

Impact of high zinc oxide supplementation or lignocellulose supplement on fecal microbiota of weaned piglets.

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This project aimed to study the effect of high zinc oxide (ZO) and fiber supplementation from lignocellulose (LIGCEL) on the fecal microbiota of weaned piglets. At weaning (21 days), 150 piglets (6.39 ± 0.28 kg) were transferred to a nursery farm, and divided into 30 pens of 5 piglets each according to their weaning weight. Each pen was assigned to one of the following treatments: Control (CON, 150 mg/kg of zinc), ZO (2,500 mg/kg of zinc), and LIGCEL (CON + 3% lignocellulose). fed to the animals administered for 14 days. In each pen, 2 to 3 fresh fecal samples were collected and pooled on day 14. The 16s RNA gene amplification and sequencing were performed at the Genomic Analysis Platform (Université Laval, Québec, Canada). Amplicon sequence variants (ASVs) were generated using the DADA2 pipeline, considering the SILVA rRNA database. Alpha diversity was calculated on normalized data (evenness: Shannon and Simpson). For the beta diversity, the Bray Curtis distance matrix was calculated and plotted using a Nonmetric multidimensional scaling (NMDS) plot. The effects of diets were tested using a non-parametric PERMANOVA model, with 999 permutations. The differential abundance analysis on the different taxa was performed using Linear discriminant analysis Effect Size (LEfSe) at family level. An LDA score of 3 was used as a cutoff value. A total of 11,083 ASVs were obtained. The ASVs were associated with 23 phyla and 107 families. The most abundant phyla were Firmicutes ($71 \pm 0.08\%$), Bacteroidota ($25 \pm 0.07\%$), and Proteobacteria ($2 \pm 0.04\%$); the most abundant families were Lachnospiraceae ($30 \pm 0.11\%$), Lactobacillaceae ($26 \pm 0.13\%$), and Prevotellaceae ($20 \pm 0.08\%$). Alpha diversity was not affected by diet. Diet significantly affected the Adonis test ($R^2 = 0.20$, $P = 0.001$). Piglets receiving the LIGCEL supplement were characterized by a higher abundance of Lactobacillaceae ($P_{\text{adj}} = 0.0007$, $\text{LDA_score} = 5.20$), Veillonellaceae ($P_{\text{adj}} = 0.026$, $\text{LDA_score} = 4.38$), Rikenellaceae ($P_{\text{adj}} = 0.009$, $\text{LDA_score} = 3.78$), Succinivibrionaceae ($P_{\text{adj}} = 0.001$, $\text{LDA_score} = 3.68$), and Selenomonadaceae ($P_{\text{adj}} = 0.038$, $\text{LDA_score} = 3.61$). CON piglets were characterized by a higher abundance of Enterobacteriaceae ($P_{\text{adj}} = 0.046$, $\text{LDA_score} = 4.35$), Christensenellaceae ($P_{\text{adj}} = 0.0003$, $\text{LDA_score} = 4.09$), and Spirochaetaceae ($P_{\text{adj}} = 0.0003$, $\text{LDA_score} = 4.09$). Piglets receiving the ZO diet were characterized by a higher abundance of Lachnospiraceae ($P_{\text{adj}} = 0.0002$, $\text{LDA_score} = 5.11$), Prevotellaceae ($P_{\text{adj}} = 0.011$, $\text{LDA_score} = 4.88$), Ruminococcaceae ($P_{\text{adj}} = 0.0009$, $\text{LDA_score} = 4.30$), Clostridiaceae ($P_{\text{adj}} = 0.045$, $\text{LDA_score} = 4.00$), and Butyricicoccaceae ($P_{\text{adj}} = 0.015$, $\text{LDA_score} = 3.76$). The addition of ZO clearly modified the fecal microbiota of piglets, highlighting families positively associated with intestinal

health, as Lachnospiraceae, Prevotellaceae, and Ruminococcaceae. The lignocellulose supplement also promoted the development of favorable families, namely Lactobacillaceae and Rikenellaceae. In contrast, CON piglets were characterized by the presence of bacteria from the Enterobacteriaceae family.

Keywords: piglet, weaning, zinc oxide, fiber, microbiota.