



Effects of *Lactobacillus acidophilus* supplementation in different energy and nutrient density diets on growth performance, nutrient digestibility, blood characteristics, fecal microbiota shedding, and fecal noxious gas emission in weaning pigs



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ABSTRACT

This study was conducted to evaluate the effects of *Lactobacillus acidophilus* supplementation in different energy and nutrient density diets on growth performance, nutrient digestibility, blood characteristics (blood urea nitrogen and creatinine), fecal microbiota shedding, and fecal noxious gas emission in weaning pigs. A total of 140 crossed [(Landrace × Yorkshire) × Duroc] weaning pigs with an initial body weight (BW) of 7.51 ± 0.79 kg were used in a 42-day trial. Pigs were randomly allocated into one of four treatment groups in a 2×2 factorial arrangement with two levels of energy and nutrient density (Phase1: 3750 and 3900 kcal/kg; Phase2: 3550 and 3700 kcal/kg) and *L. acidophilus* (0 and 0.1%) according to sex and BW (7 replicates each with 2 gilts and 3 barrows). Pigs fed high energy and nutrient density diets increased ($P < 0.05$) average daily gain (ADG), gain:feed (G:F) ratio, as well as H₂S and acetic acid emission than those fed low energy and nutrient density diets. Pigs fed *L. acidophilus* supplementation diets increased ADG and G:F ($P < 0.05$) compared with pigs fed diets without *L. acidophilus* supplementation. Moreover, *L. acidophilus* supplementation led to a significant ($P < 0.05$) increase in DM digestibility on day 14 and shift microbiota by increasing fecal *Lactobacillus*, while decreasing *E. coli* counts, as well as a significant ($P < 0.05$) decrease in serum BUN concentration and fecal noxious gas emission. The interactive effects ($P < 0.05$) of dietary energy and nutrient density and *L. acidophilus* supplementation were observed on the fecal H₂S and acetic acid gases emission. In conclusion, dietary supplementation of *L. acidophilus* improved growth performance, ATTD of DM, shifted microbiota by increasing fecal *Lactobacillus* and decreased *E. coli* counts, decreased BUN concentration and fecal noxious gas emission in weaning pigs. No interactive effects of *L. acidophilus* supplementation and dietary energy and nutrient density on growth performance, nutrient digestibility (DM, N, and GE), blood characteristics (BUN and creatinine) and microbiota (*Lactobacillus* and *E. coli*). While the beneficial effects of *L. acidophilus* supplementation on H₂S and acetic acid gases emission are more dramatic with high energy and nutrient density diets.

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Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BUN, blood urea nitrogen; BW, body weight; DM, dry matter; N, nitrogen; GE, gross energy; G:F, gain:feed ratio.

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1. Introduction

Antibiotics have been commonly used as growth promoters in animal feed since 1950s (Castanon, 2007), but antibiotic resistance and food safety are growing issues (Asai et al., 2011; Bélanger et al., 2011; Dheilly et al., 2011). Therefore, it is urgent to find antibiotics alternatives, especially since the use of antibiotics was completely banned in Europe since 2006. The researchers are focused on the development of antibiotic alternatives with the aim to maintain animal health and performance (Chen et al., 2005; Giang et al., 2012). Particular interest is now being paid to probiotics to benefit the pigs by improving growth performance, nutrient digestibility, gut health, and immunity function (Fuller, 1989; Min et al., 2004; Meng et al., 2010; Yang et al., 2012; Lee et al., 2016). According to Giang et al. (2010), weaning pigs fed with probiotic complex diets had higher feed intake, daily gain, feed conversion ratio and decrease diarrhea incidence. Likewise, inclusion of *L. plantarum* and *L. reuteri* complex improved average daily gain and decreased diarrhea score in weaning pigs (Zhao and Kim, 2015). However, other studies report that no significant effects were observed on digestibility in nursery pigs (Xuan et al., 2001) and growing pigs (Wang et al., 2009) fed *bacillus*-based probiotics. The effect of probiotics supplementation is highly inconsistent because of the different diet composition, strains, age of animals, administration level, as well as its interactions with environmental factors (Xuan et al., 2001; Min et al., 2004; Wang et al., 2009). Mountzouris et al. (2010) reported that the beneficial function of probiotic may have an energy and nutrient cost because probiotic have nutrient requirements for their growth and proliferation. Our previous studies have confirmed that the efficacy of probiotics was influenced by dietary energy and nutrient density in growing-finishing pigs (Meng et al., 2010; Yan and Kim, 2013). Therefore, we hypothesize that the efficacy of *L. acidophilus* may be enhanced by feeding high energy and nutrient density diet in weaning pigs. This study was aimed to evaluate the effects of *L. acidophilus* supplementation in different energy and nutrient density on growth performance, nutrient digestibility, blood characteristics, fecal microbiota shedding, and fecal noxious gas emission in weaning pigs.

2. Materials and methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of *Lactobacillus acidophilus*

Dried *L. acidophilus* fermentation product was kindly provided by a commercial company (Diamond V, Cedar Rapids, IA, USA). The *L. acidophilus* fermentation product derived from *L. acidophilus* in an anaerobic fermentation technology platform to produce beneficial microbial metabolites. *L. acidophilus* was grown in solid state fermentation, and traditional feed ingredients was used as the substrate. This product is composed of a mixture of spray-dried *L. acidophilus* and other metabolites, such as organic acids and peptidoglycans, which help promote the activity of the beneficial bacteria in the gastrointestinal tract. There are at least 5×10^{10} cfu/g *L. acidophilus* in spray-dried powder. The bacterial concentration was determined by an ultraviolet spectrophotometer (Nano Drop, Thermo Fisher, America) at an OD of 550 nm.

2.2. Experiment design, animals, housing and diets

A total of 140 weaning pigs [(Landrace \times Yorkshire) \times Duroc] with an average body weight (BW) of 7.51 ± 0.79 kg (28 d of age) were used in a 42 d experiment. Pigs were randomly allotted to 1 of 4 treatments in a 2×2 factorial arrangement with 2 levels of energy and nutrient density, and 0 or 0.1% *L. acidophilus* according to their sex and BW (7 replicate pens with 5 pigs, 3 gilts and 2 barrows). Pigs were subjected to a 2-period feeding program, consisting of Phase 1 (d 0–14) and phase 2 (d 15–42). All diets were formulated to meet or exceed the NRC (1998) nutrient requirements (Table 1). All pigs were housed in an environmentally controlled room with a slatted plastic floor. Each pen was equipped with a 1-sided self-feeder and a nipple waterer to allow the pig ad libitum access to feed and water throughout the experimental period.

2.3. Growth performance and nutrient digestibility

Individual BW was determined at the beginning of the experiment, on d 0, 14 and 42 of the experiment. Feed intake was recorded on a pen basis throughout the experiment to calculate average daily feed intake (ADFI), and gain:feed ratio (G:F). From d 8 to 14 and d 36 to 42, pigs were fed the diets mixed with 0.2% chromic oxide as an indigestible marker to determine apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and gross energy (GE). On d 14 and 42, about 50 g fecal samples were collected from all pigs in each pen via rectal massage and pooled within pen. Fecal and feed samples were stored at -20°C until further analysis. For chemical analysis, fecal samples were dried at 70°C for 72 h and finely ground to pass through a 1 mm screen. The procedures used for the determination of DM, N, and GE digestibility were in accordance with the methods established by AOAC (2005). Chromium concentrations were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the ATTD of DM, N, and energy was calculated using the methods described by Fenton and Fenton (1979). The digestibility was calculated according to the following formula: $\text{ATTD} = [1 - \{(N_f \times C_d) / (N_d \times C_f)\}]$, where N_f = nutrient concentration in feces (%DM), N_d = nutrient concentration in diets (%DM), C_f = chromium concentration in feces (%DM), and C_d = chromium concentration in diets (%DM). GE was determined by measuring the heat of combustion in

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