Chenodeoxycholic acid reduces intestinal permeability in newly weaned piglets

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ABSTRACT: Piglets are highly susceptible to gut health-related problems. Intravenously administered chenodeoxycholic acid (CDCA) affects gut health mediated through glucagon-like peptide 2 (GLP-2). To test whether CDCA is a suitable feed additive for improving gut health, a trial was performed with newly weaned (21 d) piglets offered a diet with or without 60 mg CDCA/kg feed (n = 24/treatment). Upon weaning, piglets were fasted for 16 h and then intragastrically dosed with 20 g test feed in 40 g water. Subsequently, a jugular blood sample was taken on 45, 90, 135, or 180 min for analysis of GLP-2, peptide YY (PYY), and glucose. Afterwards, piglets were offered the experimental diets ad libitum. On days 3.5, 7.5, and 10.5 after weaning, serum responses to an intragastric dose of lactulose and Co-EDTA were tested at 2 h after dosing in 8 piglets per treatment. Immediately thereafter, piglets were euthanized, intestines were harvested, and permeability was measured ex vivo using the everted gut sac technique with 4 kDa fluorescein isothiocyanato (FITC)-dextran as marker at 25, 50, and 75% of the length of the small intestine. Dietary CDCA did not affect (P > 0.05) ADFI, ADG, G:F, blood glucose, and plasma GLP-2 and PYY. Serum cobalt and lactulose at day 10.5 tended to be lower in CDCA pigs compared with control pigs. Serum cobalt and lactulose concentrations were positively correlated (r = 0.67; P < 0.01). In conclusion, CDCA tended to reduce intestinal permeability at 10.5 d after weaning when fed to newly weaned piglets, implying that CDCA deserves further study as a means for improving intestinal health. The positive correlation found between Co-EDTA and lactulose indicates that both marker molecules measure similar change in permeability.

Key words: chenodeoxycholic acid, everted gut sac technique, gut health, intestinal permeability, piglet

INTRODUCTION

Piglets that are weaned early at 3 wk of age commonly suffer from gut health-related problems such as shortening of villi and decreased mucosal development. This affects nutrient absorption capacity and predisposes the animals to infections such as Escherichia coli (Cera et al., 1988; Pluske et al., 1997). Recently, in newborn piglets fed via total parenteral nutrition (TPN), the bile acid chenodeoxycholic acid (CDCA) increased intestinal mucosal growth mediated through glucagon-like peptide-2 (GLP-2) (Jain et al. 2012). The present study was designed to test whether CDCA as a feed additive could improve gut health in early-weaned piglets.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee of Wageningen Livestock Research Institute, The Netherlands. In total, 48 (Talent × Topigs 20; Topigs, Vught, The Netherlands) barrows were blocked by BW (6.0 ± 0.59 kg) and housed individually during the 11-d experiment, starting with weaning at 21 d of age. Treatments consisted of a regular cereal-based diet for weaned pigs [containing
barley (*Hordeum vulgare*), wheat, and corn (*Zea mays*) in a ratio of 5:3:2; ME = 14.3 MJ/g; CP, crude fat, Ca, and P were 196, 63, 7.6, and 8 g/kg, respectively] and a CDCA diet (control diet with 60 mg/kg maize starch replaced by CDCA; Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands).

Pigs did not have access to creep feed before weaning. After weaning, piglets were fasted for 16 h. Subsequently, 20 g of test diet mixed with 40 g of water was administered to the piglets by tube feeding. Blood samples (10 mL) were taken from the jugular vein on 45, 90, 135, or 180 min after tube feeding. Each pig was sampled once (n = 6 per time point) and 2 extra pigs per time point were used for plasma glucose, peptide YY (PYY), and GLP-2 fasting-level measurements. Blood samples (10 mL) were drawn into chilled EDTA tubes and glucose was measured immediately with a glucometer. Diprotin A was added to stabilize GLP-2 (final concentration 0.1 mM Diprotin A) and blood samples were centrifuged (3,000 × g for 12 min). Plasma was stored at −20°C for analysis of plasma GLP-2 and PYY by RIA as described by Taylor-Edwards et al. (2011). After blood sampling, treatment diets were offered ad libitum.

Piglets were weighed at weaning, days 1, 4, 8, and 11, or at euthanasia. Feed intake was determined daily for days 0 to 4, days 5 to 7, and days 7 to 10. The ADG and G:F were calculated for the same periods. Sixteen piglets were selected to be euthanized for permeability measurements at days 3 and 4, days 7 and 8, and days 10 and 11 (8 piglets/d; 4 of each treatment). Two hours before euthanizing, piglets were administered with a marker cocktail (1.3 g of $9.5 \times 10^{-10}$ m lactulose and 0.6 g of $10.0 \times 10^{-10}$ m Co-EDTA dissolved in demineralized water until 15 g) by tube feeding. One minute before euthanizing, a blood sample (10 mL) was taken for analysis of serum lactulose and cobalt by isocratic cation-exchange liquid chromatography-mass spectrometry as described in van Wijck et al. (2011) and inductive coupled plasma spectrometer, respectively. After euthanizing, 15-cm segments were harvested at 25, 50, and 75% of length of the small intestine to measure permeability by the “everted gut sac” technique (Wilson and Wiseman, 1954). In brief, segments were inverted, filled with PBS containing 5 mM glucose, and sealed. Sacs were inserted for 60 min in an aluminum foil-covered bath with aerated
PBS containing 125 μM fluorescein isothiocyanato (FITC)-dextran (14.0 × 10⁻¹⁰ m) and maintained at 39°C. Thereafter, sac content and medium were analyzed for FITC by a fluorometer.

Data were analyzed using the GLM procedure of IBM SPSS statistics 19 (IBM Corporation, Armonk, NY) with piglet as experimental unit. Data from each time period (days 3.5, 7.5, or 10.5) were analyzed separately, with treatment and block as fitted variables and ADFI as covariable. Correlation was tested using the Pearson correlation test. Values were considered significant at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

Dietary CDCA did not affect ADFI, ADG, and G:F (\( P > 0.05 \); data not shown). Dietary CDCA did not affect (\( P > 0.05 \)) blood glucose levels and plasma PYY and GLP-2. The CDCA was offered in an amount of 60 mg/kg feed (resulting in about 1.5 mg CDCA/(kg BW·d⁻¹) with an ADFI of 25 g/(kg BW·d⁻¹), a considerably lower dose compared with the study of Jain et al. (2012) in which 30 mg CDCA/(kg BW·d⁻¹) was TPN fed to newborn piglets. This lower dose may explain the lack of difference.

Plasma Co-EDTA and lactulose levels data for intestinal permeability tended both to be lower for CDCA at day 10.5 after weaning (\( P = 0.054 \) and \( P = 0.089 \) in Figure 1a and 1b, respectively). Hence, intestinal permeability was lower for piglet fed CDCA diet compared with control diet, indicating that administration of CDCA increases intestinal health, in line with our hypothesis. Interestingly, cobalt and lactulose responses in the present study were correlated (\( r = 0.67 \); \( P < 0.01 \); Figure 2), indicating that both markers served a similar purpose.

Dietary CDCA did not affect the FITC-dextran concentrations in the everted intestinal sacs (\( P > 0.05 \)). These data were also not associated with Co-EDTA and lactulose permeability measurements. The FITC analysis of the buffer revealed that the FITC concentrations dropped quickly (half-life ± 3 h), even though exposure to light was avoided by aluminum foil. This is raising doubts about the validity of the test and makes data interpretation difficult.

In conclusion, serum Co-EDTA and lactulose concentrations at day 10.5 tended to be lower in CDCA pigs compared with the control pigs, implying that CDCA deserves further study as a means for improving intestinal health. A correlation found between Co-EDTA and lactulose suggests that both marker molecules measure similar change in permeability and could be well used in future to determine intestinal permeability.

**LITERATURE CITED**


