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Effects of diosmectite-*Lactobacillus acidophilus* on growth performance, intestine microbiota, mucosal architecture of weaned pigs

Shuting Cao[a,1], Li Wang (Dr.)[b,*,1], Lefei Jiao[a], Fanghui Lin[a], Kan Xiao[a], Caihong Hu (Dr.)[a,*]

[a] *Animal Science College, Zhejiang University, The Key Laboratory of Molecular Animal Nutrition, Ministry of Education, Hangzhou, 310058, China*

[b] *Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangzhou, 510640, China*

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ABSTRACT

Diosmectite-*Lactobacillus acidophilus* composite (DS-*L. acidophilus*) was prepared by immobilization of *L. acidophilus* onto DS. The surival rate of *L. acidophilus* in DS-*L. acidophilus* were determined after simulated gastric juice and intestinal fluid. A total of 180 piglets, weaned at 21 ± 1 d age, were used to investigate the effects of DS-*L. acidophilus* in intestinal function. The *vivo* trial included five groups: (1) control; (2) *L. acidophilus*; (3) DS; (4) DS-*L. acidophilus*; (5) The mixture of diosmectite and *L. acidophilus* (DS + *L. acidophilus*). The amount of DS or *L. acidophilus* in each group was equivalent. The results *in vitro* showed that DS-*L. acidophilus* increased ($P < 0.05$) the survival rate of *L. acidophilus* in simulated gastric juice for 80 min and intestinal fluid for 240 min, as compared with the free *L. acidophilus*. The results *in vivo* showed that, as compared with control, DS-*L. acidophilus* increased ($P < 0.05$) average daily gain, intestinal *Lactobacillus*, the ratio of villus height and crypt depth, the jejunal and colonic transepithelial electrical resistance. The DS-*L. acidophilus* addition decreased ($P < 0.05$) the paracellular permeability of fluorescein isothiocyanate dextran 4 kDa in jejunum and colon. However, DS, *L. acidophilus* or DS + *L. acidophilus* had no ($P > 0.05$) effect. The results indicated that DS-*L. acidophilus* was more effective in improving growth performance and intestinal function of weaned pigs than DS, *L. acidophilus* or DS + *L. acidophilus.*

1. Introduction

Probiotics are living microorganisms, which confer a health benefit on the hostwhen administered in adequate amounts (FAO/WHO, 2002; Mennigen and Bruewer, 2009). *Lactobacillus acidophilus* (*L. acidophilus*) have already been used for health promoting additives in weaned pigs (Guerra et al., 2007). A number of studies demonstrated that *L. acidophilus* can alleviate

Abbreviations: FD4, fluorescein isothiocyanate dextran 4 kDa; TER, transepithelial electrical resistance.

* Corresponding authors.
E-mail addresses: wanglineau@163.com (L. Wang), chhu@zju.edu.cn (C. Hu).

[1] Both authors contributed equally to the work.

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intestinal inflammation and increases intestinal integrity. Introduction of *L. acidophilus* into the gastrointestinal tract of animals restores mucosal adherent probiotic populations by producing immunoregulatory factors that may enhance colonization or survival in the host (Lewis and Burmeister, 2005; Chen et al., 2009; Qiao et al., 2015). However, some studies reported that no significant effects were observed (Wang et al., 2009; Lan et al., 2016; Zhao and Kim, 2015). One of the major problems in the efficacy of probiotics is the low survival rate in gastric pH and high concentrations of bile salts in the intestine (Simon et al., 2001; Sabikhi et al., 2010). Ingested probiotic bacteria must survive through the gastrointestinal tract, tolerating acid, bile and gastric enzymes, and then show viability at the site of action (Huang and Adams, 2004; Ding and Shah, 2007). In this regard, stabilization and protection of probiotics against harsh gastrointestinal conditions to increase survivability plays an important role in the beneficial effect of probiotics.

Diosmectite (DS) is an aluminosilicate clay mineral (Hu et al., 2013a,b). Due to its excellent adsorbent properties based on the high aspect ratio, research on DS as a support to adsorb and immobilize probiotic cells has increased interest (Sun et al., 2008; Li et al., 2014). DS has been researched as an effective drug delivery carrier for controlled-release of bioactive molecules, drugs and nutrients, such as epidermal growth factor (EGF) (Vaiana et al., 2011), ibuprofen (Zheng et al., 2007) and vitamin B6 (Joshi et al., 2009). Li et al. (2014) demonstrated that lactic acid bacteria was adhered to the surface of DS and were covered or buried by DS particles. Therefore, we hypothesis that DS provide a physical barrier for *L. acidophilus* against harsh environmental conditions, and then release the *L. acidophilus* in the GI tract. The adsorption of *L. acidophilus* onto DS and the biological effects *in vivo* were not previously reported. In this study, diosmectite-*Lactobacillus acidophilus* composite (DS-*L. acidophilus*) was prepared by immobilization of *L. acidophilus* onto DS. The surival rate of *L. acidophilus* in DS-*L. acidophilus* in simulated gastrointestinal juices and the effects on weaned pigs were investigated.

2. Materials and methods

2.1. Preparation of DS-L. acidophilus

The DS content was 99.0%, and the cation exchange capacity (CEC) was 1.30 mmol/kg. The CEC values of the diosmectite were determined with the $[Co(NH3)6]^{3+}$ method (Zhu et al., 2007). The *L. acidophilus* (CGMCC 1.1878) was purchased from China General Microbiological Culture Collection Center. This strain was stationarily cultivated in MRS medium (Difco, USA) at 37 °C for 24 h. The DS-*L. acidophilus* was prepared as described previously (Li et al., 2014). Preparation was conducted by mixing 10 g/L of DS and 1×10^8 CFU/mL of *L. acidophilus* in 0.01 mol/L $NaNO_3$ (pH = 7.0) at 30 °C with shaking at 90 rpm for 2.0 h. The separation of the unattached bacteria from the fraction containing mineral power and attached bacteria was accomplished by injecting a sucrose solution (60% by weight) into the bottom of the DS-*L. acidophilus* suspension (Guo et al., 2011). The mineral powder with any adsorbed bacteria sank to the bottom of the test tube, and the unattached bacteria and aqueous solution floated on top of the sucrose layer. After the sucrose separation, the suspension of unattached bacteria in the supernatant was discharged. Then DS-*L. acidophilus* was acquired according to treatment in a vacuum freeze-drying machine (Tofflon, Shang Hai, China), and *L. acidophilus* content in DS-*L. acidophilus* was 5×10^8 CFU/g.

2.2. Survival rate of L. acidophilus in simulated gastrointestinal juices

In *vitro* trial included three groups: (1) *L. acidophilus*; (2) DS-*L. acidophilus*; (3) the mixture of diosmectite and *L. acidophilus* (DS + *L. acidophilus*). The survival rate of *L. acidophilus* in simulated gastrointestinal juices was determined according to the procedure described by Guerra et al. (2007). Simulated gastric juice and intestinal fluid were prepared fresh by dissolving respectively 3 g/L pepsin from porcine stomach mucosa and 1 g/L pancreatin from porcine pancreas in 5 g/L sterile saline, the pHs of the gastric juice and intestinal fluid were adjusted to 2.0 and 8.0, respectively. All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA). Add 10^9CFU of *L. acidophilus*, DS + *L. acidophilus* and DS-*L. acidophilus* to 10 mL of gastric juice or intestinal fluid and incubated at 37 °C after brief vortexing. The number of viable *L. acidophilus* was determined by the method of Hrenovic et al. (2009). When assaying gastric transit tolerance, aliquots of 100uL were removed after 40 and 80 min for determination of survival rate. When assaying for small intestinal transit tolerance, the sampling times were 120 and 240 min. The experiment was repeated triplicate.

2.3. Experimental design and samples collection

All procedures were approved by the Zhejiang University Animal Care and Use Committee. A total of 180 weaned piglets (Duroc × Landrace × Yorkshire), with an average initial weight of 6.2 kg weaned at 21 ± 1 d, were allocated to five treatment groups for three weeks, each of which was replicated six times with six pigs per replicate. The dietary treatments were as follows: (1) control (piglets fed the basal diet); (2) *L. acidophilus* (piglets fed the basal diet supplemented with *L. acidophilus*); (3) DS (piglets fed the basal diet supplemented with the DS); (4) DS-*L. acidophilus* (piglets fed the basal diet supplemented with the DS-*L. acidophilus*); (5) DS + *L. acidophilus* (piglets fed the basal diet supplemented with the mixture of DS and *L. acidophilus*). The amount of DS or *L. acidophilus* in each group was 1.5 g/kg and 7.5×10^8 CFU/kg, respectively. Diets were formulated to meet or exceed requirements suggested by the National Research Council (2012), and their compositions are shown in Table 1. The crude protein (method 984.13), lysine (method 994.12), methionine (method 994.12), calcium (method 935.13), phosphorus (method 964.06) and zinc (method 986.15) in the feed were determined according to the