

# Real-Time PCR Detection of Lactic Acid Bacteria in Cecal Contents of *Eimeria tenella*-Infected Broilers Fed Soybean Oligosaccharides and Soluble Soybean Polysaccharides

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**ABSTRACT** This experiment was conducted to test whether dietary soybean meal oligosaccharides (SMO) and water-soluble polysaccharides (SMP) can assist broiler chickens in resisting *Eimeria tenella*, and to determine the survival of lactic acid bacteria in cecal contents postinfection. All birds received a soybean meal-free diet. The 6 experimental treatments were as follows: positive (COR) and negative (COW) control groups, 2 groups fed diets containing either 1% SMO or 0.5% SMP from 1 to 11 d of age; a vaccinated group (VAC), and an anticoccidial medicated group (ANT). Chickens of all treatments except COW were orally infected with 1,000 sporulated oocysts of *E. tenella* on d 15. Fecal oocyst shedding was

monitored per treatment group between d 5 and 13 postinfection. Lactic acid bacteria (LAB) in cecal contents were evaluated by a real-time PCR technique on d 7 postinfection. The results showed that the SMO and SMP groups had a lower number of oocysts per gram of feces during the monitoring period than the COR group. Threshold cycles were 22.21, 27.68, 13.99, 14.92, 12.97, and 14.85, for COW, COR, SMO, SMP, VAC, and ANT groups, respectively; specific PCR products were confirmed by the results of melting curve analysis and agarose gel electrophoresis. The results suggest that these LAB communities were promoted by SMO and SMP and have a competitive exclusion function when broiler chickens are infected with *E. tenella*.

(Key words: broiler, cecal contents, lactic acid bacteria, melting curve analysis, real-time PCR)

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## INTRODUCTION

Broiler chickens have been selected for a high rate of gain within the intensive poultry industry. They are assumed to have poor immunocompetence, and are considered more susceptible to various types of infections (Havenstein et al., 1994). Coccidiosis infection is one of the major contributors to production loss, especially in young chickens. Generally, a complex community of microorganisms colonizes the broiler's distal intestine, and plays an important role in reducing the risk of infection with enteropathogens, a phenomenon known as competitive exclusion (Nurmi and Rantala, 1973). Lactic acid bacteria (LAB), as part of the normal gut bacterial flora, can be modulated and stimulated by the presence of prebiotics (Buddington and Weiher, 1999; Gibson, 1999; Jenkins et al., 1999; Rao, 1999; Reddy, 1999; Brady et al., 2000; Menne and Guggenbuhl, 2000). Furthermore, LAB showed a competitive exclusion

effect in broiler chickens (Kaldhusdal et al., 2001; Hofacre et al., 2002; van der Wielen et al., 2002). Experimental results from one of our research studies have shown that the cecal LAB communities of broiler chickens were stimulated by dietary soybean oligosaccharides (SMO) and water-soluble polysaccharides (SMP), especially when added to the diet for 2 wk posthatching (unpublished data). It was hypothesized that LAB communities stimulated by SMO and SMP could exert a competitive exclusion function for broiler chickens infected by *E. tenella*. This work describes an experiment designed to test this hypothesis.

## MATERIALS AND METHODS

### *Isolation of Oligosaccharides and Water-Soluble Polysaccharides from Soybean Meal*

Soybean meal oligosaccharides SMP were extracted from soybean meal as follows. One kilogram of soybean meal

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**Abbreviation Key:** ANT = anticoccidial treatment group; COR = positive control group; COW = negative control group; LAB = lactic acid bacteria; SMO = soybean meal oligosaccharides; SMP = soybean meal polysaccharides; Tm = melting temperature; VAC = vaccinated group.

was refluxed twice in 10 L of alcohol [80% (vol/vol)] at 75°C for 1 h. The supernatant was used for SMO isolation and the residue for SMP isolation.

The supernatant was condensed by Rotavapor (Büchi RE 120)<sup>2</sup> and protein was subsequently removed with 5% trichloroacetic acid. The liquid was freeze-dried after passing a column filled with Amberlite 200 and Amberlite IRA-900<sup>3</sup> to remove trichloroacetic acid and amino acids from the supernatant. Replicate SMO products extracted at different times were mixed together and stored at -20°C.

The residue was hydrolyzed in a 0.2 M NaOH solution at 80°C for 2 h and filtered using cheesecloth. The filtrate was neutralized with 30% acetic acid and freeze-dried. The freeze-dried sample, which was a mixture of several extractions, was dissolved in 400 mL of water. Protein was removed with 5% trichloroacetic acid. After 1 h, the supernatant was centrifuged at 13,000 × *g* for 30 min. To precipitate SMP, alcohol was added to the supernatant until the alcohol concentration was 80% (vol/vol), and the mixture was stored at 4°C overnight. The polysaccharide precipitate was centrifuged at 2,500 × *g* for 10 min. The polysaccharide product was freeze-dried. Replicate SMP products extracted at different times were mixed together and stored at -20°C.

## Birds and Diets

The experimental protocol was approved by Experimental Animal Committee of China Agricultural University. Two hundred forty 1-d-old male Arbor Acres broilers were obtained from a commercial producer. They were divided into 6 groups with similar mean weight ( $P > 0.05$ ) and housed per group in cages (100 × 100 × 60 cm) until 30 d of age. The basal diet free of soybean meal, the experimental diet formulation, and its nutrient contents are listed in Table 1. The basal diet was heated to 80°C for 1 h to kill possible coccidia, and then mixed with SMO, SMP, or the anticoccidial drug, maduramicin, after which the diet was cooled to room temperature. The diets were kept at -25°C until the day before use, and then brought to a temperature of 31°C.

The 6 experimental treatments are shown in Table 2, including negative (COW) and positive controls (COR). The treatment groups included diets with soybean meal oligosaccharides (SMO) and water-soluble polysaccharides (SMP), a vaccination group (VAC), and an anticoccidial medicated group (ANT). In the SMO and SMP groups, diets were formulated by diluting the basal diets with 1% SMO and 0.5% SMP, respectively. The amount of SMO or SMP was matched to the normal content of corn-soybean meal basal diet for broilers of the same age. The birds received this diet from 1 to 11 d of age. Thereafter, to prevent birds suffering from the stress caused by sudden feed change, the experimental diets were substituted by a

TABLE 1. The diet formulations and nutrient content<sup>1</sup>

Ingredient	Basal diet (% as fed)
Ground corn	47.90
Soybean meal	—
Corn germ meal	15.00
Corn gluten meal	24.0
Fish meal	7.00
CaHPO <sub>4</sub>	0.70
Limestone	1.60
Salt	0.30
Sunflower oil	2.50
Vitamin-mineral mixture <sup>2</sup>	1.00
Crude protein	20.21
Metabolizable energy (kcal/kg)	3,019
Ca	1.00
P	0.64
Met	0.48
Lys	0.72
Trp	0.04
Arg	1.06
Leu	3.09
Ile	1.00
Phe	1.30
Thr	0.86
Val	1.19
His	0.55

<sup>1</sup>Soybean meal oligosaccharides (SMO) and soybean meal water-soluble polysaccharides (SMP) were used in the basal diet, diluting basal diet by 1% SMO and 0.5% SMP, respectively. Soybean meal oligosaccharides and SMP consisted of 724 and 275 g/kg total sugar, and 15.7 and 504.6 g/kg of protein, respectively.

<sup>2</sup>Provided vitamins and minerals as previously described (Lee and Leeson, 2001). Provided per kilogram of diet: vitamin A, 8,800 IU; vitamin D<sub>3</sub>, 3,300 IU; vitamin E, 40 IU; vitamin K<sub>3</sub>, 3.3 mg; thiamine, 4.0 mg; riboflavin, 8.0 mg; panthothenic acid, 15 mg; niacin, 50 mg; pyridoxine, 3.3 mg; choline, 600 mg; folic acid, 1 mg; biotin, 220 mg; vitamin B<sub>12</sub>, 12 mg; ethoxyquylin, 120 mg; manganese, 70 mg; zinc, 70 mg; iron, 60 mg; copper, 10 mg; iodine, 1.0 mg; and selenium, 0.3 mg.

30% basal diet at d 12; 60% at d 13; 90% at d 14, and 100% by d 15. In the VAC group, the birds were vaccinated with 100 oocytes of *E. tenella* at 3 d of age. In the ANT group, maduramicin (5 ppm) was added to the diet from 5 d before infection up to and including d 7 postinfection.

Chickens had access to feed and water ad libitum. Room temperature was approximately 31°C for the first 5 d and was then gradually reduced to around 27°C and kept at this temperature until the end of the experiment. Light was provided continuously throughout the study.

## Parasite and Experimental Infections

The strain of *E. tenella* used was developed from a single oocyst, multiplied to ensure that coccidia used for infection were all *E. tenella*. Oocysts originally came from the *E. tenella* Houghpon strain maintained at the Laboratory of Parasitology and Parasitological Disease, College of Veterinary Medicine, China Agricultural University, China. Chickens, excluding those in the COW group, were orally infected with 1,000 sporulated oocysts of *E. tenella* at 2000 h on d 15.

## Oocyst Counting

Fecal oocyst shedding was monitored following infection per treatment group between the d 5 and 13 after infection

<sup>2</sup>Rotavapor, BÜCHI Labortechnik AG, Postfach, Switzerland.

<sup>3</sup>Amberlite 200 and Amberlite IRA-900, Polysciences, Inc., Warrington, PA.

TABLE 2. Experimental treatment groups

	COW <sup>1</sup>	COR <sup>2</sup>	SMO <sup>3</sup>	SMP <sup>3</sup>	VAC <sup>4</sup>	ANT <sup>5</sup>
Basal diet	yes	yes	yes	yes	yes	yes
Soybean meal oligosaccharides (SMO)	no	no	yes	no	no	no
Soybean meal polysaccharides (SMP)	no	no	no	yes	no	no
Vaccinated with <i>E. tenella</i>	no	no	no	no	yes	no
Infected with <i>E. tenella</i>	no	yes	yes	yes	yes	yes
Anticoccidial medicine	no	no	no	no	no	yes

<sup>1</sup>COW = negative control.

<sup>2</sup>COR = positive control group.

<sup>3</sup>SMO and SMP = treatment diets containing soybean meal oligosaccharides (SMO) and water-soluble polysaccharides (SMP); these diets were formulated by diluting the basal diets with 1% SMO and 0.5% SMP respectively. The amount of SMO or SMP matched the normal content of corn-soybean meal basal diet for broilers of the same age. The birds received this diet from 1 to 11 d of age. Thereafter, in order to prevent birds suffering from the stress caused by feed sudden change, the experimental diets were substituted by a 30% basal diet at d 12; 60% at d 13; 90% at d 14 and 100% by d 15.

<sup>4</sup>VAC = vaccination group, in which the birds were vaccinated with 100 oocytes of *E. tenella* at 3 d of age.

<sup>5</sup>ANT = anticoccidial medicated group, in which maduramicin (5 ppm) was added to the diet from d 5 before infection up to and including d 7 after infection.

by the McMaster's method (Hodgson, 1970). Fecal samples were collected by group for 24 h. Samples were homogenized in a mixer (CDE-300A)<sup>4</sup> for 15 min. Two grams of sample was suspended in 10 mL of water and then diluted in 50 mL of a saturated salt solution. The oocysts were counted microscopically in a McMaster chamber. Oocyst number per gram of feces was calculated by number of oocysts counted/chamber ×100 (Xun and Guo, 1998).

### Sampling and DNA Extraction

Forty-eight birds, 8 from each of the 6 treatments, were euthanized by cervical dislocation on d 7 after infection. Cecal contents were examined for the presence of LAB. To reduce the effect of individual differences on the experimental result, the cecal contents of the birds from each treatment group were combined and mixed in a sterilized bottle immediately after death. The samples were kept at -80°C pending further analysis. Cecal content bacteria were purified and total DNA was extracted using the methods of Lewington et al. (1987) and Wang et al. (1996).

### Primers and Real-Time Quantitative PCR

Primers specific for LAB of the genera *Lactobacillus*, *Pediooccus*, *Weissella*, and *Leuconostoc* were used for this work (Walter et al., 2001). The primers included forward primer Lac1 (5'-AGC AGT AGG GAA TCT TCC A-3') and reverse primer Lac2GC (5'-GC-Clamp-ATT YCA CCG CTA CAC ATG-3'). Polymerase chain reaction with primers Lac1 and Lac2GC amplified a 340-bp fragment of the V3 region of the 16S rRNA gene.

Real-time PCR and melting curve analysis were performed using a Light Cycler rapid thermal cycler instrument (BIORAD cycler)<sup>5</sup> according to the method described

by Komurian-Pradel et al. (2001). Reactions were performed in a 20- $\mu$ L volume with 2.5 nM each of Lac1 and Lac2GC primers, 2.5 nM of Mg<sup>2+</sup>, 20  $\mu$ M of each dNTP, 0.2  $\mu$ L of Taq DNA polymerase, 1  $\mu$ L of SYBR Green I, and 1  $\mu$ L of extracted DNA. Melting curve determinations were done immediately after PCR was completed. Polymerase chain reaction amplified product melting temperature (T<sub>m</sub>) was determined by calculating the derivatives of the curve with the Light Cycler software and visualized by plotting the negative derivative against the temperature (Komurian-Pradel et al., 2001). Each sample had 3 repeats in a real-time PCR run. Subsequently, the PCR products were further confirmed using agarose gel electrophoresis.

### Data Analysis

The results were compared as means using a 1-way ANOVA model, the significant difference between groups were analyzed by LSD *t*-test, performed using SPSS 8.0 (SPSS, Inc., 1997) statistical software.

## RESULTS

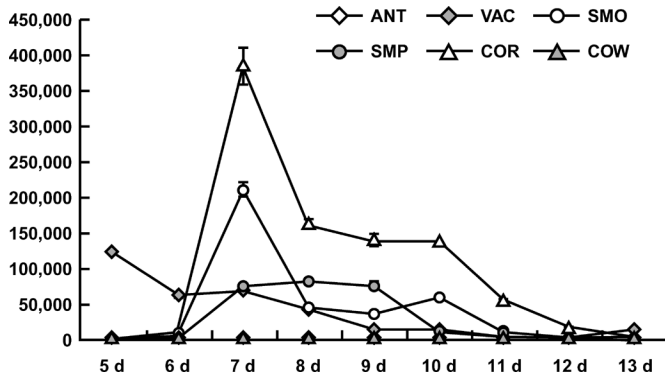
### Fecal Oocyst Shedding in Broilers

Fecal oocyst shedding was monitored per group between 5 and 13 d following infection. Figure 1 shows the plot of oocyst number per gram of feces vs. days postinfection. There were no oocysts found in feces from birds in the negative control group (COW) throughout the study period.

Compared with the positive control group (COR), the anticoccidial treatment group (ANT) had negligible numbers of oocysts in feces; the VAC, SMO, and SMP groups had lower numbers of oocysts between 7 and 13 d postinfection ( $P < 0.05$ ). The VAC group had a higher number of oocysts in feces than the others at d 5 and 6 postinfection ( $P < 0.05$ ), and the curve of the VAC group shows that the number of oocysts per gram of feces declined throughout

<sup>4</sup>Ouke Electrics Co., Ltd., GuangDong, China.

<sup>5</sup>Light Cycler rapid thermal cycler, Biochip Technical Center, Academy of Military Medical Science of China, Beijing, China.



**FIGURE 1.** Number of oocysts per gram of feces (y-axis) against days postinfection (x-axis), showed fecal oocyst shedding of broilers infected with  $10^3$  sporulated oocysts of *Eimeria tenella* at 15 d of age. Mean came from 3 counts for each sample. Error bars represent standard deviation between mean. COW = negative control group; COR = positive control group; SMO = diet with soybean meal oligosaccharides; SMP = diet with water-soluble soybean meal polysaccharides; VAC = vaccination group; ANT = anticoccidial medicated group.

the study following infection. The SMP group did not show a significant peak.

### Melting Curve Analysis

To demonstrate that the amplification was specific for the target LAB gene, a melting curve analysis was performed immediately after the real-time PCR procedures. Melting curve analysis showed COW, SMO, SMP, VAC, and ANT with  $T_m$  (melting peaks) at 91.2, 91.3, 91.2, 90.9, and 91.3°C, respectively (Figure 2), and each group had one predominant peak. There were no significant differences in  $T_m$  between groups ( $P > 0.05$ ,  $SME = 0.08$ ). Meanwhile, the agarose gel electrophoresis analysis (Figure 2) demonstrated that this peak corresponded to a single band. In the case of the COR group, the melting curve did not show a clear peak around 91°C, and the electrophoresis result showed only a dim band.

### Real-Time PCR Amplification

Real-time PCR amplification of LAB V3 region gene of the genera *Lactobacillus*, *Pediococcus*, *Weissella*, and *Leuconostoc* was carried out by a real-time PCR technique performed on a Light Cycler instrument 7 d after *E. tenella* infection of the birds. Figure 3 is a plot of fluorescence vs. threshold cycle derived from 6 groups using the Lac1 and Lac2GC group specific primers. Threshold cycles were 22.21, 27.68, 13.99, 14.92, 12.97, and 14.85, for COW, COR, SMO, SMP, VAC, and ANT groups ( $SEM = 1.31$ ), respectively. The threshold cycles of SMO, SMP, VAC, and ANT groups were lower than those of COW and COR groups ( $P < 0.05$ ), and threshold cycle in COW was lower than that in COR ( $P < 0.05$ ). In other words, a comparison of the LAB population in cecal contents in term of treatment groups was  $VAC > SMO > ANT > SMP > COW > COR$ .

## DISCUSSION

### Competitive Exclusion of LAB

*Eimeria tenella* undergoes schizogony and gametogony in the cecal wall of broiler. The oocyst, which is shed in the feces, is the mature form of the infective coccidia. Merozoites of the first generation schizonts invade the cecal lamina propria via the crypt epithelial cells. The second-generation schizonts develop in the lamina propria within the crypt cells, and the new merozoites invade other cecal cells directly through the connective tissue or via the cecal epithelial cells (Fernando et al., 1983). Generally, the number of oocysts shed is dependent on the number of sporozoites and merozoites that penetrate the enterocytes for a given inoculum dose, and represents the infection-resistant ability of broilers. Cecal LAB have been shown to play an important role in competitive exclusion of pathogenic bacteria by several mechanisms during bacterial infection (Fukata et al., 1999; van der Wielen et al., 2002); these results suggest that the same may also be true for coccidia, including *E. tenella*.

The results indicate (Figure 1) that the number of oocysts per gram of feces in all experimental groups was much lower during the monitoring period (7 to 13 d postinfection) when compared with the COR group. However, the inhibitory mechanism may have been different for the different groups. In the case of the SMO and SMP groups, there are several possible mechanisms involved. Firstly, the results of another experiment in our laboratory showed that SMO and SMP stimulated the development of cecal LAB when these 2 nondigestible carbohydrates were used in the diets of the 1- to 11-d-old broilers; other gut bacteria may have been stimulated by SMO and SMP concurrently with LAB. Indigenous bacteria occupy gut mucosal surfaces and may prevent the coccidial parasite from attaching to the mucosa, rendering it unable to penetrate the surface epithelial layer. This is one of the mechanisms involved in competitive exclusion. Second, the fact that birds have relatively short intestines and can eat as much as their gut capacity allows, means that a large quantity of undigested carbohydrates and other substrates can reach the hindgut. Volatile fatty acids represent a major product when microorganisms ferment these carbohydrates, and can lead to a lower pH of cecal contents. This low pH may have negative effects on *E. tenella* growth. Thirdly, some strains of LAB, particularly *Lactobacilli*, can augment the nonspecific defense mechanism of the host (in rat; Bloksma et al., 1979), or modulate the specific immune response in a different fashion (in humans; Vidal et al., 2002). It is apparent that the negligible number of oocysts in the ANT group was caused by the inhibition activity of the anticoccidial medicine in the diet. However, in the case of the VAC group, *E. tenella*-specific antibodies were produced after the birds were vaccinated with *E. tenella* at d 3, and some memory immune cells were produced after the vaccination. Therefore, *E. tenella*-specific antibody secretion plays a key role in *E. tenella* exclusion when the birds are infected on d 15 of life. These results will be reported separately.

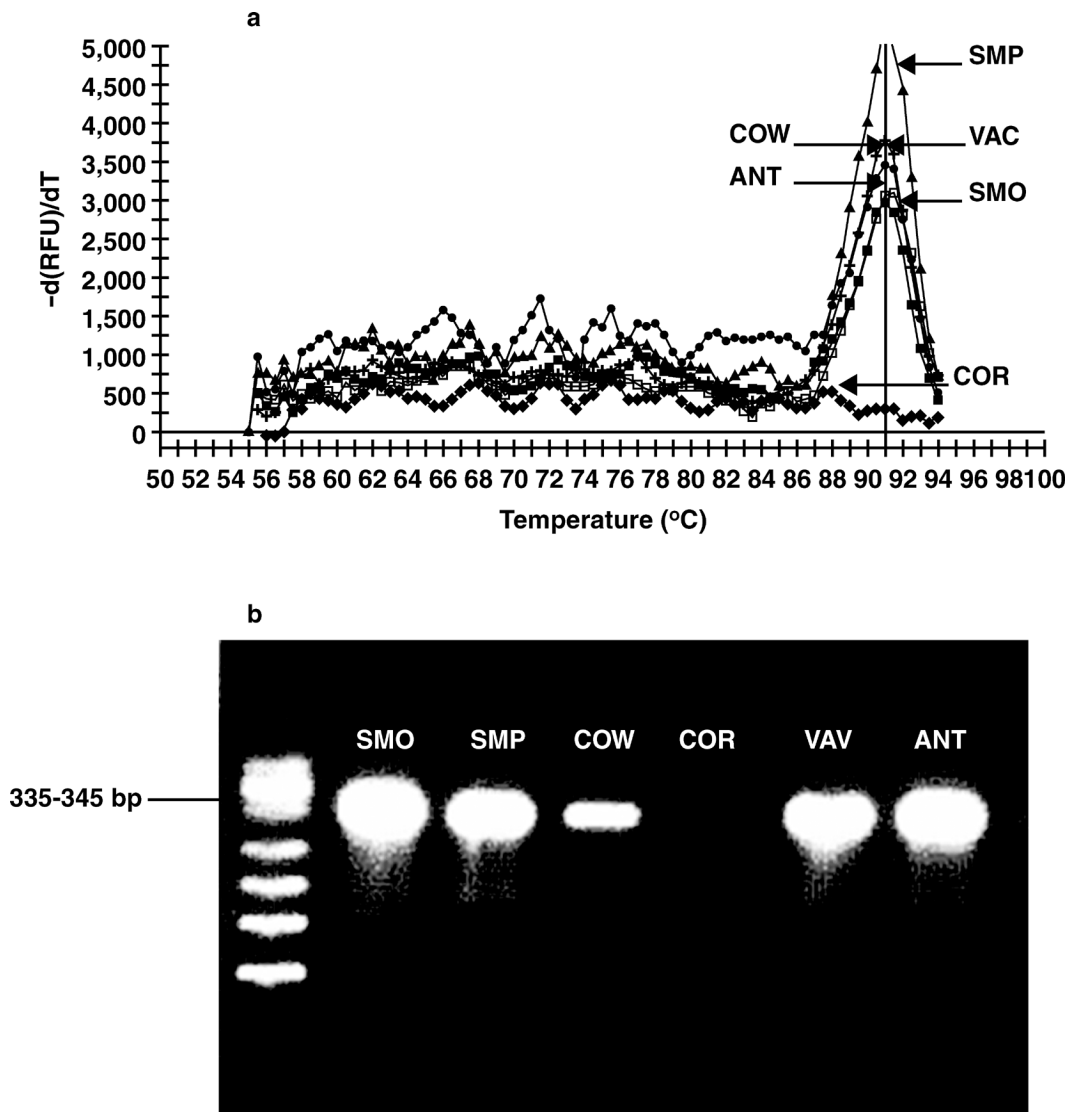


FIGURE 2. a) Plot of the negative derivative of the melting curves (y-axis: fluorescence  $-dF/dT$ ) vs. melting temperature (x-axis:  $T_m$  in  $^{\circ}\text{C}$ ); b) Agarose gel electrophoresis analysis. COW = negative control group; COR = positive control group; SMO = diet with soybean meal oligosaccharides; SMP = diet with water-soluble soybean meal polysaccharides; VAC = vaccination group; ANT = anticoccidial medicated group.

### Diet and *E. Tenella* Infection

In this experiment, a soybean meal-free basal diet was used, and other than the SMO and SMP groups, this diet was not changed throughout the study. Soybean meal oligosaccharides and SMP were used in the diets from 1 to 11 d of age. To eliminate the interaction between SMO, SMP, and coccidia infection, the experimental diets were totally substituted by the basal diet 24 h before infection, because it has been reported that polysaccharides can block the receptor-mediated invasion of some pathogens (Dalton et al., 1991). That means the competitive exclusion ability of the birds in SMO and SMP groups is caused by dietary soy carbohydrate during the early stage posthatching. Newly hatched chicks are particularly vulnerable to invasion by pathogens before their intestinal microbial ecosystem becomes established. Therefore, accelerating the process of gut microbial community development, especially to increase the beneficial bacteria population, is important

for young broiler chickens. Soybean meal oligosaccharides and SMP, as nondigestible but fermentable carbohydrates, might help the establishment of gut microbial ecosystem. The lower oocyst output and the higher survival of the LAB population in the birds, which accepted soy soluble carbohydrates, demonstrated that dietary SMO and SMP stimulated the growth of the gut microbial community. As a result, the competitive exclusion ability was promoted when the birds were infected with *E. tenella*. Meanwhile, other mechanisms might be involved in effecting competitive exclusion, which will require further study.

### Real-Time PCR and Melting Curve Analysis

SYBR Green I dye is a nonspecific intercalating dye, and the reaction is made specific by using "hot-start" PCR and empirically determined annealing and signal acquisition temperatures for each gene-specific primer (De Preter et al., 2002). It implies that the analysis of the melting curve

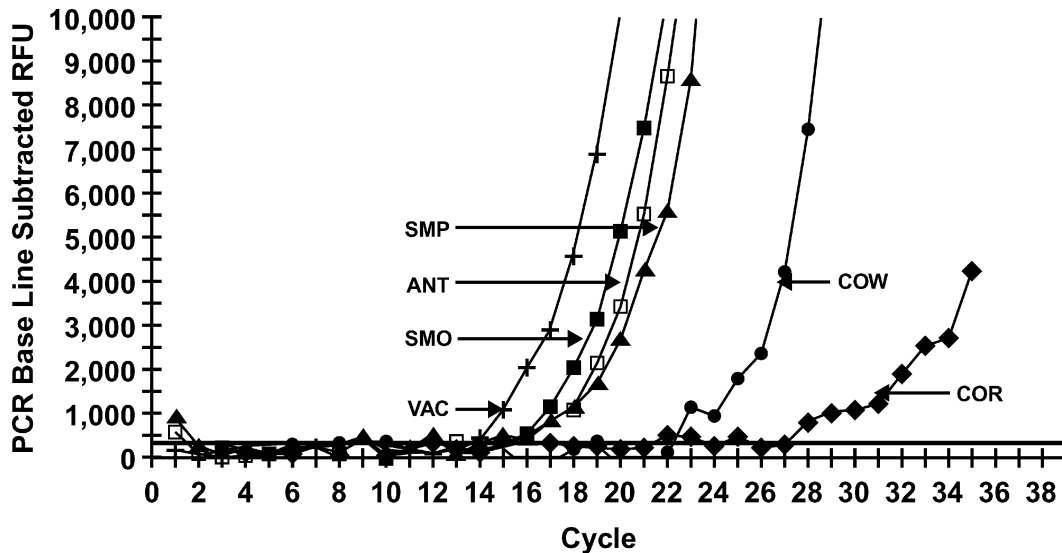


FIGURE 3. A plot of fluorescence (y-axis) against threshold cycle (x-axis) for each treatment. COW = negative control group; COR = positive control group; SMO = diet with soybean meal oligosaccharides; SMP = diet with water-soluble soybean meal polysaccharides; VAC = vaccination group; ANT = anticoccidial medicated group.

provides a check that specific amplification has occurred; it also distinguishes products from nonspecific primer-dimers and highlights any contamination (Ririe et al., 1997; Pietilä et al., 2000). In this study, the melting curve demonstrated that primers were specific for LAB of the genera *Lactobacillus*, *Pediococcus*, *Weissella*, and *Leuconostoc*, because they amplified one major specific product with a distinct melting peak. For the melting curve of the COR group, no peak was present around 91°C. This result indicated that no specific PCR product was melted at that temperature. However, a stranded PCR product was detected at amplification cycle 27.68 (Figure 3), which may have originated from nonspecific PCR products with a melting temperature below 86°C (Figure 2).

The shape and position of a DNA melting curve is characterized by its GC/AT ratio, sequence, and length (Ririe et al., 1997). During PCR amplification, nonspecific products, primer-dimers, or even the fold of Lac2GC itself, can form double-stranded DNA that can be bound by SYBR Green I. In this melting curve, there were some trivial nonspecific peaks before the predominant peaks. The nonspecific peaks were distinguishable from the specific amplification PCR product. However, no primer-dimer products were visible in the gel electrophoresis analysis of the same samples. It was therefore assumed that these peaks were associated with the DNA samples isolated directly from cecal contents and consisted of a complex DNA mixture of various bacteria. The Lac2GC reverse primer, which was originally designed for denaturing gradient gel electrophoresis analysis, is longer (68 bp) because of the 40-bp GC clamp (Wu et al., 1998) and may fold during the real-time PCR procedure. There were only small differences in the dominant amplification  $T_m$  among groups, and that may have resulted from different components of LAB DNA that were covered by the group-specific primers used in this experiment.

Real-time PCR allows continuous detection of PCR products by monitoring SYBR Green I from the initial synthesis procedure. The sensitivity of SYBR Green I analysis is more than adequate for the majority of research applications, and its quantitative nature is demonstrated by the linear relationship between the log of the template concentration and the cycle number, named threshold cycle, at which fluorescence rises above the baseline (Simpson et al., 2000; Bates et al., 2001). The relative DNA expression levels are further quantified by constructing a standard curve using target DNA dilution.

Comparing the threshold cycles, VAC, SMO, ANT, and SMP groups had lower threshold cycle than the COW and COR groups, especially the COR group. Combined with the melting curve analysis results of the COR group, it may be concluded that there was hardly any LAB DNA in the cecal contents 7 d postinfection. The results suggest that SMO and SMP can stimulate LAB growth in the ceca of broilers during the early stage posthatching (unpublished results), and that LAB may play a competitive exclusion role in coccidia infection. In fact, there was greater survival of LAB in SMP and SMO birds after the infection. The birds of the VAC and ANT group had more surviving LAB than those in the COW group, which is an interesting finding requiring further analysis.

In this case, it was impossible to quantify the DNA in samples because the amplified DNA consists of several genera of LAB and the proportion of each genus is difficult to estimate. Consequently, the amplified DNA did not have a corresponding standard curve. Whether the standard curve can be done by single species of LAB or not needs further research.

In conclusion, it has been shown that soybean oligosaccharides and water-soluble polysaccharides (SMO and SMP) can promote growth of the lactic acid bacteria popula-

tion, which can lead to competitive exclusion in the cecal contents of *E. tenella*-challenged broilers. It is clear that when SMO and SMP are used in the diet in the early stage posthatch there will be an anticoccidial effect. The results suggest that under the conditions described here, SMO and SMP can have prebiotic properties in broiler diets.

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