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Effects of dietary hop β-acids or colistin on the performance, nutrient digestibility, and intestinal health of weanling pigs

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ABSTRACT

The purpose of this study was to evaluate the effects of dietary graded levels of hop (*Humulus lupulus*) β-acids or colistin on the growth performance, nutrient and energy digestibility, diarrhea occurrence, unthrifty pigs, blood respiratory burst, organ weights, small intestine histology, and intestinal microbial diversity of weanling pigs, as well as *in vitro* bacterial sensitivity to hop β-acids. Two hundred 21-d weaned castrated male and female pigs (6.2 ± 0.33 kg initial BW) were used in a randomized complete block design experiment with 5 treatments, 8 replicates, and 5 pigs per experimental unit (pen). Pigs were fed corn-soybean meal basal diets supplemented with 0 (negative control), 120, 240, or 360 mg/kg hop β-acids, or with 40 mg/kg colistin (antimicrobial control) during a 35-d nursery feeding experiment. On d 7 and 35 of the experiment, one castrated male from each pen was slaughtered to evaluate organ weights, small intestine histology, and intestinal microbial diversity. ANOVA and orthogonal contrasts were performed to determine the dose-response of each variable to dietary hop β-acids levels (0, 120, 240, and 360 mg/kg), as well as to compare the means of antimicrobial control with the negative control and with each dietary hop β-acids level. Increasing dietary levels of hop β-acids improved linearly ($P < 0.05$) BW, ADG, G:F, and digestibility of ether extract of feed of weanling pigs. The colistin treatment improved ($P < 0.03$) BW, ADG, and G:F compared to the negative control but did not affect nutrient and energy digestibility. No difference in growth performance was observed between hop β-acids (120, 240, or 360 mg/kg) and colistin. No effect on ADFI was observed. Overall, the occurrence of diarrhea was lower ($P < 0.01$) for colistin, negative control, and 360 mg/kg hop β-acids than for 120 and 240 mg/kg hop β-acids, while no unthrifty pigs were identified throughout the entire experimental period. No effect of dietary treatments on blood respiratory burst, organ weights, small intestine histology, and intestinal microbial diversity was observed. By using the standardized paper disk diffusion method, *Staphylococcus aureus* was

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CP, crude protein; G:F, gain:feed ratio; ME, metabolizable energy; PCA, principal component analysis; PCR-DGGE, polymerase chain reaction—denaturing gradient gel electrophoresis; TTAD, total tract apparent digestibility; UPGMA, unweighted pair group method with arithmetic mean.

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sensitive to hop β-acids (33.1 ± 2.57 mm inhibition zone), while *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhimurim*, and *Enterococcus faecalis* were resistant. In conclusion, dietary hop β-acids concentrations up to 360 mg/kg improved weanling pig growth rate by affecting the efficiency of feed utilization, as well as observed for colistin.

1. Introduction

The post-weaning period of pigs has been associated with limited feed digestion and nutrient absorption, anorexia, diarrhea, and reduced growth rate (Lallès et al., 2007). Consequently, adaptive gastrointestinal responses have been observed with changes in organ weights, mucosa, microbiota, and immune activation (Pluske et al., 1997). Therefore, non-therapeutic dietary doses of antimicrobials have been used to improve the health and performance of weanling pigs. However, due to increasing restrictions of antimicrobial use as growth promoters, there is a great need for alternative feed additives.

Hop (*Humulus lupulus* L., Cannabaceae) β-acids, fraction of secondary metabolites from the female plant cones that share a basic alicyclic structure of 2.4-cyclohexadiene-1-one, to which is attributed its antimicrobial activity, is represented by the compound lupulone and its congeners (colupulone, adlupulone, prelupulone, postlupulone, and adpostlupulone) (Siragusa et al., 2008). Hop β-acids have been reported as a growth enhancer for broiler chickens (Cornelison et al., 2006; Bozkurt et al., 2009; Bortoluzzi et al., 2015), showing antimicrobial activity against *Clostridium perfringens* in broiler chicken intestines (Siragusa et al., 2008; Tillman et al., 2011; Bortoluzzi et al., 2015). Properties being attributed to hop β-acids also include antiviral (Buckwold et al., 2004), anti-inflammatory (Bortoluzzi et al., 2016; Tang et al., 2011), antioxidant (Tagashira et al., 1995), antidepressant (Zanoli et al., 2005), and anticancer (Lamy et al., 2007). In addition, hop bitter-acids have shown to affect lipid metabolism by improving hepatic fatty acid β-oxidation (Shimura et al., 2005) and reducing adipose tissue (Sumiyoshi and Kimura, 2013; Yajima et al., 2004) in mice. However, no scientific information concerning the effects of hop β-acids on pigs is available. Based on these findings, we hypothesize that hop β-acids are able to improve the intestinal health and growth performance of weanling pigs.

The purpose of this study was to evaluate the effects of dietary graded levels of hop β-acids or colistin (antimicrobial growth promoter) on growth performance, nutrient and energy digestibility, diarrhea occurrence, frequency of unthrifty pigs, respiratory burst, organ weights, and intestinal histology and microbial diversity of weanling pigs, as well as to study *in vitro* bacteria sensitivity to hop β-acids.

2. Materials and methods

The study was carried out at the Swine Research Unit of College of Agriculture "Luiz de Queiroz", University of Sao Paulo (Piracicaba, SP, Brazil). All procedures using animals were previously approved by the institutional animal care and use committee (Protocol 2012-10).

2.1. Animals, experimental design, and dietary treatments

Two hundred 21-d weaned castrated male and female pigs (6.2 ± 0.33 kg initial BW) were allotted according to initial BW and sex in a randomized complete block design experiment with 5 dietary treatments, 8 replicates per treatment, and 5 pigs per experimental unit (3 castrated males and 2 females per pen). Pigs were raised in 1.20 × 1.50 m pens with partially slatted floors equipped with a self-feeder, a nipple waterer, and a supplementary heat source in a naturally ventilated nursery room. Before pig housing, the nursery room was cleaned only with water blasting, avoiding chemical disinfectants in order to provide a low sanitary condition to pigs, similar to a model tested by Le Floc'H et al. (2006). Pigs had *ad libitum* access to water and feed in mash form throughout the 35-d nursery feeding experiment. The nutritional program consisted of three-phase feeding basal diets (Table 1) formulated to meet the nutrient requirements of pigs (Rostagno et al., 2011): pre-starter 1 (0–7 d), pre-starter 2 (7–21 d) and starter (21–35 d).

The experimental treatments were as follows: basal diet unsupplemented (negative control); basal diet supplemented with 40 mg/kg of colistin (antimicrobial control); and basal diet supplemented with graded levels of hop β-acids (120, 240, or 360 mg/kg). Because hop β-acids have strong bitter taste, a preliminary acceptance assay was performed to establish the hop β-acids levels tested in this experiment in order to have similar feed intake among all dietary treatments. In order to prevent oxidation, hop β-acids used in this study were microencapsulated with cellulose to contain 30% total active principle (2.4-cyclohexadiene-1-one based-compounds). Total hop β-acids concentration in the microencapsulated product was confirmed by solvent extraction with ethyl acetate followed by quantification using standard references in high-performance liquid chromatography-mass spectrometry accordingly EBC 7.8 Method (EBC, 2005). The stability (self-life) of the microencapsulated product was tested following a 30-d incubation period at 45 °C in the presence of light and oxygen, showing a minimum 90% total hop β-acids recovery. Both additives, hop β-acids and colistin, were supplemented in the basal diet according the concentration of active principle in each treatment.