of 80.5 ± 1.5 and 88.5 ± 1.2%, respectively.

**Fecal Aerobic and Anaerobic Bacterial Plate Counts**

There were no differences in anaerobic or aerobic fecal bacteria colony forming unit counts for the 4 treatments evaluated on experimental d 0 and 49 in the current experiment (data not shown). Therefore, negative control treatments SP-1 and CS-1 were not different from treatments SP-3 and CS-2 that received dietary avidin and cecal OTC infusion. The overall experimental means for total aerobic and anaerobic fecal bacteria samples ($\log_{10} \text{cfu/g dry feces}$) were 7.60 ± 0.40 and 9.10 ± 0.25 on d 0, respectively, and 7.30 ± 0.25 and 8.75 ± 0.30 on d 49, respectively.

**Daily Observation of Gilts and Tissue Sample Evaluation**

No treatment differences were observed for dermatitis, hair loss, or lameness during the daily observation of gilts from d 0 to 49 in the current experiment. Also, histological evaluation of cross-sections of kidney and epithelium from tongue and skin found that the tissues were normal for all gilts in the experiment, with no abnormalities or lesions associated with a biotin deficiency. Furthermore, there were no differences in the treatment responses for fresh liver weight or liver PC activity, with overall experimental means of 1,945 ± 125 g for fresh liver weight and 0.50 ± 0.23 for liver PC activity ($\mu$mol of $^{14}\text{CO}_2$ incorporated/g of fresh liver/min).

**DISCUSSION**

The apparent digestibility of biotin in corn and soybean meal (SBM) for swine were 100 and 85.5%, respectively, in one experiment (Misir and Blair, 1988), and much less in another experiment (Sauer et al., 1988). Experiments with broiler chicks also found apparent biotin digestibilities of about 100% for both corn and SBM (Whitehead et al., 1982; Blair and Misir, 1989). The microbiological assay used for biotin in the current experiment and the experiment by Misir and Blair (1988) may have greater biotin values for plant ingredients than the chick bioassay because of greater free biotin (%) in plant ingredients than animal ingredients (Scheiner and De Ritter, 1975). In the current experiment, gilts on the CS treatments consumed about 0.18 mg of biotin/d from corn and SBM, with 0.17 mg of biotin absorbed/d (Misir and Blair, 1988), providing 42.5% of the daily gestation biotin requirement of 0.4 mg (NRC, 1998). However, when the smaller biotin concentrations in corn and SBM from NRC (1998) are used in these calculations, biotin
intake by gilts on the CS treatments is 0.14 mg/d with 0.13 mg of biotin absorbed/d, providing 32.5% of the daily gestation biotin requirement. Therefore, even with biotin digestibilities of 100% for corn and 85.5% for SBM (Misir and Blair, 1988), most of the biotin requirement for gilts on the CS treatments in the current experiment would need to be provided by dietary supplementation or by microbial synthesis of biotin in the intestinal tract. Gilts on the SP treatments in the current experiment consumed and absorbed about 0.01 mg of biotin/d from casein with an apparent ileal biotin digestibility of 95% (Kopinski et al., 1989b), providing only 2.5% of the daily gestation biotin requirement.

A diverse and extensive microflora exists in the hind-gut of growing pigs (Russell, 1979; Robinson et al., 1981; Leser et al., 2002) and sows (Salanitro et al., 1977), although fecal bacteria colony forming unit count data were not found for gilts or sows to compare with the gilt data in the current experiment. However, total anaerobic fecal bacteria counts for the gilts in the current experiment are similar to the anaerobic fecal bacteria counts for growing swine in other experiments (Jensen and Jørgensen, 1994; Nagamine et al., 1998), and similar to or greater than the values for weanling pigs (Muhl and Liebert, 2007; Leonard et al., 2011). This indicates that the daily cecal infusion of OTC in the current experiment had little or no effect on total aerobic and anaerobic bacterial populations in the hindgut of gilts. Other experiments also found that the addition of sulfaguanidine or sulfasuxidine to a semipurified diet with casein as the protein source had no effect on the biotin status of growing pigs, whereas the addition of sulfathalidine caused a biotin deficiency (Lindley and Cunha, 1946; Cunha et al., 1948). Also, the addition of a therapeutic amount of sulfamethazine to a semipurified diet with EA as the protein source in a different experiment had no effect on liver measurements, PUN concentrations, or the growth performance of young pigs compared with pigs fed the semipurified diet without sulfamethazine (Hamilton et al., 1983).

Feeding subtherapeutic quantities of OTC and other antibiotics for growth promotion, and therapeutic use of antibiotics for disease purposes has increased enteric bacterial resistance to antibiotics over time (Dawson et al., 1984a; Gellin et al., 1989; Mathew, et al., 1999), with a greater bacterial resistance to OTC in older pigs.

<table>
<thead>
<tr>
<th>Treatments1</th>
<th>SP2 dietary treatments</th>
<th>CS1 dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP-1</td>
<td>SP-2</td>
<td>SP-3</td>
<td>SP-4</td>
</tr>
<tr>
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<td>4</td>
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<tr>
<td>Diet intake (d 0 to 49), kg/d</td>
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<td>1.86</td>
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<td>Biotin, μg/kg diet intake</td>
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<td>5</td>
<td>705</td>
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<td>Ileal</td>
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<td>49a</td>
<td>50a</td>
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<tr>
<td>Fecal</td>
<td>284ab</td>
<td>95a</td>
<td>263ab</td>
<td>817c</td>
</tr>
<tr>
<td>Fecal - ileal</td>
<td>269ab</td>
<td>46a</td>
<td>218a</td>
<td>330b</td>
</tr>
<tr>
<td>N</td>
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<tr>
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<td>32.1</td>
<td>45.0</td>
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<td>85.5b</td>
<td>84.1b</td>
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<tr>
<td>Fecal ME, %</td>
<td>85.2b</td>
<td>88.1b</td>
<td>86.0b</td>
<td>84.6b</td>
</tr>
</tbody>
</table>

1Within a row, means lacking a common superscript letter differ (P ≤ 0.05).
2The basal SP and CS diets (without avidin) were fed at 1.86 and 1.80 kg/d, respectively. Spray-dried egg albumen (EA) was the avidin source, with 100 g of EA added to the daily ration of each gilt fed diets SP-3, SP-4, CS-2, or CS-3, that provided 50 mg of avidin/gilt daily (Osuga and Feeney, 1977; Li-Chan et al., 1995). Crystalline D-(+)-biotin premix (DSM Nutritional Products, Basel, Switzerland) was added to diets SP-2, SP-4, and CS-3.
3The OTC was infused into the cecum through a cecal fistula (Hamilton et al., 1985). The total daily OTC dose of 2.56 g was divided into 2 doses of 1.28 g that were infused after feeding at 0800 and 1700 h daily. At each feeding 1.28 g of OTC and 3.0 g of sucrose were mixed in 25.0 mL of distilled water immediately before infusion into the cecum. Gilts not infused with OTC received a sham infusion of 3.0 g of sucrose with 25.0 mL of distilled water after each feeding.
and sows than in young pigs (Mathew et al., 2001). Conversely, feeding a subtherapeutic level of OTC did not increase the number of OTC-resistant *Salmonella typhimurium* in young swine challenged orally with *S. typhimurium* compared with swine fed the diet without OTC (Evangelisti et al., 1975). However, even after prolonged antibiotic feeding and treatment, large and complex anaerobic bacterial populations are maintained in the cecum and colon of swine (Dawson et al., 1984b).

In the current experiment, the overall (experimental) mean concentration of biotin in feces was 2.6-fold greater than the overall mean concentration of biotin in ileal digesta. Other experiments also found considerable microbial synthesis of biotin in pig intestine that was independent of dietary biotin intake, with greater biotin concentrations in feces than in ileal digesta (Sauer et al., 1988; Kopinski et al., 1989a; Mosenthin et al., 1990). However, the microbial population in the distal small intestine of swine (Broom et al., 2006; Muhl and Liebert, 2007) makes an important contribution toward meeting the nutrient requirements of swine (Jensen and Jørgensen, 1994; Torrallardona et al., 2003). Microbial synthesis of nutrients also occurs in the small intestine of other mammals (Bowman and Rosenberg, 1987; Metges, 2000) and poultry (Bryden, 1989). Therefore, the dietary requirement for some B vitamins is less than the metabolic requirement because of microbial vitamin synthesis in the distal small intestine (Torrallardona et al., 2003). Other experiments also found that the microbial synthesis of vitamins in the intestinal tract contributes toward meeting the B vitamin requirements of growing swine (Mahan et al., 2007; Ivers and Veum, 2012).

In the current 49-d experiment, the microbial synthesis of biotin in the intestinal tract of gilts on all treatments was adequate to meet the biotin requirement for normal tissue growth, cellular metabolism, and the prevention of any symptoms associated with biotin deficiency (Cunha et al., 1946; Bryant et al., 1985a; Misir et al., 1986). A longer-term experiment with growing gilts from weaning to 92 kg BW also found that biotin supplementation of CS diets did not improve hair, skin, and soundness scores or growth performance (Bryden, 1989). Thus, supplementation of practical grain-soybean meal diets did not increase the number of OTC-resistant *Salmonella typhimurium* in young swine challenged orally with *S. typhimurium* compared with swine fed the diet without OTC (Evangelisti et al., 1975). However, even after prolonged antibiotic feeding and treatment, large and complex anaerobic bacterial populations are maintained in the cecum and colon of swine (Dawson et al., 1984b).

In another experiment with adult mini pigs fed a SP casein-protein diet supplemented with 25% of the biotin requirement, the cecal infusion of avidin (18 mg/d) did not cause a biotin deficiency (Scholtissek et al., 1990).

However, in a different experiment where EA was the sole source of protein in the SP diet (no added biotin), growing pigs began to develop biotin deficiency symptoms in 2.5 wk because the dietary and microbial sources of biotin were bound by the avidin in EA (Cunha et al., 1946). Conversely, autoclaving the EA used in the SP diet for weanling pigs in a different experiment eliminated the metabolic biotin deficiency symptoms observed in pigs fed the diet with nonautoclaved EA (Hamilton et al., 1983).

The avidin-biotin complex is the strongest biochemical (noncovalent) bond known (Gitlin et al., 1987; Haugland and You, 2008), and is resistant to proteolysis in the digestive tract (Green, 1975). One mol of avidin (MW of about 67,000) will bind 4 mol of biotin (MW of 244.3 × 4 = 977; Green, 1964, 1966). Therefore, in the current experiment, each gilt fed 100 g of EA/d on treatments SP-3, SP-4, CS-2, and CS-3 consumed 50 mg of avidin/d (Osuga and Feeney, 1977; Li-Chan et al., 1995) that bound 0.73 mg of biotin/d (50 mg of avidin × 977/67,000 = 0.73 mg of biotin bound/d). Also, treatments SP-4 and CS-3 were supplemented with 0.70 mg of biotin/kg of diet, and gilts on those treatments consumed about 1.3 mg of supplemental biotin/d (1.86 kg of SP diet × 0.7 mg biotin/kg = 1.3 mg biotin/d). Therefore, about 0.57 mg of supplemental biotin was available for absorption/d by gilts on treatments SP-4 and CS-3 (1.3 mg of biotin intake/d − 0.73 mg of avidin-bound biotin/d = 0.57 mg), which slightly exceeds the daily NRC (1998) gestation biotin requirement of 0.4 mg.

There was considerable variation in plasma biotin concentration within treatments for gilts in the current experiment, which is in accord with the wide variation in plasma biotin concentrations found in other experi-
ments with gilts and sows (Misir and Blair, 1986a; Misir et al., 1986) and growing pigs (Kopinski et al., 1989c). In other experiments, biotin supplementation increased body tissue and plasma biotin concentrations of gilts and sows (Bryant et al., 1985b,c; Misir and Blair, 1986a) and growing pigs (Kopinski et al., 1989c). Plasma biotin concentrations of gilts in the current experiment were greater than the plasma biotin concentrations for gilts in a different experiment (Mevius et al., 1990), and were within the range of plasma biotin concentrations for gilts in other experiments (Bryant et al., 1985b,c; Misir and Blair, 1986a).

For liver PC activity, the lack of a biotin treatment response in the current experiment is in agreement with the results of another experiment where the biotin supplementation of corn- or wheat-based diets for sows did not increase liver PC activity (Bryant et al., 1985c). However, in a different experiment with weanling pigs, liver PC activity was greater for the pigs fed the SP diet containing autoclaved EA than for the pigs fed the diet containing nonautoclaved EA, either with or without added biotin (Hamilton et al., 1983).

The greater apparent ileal and total tract N digestibility for the SP treatments with casein as the protein source in the current experiment may be attributed to the greater AA availability for casein compared with corn and soybean meal (Kies et al., 1986; Chung and Baker, 1992). The EA used as a source of avidin in the current experiment contained protease inhibitors (Rhodes et al., 1960; Kassell, 1970) that may reduce protein digestibility when EA is the primary dietary protein (Imondi et al., 1973; Hamilton et al., 1983; Watkins and Veum, 2010). However, feeding 100 g of EA/d in the current experiment had no effect on the ileal or fecal digestibilities (％) of N and energy of gilts on treatments with the same dietary protein source (SP or CS). This indicates that the protease inhibitor effect was negated because EA was ≤ 30％ of the total daily protein intake, and because of the high apparent AA digestibility of EA (Watkins and Veum, 2010).

In the current experiment, the increases in urinary N excretion and PUN concentration that occurred with cecal OTC infusion with the SP treatments, but not with the CS treatments, may be associated with the antianabolic effect of OTC on kidney function reported in feedlot cattle (Griffin et al., 1979) and in human patients who also had increased urinary N excretion and increased PUN concentrations (Faloon et al., 1957; Whelton, 1978; Babu et al., 2002). To our knowledge, the negative effect of cecal OTC infusion on N retention in swine has not been reported previously. For swine, the excretion of OTC occurs primarily by glomerular filtration and tubular secretion in the kidney, with about 70 to 75％ of an orally administered dose excreted within a week (Xia et al., 1983; Black and Gentry, 1984; Mevius et al., 1986). In animal nutrition, increases in PUN concentration and urinary N excretion usually are indicators of a reduced quality dietary protein with a poor AA balance (Eggum, 1970; Brown and Cline, 1974; Kohn et al., 2005), although that was not the situation in the current experiment.

In conclusion, the biotin requirement of nongravid gilts in the current experiment was met primarily by the synthesis and absorption of microbial biotin from the distal small intestine and the large intestine. Also, endogenous biotin in corn and SBM contribute toward meeting the daily gestation biotin requirement. No gilts developed symptoms of biotin deficiency, and there were no biotin treatment differences in fecal bacteria counts, apparent ileal and total tract N and energy digestibility, plasma glucose concentration, liver PC activity, kidney and epithelial tissue histology, and BW gain. Therefore, supplementation of practical diets for gilts that enter the breeding herd with a vitamin premix that provides 100％ of the NRC (1998) biotin requirement for sows is more than adequate to maximum performance when the diet meets all other nutrient requirements.

LITERATURE CITED


