Livestock Science 178 (2015) 251–254

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effects of *Lactobacillus brevis* preparation on growth performance, fecal microflora and serum profile in weaned pigs

H. Liu, H.F. Ji*, D.Y. Zhang, S.X. Wang, J. Wang, D.C. Shan, Y.M. Wang

Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form
22 May 2015
Accepted 3 June 2015

Keywords:
Lactobacillus brevis
Growth performance
Fecal microflora
Serum profile
Weaned pigs

ABSTRACT

This study was conducted to evaluate the effects of *Lactobacillus brevis* ZLB004 on growth performance, fecal microflora, and serum profile in weaned pigs. A total of 144 weaned pigs (Duroc × Yorkshire × Landrace) with an average initial body weight of 15.60 ± 0.13 kg were randomly assigned to 3 treatments, with 4 replicate pen per treatment and 12 pigs per pen. Pigs were fed the basal diet supplemented with 0, 0.4, and 0.8 g/kg of *L. brevis* ZLB004. All pigs were given free access to feed and water for 30 d. The results showed that pigs fed diets with *L. brevis* ZLB004 increased average daily gain, average daily feed intake, and gain to feed ratio ($P=0.026$, 0.031, and 0.022 respectively), while decreased diarrhea incidence ($P=0.044$) compared with the control group. On d 30, dietary *L. brevis* ZLB004 increased lactobacillus populations ($P=0.001$), reduced fecal coliform populations ($P=0.022$). Supplemental *L. brevis* ZLB004 increased serum interferon-γ and total protein concentrations ($P=0.024$ and 0.044, respectively), while decreased serum haptoglobin and blood urea nitrogen ($P=0.014$ and 0.040, respectively). The results showed that *L. brevis* ZLB004 had beneficial effects on the improvement of intestinal microflora balance, immunity, and growth performance of weaned pigs.

1. Introduction

Since the 2006 European ban on the use of in-feed antibiotic growth promoters, probiotics have received increasing interest as an alternative means to improve animal health and protect against infectious challenges. Probiotics are defined as live microorganisms that, when administered in adequate amounts, conferring a health benefit on the host (Reid et al., 2003). The mechanisms by which probiotics exert their beneficial actions involve competitive exclusion through mucosal binding, production of bacteriocins/defensins, reduction of luminal pH, modulation of mucosal and systemic immune response and reinforcement of nonspecific intestinal barrier (Ng et al., 2009).

Lactobacillus brevis is a heterofermentative gram-positive organism and widely found in fermented foods of both plant and animal origin (Mante et al., 2003; Sánchez et al., 2000) as well as in intestinal flora (Delgado et al., 2005; Gu et al., 2008). As a member of the genus *Lactobacillus* and due to its long-term use in various traditionally fermented food products, *L. brevis* is granted a generally recognized as safe status by the FDA (2003). Since Kishi et al. (1996) reported a *L. brevis* strain as a potential probiotic, several laboratories have reported the utility of *L. brevis* as a probiotic (Rönkä et al., 2003; Soo et al., 2008; Yakabe et al., 2009). In those studies, the strains showed several properties including high adhesion ability to low pH, bile salts and pancreatic fluid, high adherence to Caco-2 cells, as well as competitiveness against *Salmonella* and *Escherichia coli*. Although these properties made it a promising probiotic feed additive, experiments using *L. brevis* as a feed additive in pigs were scarce. Therefore, the aim of the current study was to evaluate the effects of a probiotic strain (*L. brevis* ZLB004) on growth performance, fecal microorganisms, and serum profile in weaned pigs.

2. Material and methods

2.1. Strains

The probiotic preparation used in the current study was manufactured in our laboratory and contained 5.50×10^9 cfu/g live bacteria of *L. brevis* ZLB004. *L. brevis* ZLB004 was isolated from the gastrointestinal mucosa of healthy weaned piglets in our laboratory and identified by the China Center of Industrial Culture Collection (Beijing, China). Previous study has shown that the strain had good resistance to heat, low pH, bile salts, and antagonism to pathogenic bacteria such as *Salmonella* and *E. coli* (H. Liu, unpublished data).

* Corresponding author. Fax: +86 10 88433070.
E-mail address: jhf207@126.com (H.F. Ji).

http://dx.doi.org/10.1016/j.livsci.2015.06.002
1871-1413/

Table 1
Ingredient and chemical composition of the basal diet (as-fed basis).

Item	Content
Ingredient (%)	
Corn	64
Soybean meal	26
Wheat bran	6
Limestone	1.1
Dicalcium Phosphate	0.9
Salt	0.4
Vitamin and mineral premix[a]	1.6
Chemical composition	
DE (MJ/kg)[b]	13.20
CP (%)[c]	16.50
Ca (%)[c]	0.60
P (%)[c]	0.52
Lys (%)[b]	0.85
Met (%)[b]	0.37

[a] Provided per kilogram of complete diet: vitamin A, 5000 IU; vitamin D_3, 2000 IU; vitamin E, 20 mg; vitamin K_3, 2.5 mg; vitamin B_1 2.5 mg; vitamin B_2, 6.6 mg; vitamin B_6, 3 mg; vitamin B_{12}, 0.025 mg; biotin, 0.2 mg; niacin, 25 mg; pantothenic acid, 13 mg; choline chloride, 200 mg; Cu, 12 mg; Fe, 150 mg; Mn, 55 mg; Zn, 120 mg; I, 0.5 mg; and Se, 0.3 mg.
[b] Calculated value.
[c] Analyzed value.

2.2. Animals, diets and experimental design

A total of 144 (Duroc × Yorkshire × Landrace) weaned pigs (49 ± 2 d of age) with an average initial body weight (BW) of 15.60 ± 0.13 kg were randomly assigned to 3 treatments balanced for sex, weight and litter origin and each treatment had 4 replicate pen with 12 pigs per pen. Pigs were fed the basal diet supplemented with 0, 0.4, and 0.8 g/kg of *L. brevis* ZLB004. All pigs were given free access to feed and water for 30 d. The basal control diet was formulated according to the nutrient requirement recommended by NRC (1998). The feed composition of the basal diet is shown in Table 1.

2.3. Measurements and analyses

Individual weaned piglet BW and feed consumption from each pen were recorded on d 0 and 30 during the study to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

To evaluate the frequency of diarrhea, the number of diarrheic piglets per pen was collected daily throughout the study. Diarrhea incidence (%)=(Total number of pigs with diarrhea)/(whole number of pigs × experimental days) × 100 (Wang et al., 2007).

On the final day of the study, fresh fecal samples were randomly collected from 6 pigs of each pen for enumerating the fecal microbial counts. The microbiological assay of fresh fecal samples was carried out as previously described by Torrallardona et al. (2003). The colony counts were counted after culturing in Petri plates with the corresponding culture media: MacConkey agar for coliforms and MRS agar for lactobacilli. The plates were incubated at 37 °C for 48 h. Bacterial counts were expressed as $\log_{10}$ cfu/g.

Blood samples were collected on the final day by precaval vein puncture into 10-mL vacuette tubes. Six samples were randomly collected from each pen. Serum was collected after centrifugation at 3000*g* for 15 min at 4 °C and stored at −20 °C until analysis. The serum concentrations of albumin (ALB), globulin (GLOB), total protein (TP), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), and the serum activities of alanine amiotransterase (ALT) and aspartate amiotransterase (AST) were determined using automatic biochemical analyzer (Model 7020; Hitachi, Tokyo, Japan) with corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Haptoglobin (HP) and interferon-γ (IFN-γ) were determined using commercially available porcine ELISA kits (Immunology Consultants Laboratory, Newberg, OR).

Table 2
Effects of *Lactobacillus brevis* preparation on growth performance of weaned pigs.

Item	*L. brevis* (g/kg)			SEM[1]	*P*-value
	0	0.4	0.8		
Initial body weight (kg)	15.50	15.75	15.54	0.48	0.719
Final body weight (kg)	22.89[b]	26.57[a]	25.08[a]	0.75	0.024
Average daily gain (g)	246.3b	360.7[a]	318.0[a]	16.7	0.026
Average daily feed intake (g)	590.5[b]	679.4[a]	677.8[a]	23.3	0.031
Gain to feed ratio (g/g)	0.417[a]	0.531[b]	0.469[b]	0.041	0.022
Diarrhea incidence (%) [2]	3.16[a]	1.95[b]	2.33[b]	0.15	0.044

[a,b] Means within row with different superscripts differ ($P < 0.05$).
[1] SEM=standard error of means.
[2] Diarrhea incidence (%)=(Total number of pigs with diarrhea)/(whole number of pigs × experimental days) × 100.

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Each pen served as the experimental unit. Differences among means were tested using Tukey's test and *P*-values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Growth performance

Effects of lactobacillus preparation on growth performance are shown in Table 2. No differences in initial BW were observed among the three groups. Pigs fed diets with 0.4 and 0.8 g/kg of *L. brevis* ZLB004 increased ADG, ADFI, and G:F (P=0.026, 0.031, and 0.022 respectively), and decreased diarrhea incidence (P=0.044), compared with the control group. No difference was observed in the performance between dietary treatments.

3.2. Fecal microbial populations

Effects of lactobacillus preparation on fecal microbial populations are shown in Table 3. Compared with the control group, dietary supplementation with *L. brevis* ZLB004 preparation increased lactobacillus populations (P=0.001) and reduced fecal coliform populations (P=0.022), compared with the control group. No difference was observed in fecal microbial populations between dietary treatments.

3.3. Serum profile

Effects of lactobacillus preparation on serum indices are presented in Table 4. Compared with the control group, supplemental *L. brevis* ZLB004 increased serum IFN-γ and TP concentrations (P=0.024 and 0.044, respectively), while decreased serum HP and BUN (P=0.014 and 0.040, respectively). There were no differences in the levels of GLOB, A/G, GLU, ALT, AST, TC and TG among the groups during the experiment.