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RESEARCH ARTICLE

Anti-Diarrheal Effects of a Combination of Korean Traditional Herbal Extracts and Dioctahedral Smectite on Piglet Diarrhea Caused by *Escherichia coli* and *Salmonella typhimurium*

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ABSTRACT

The present study evaluated anti-diarrheal effects of a mixture of *Coptidis rhizoma, Lonicerae flos,* and *Paeonia japonica* (1:1:1, v/v/v) methanol extracts and dioctahedral smectite (CLPD) on piglet diarrhea caused by *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*). Diarrhea index of group 1 administered by 0.5% CLPD mixed with feed, decreased with the passage of time and was insignificantly differed compared to that of control. In group 2 administered by 1.0% CLPD mixed with feed, diarrhea index was significantly decreased compared to that of control and group I during overall experimental periods (P<0.05). After administration of CLPD mixed with feed, the number of *E. coli* and *S. typhimurium* in piglet feces of group 1 except for the 1st day was significantly decreased compared to that of the control group (P<0.05), and the number of *E. coli* and *S. typhimurium* in piglet feces of group 2 except for the 1st day was significantly decreased compared to that of the control group and group I (P<0.05). This study showed that CLPD had anti-diarrheal effect on *E. coli* and *S. typhimurium* causing diarrhea in piglets. CLPD could be an effective candidate for the treatment of enteric bacterial infections in piglets.

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INTRODUCTION

Pathogenic *Escherichia coli* (*E. coli*) has caused scouring, increased mortality, and poor performance in the postweaning period (Bhandari *et al.*, 2008). *Salmonella typhimurium* (*S. typhimurium*) is one of the most frequently isolated pathogen from pig farms, slaughtered swine and human food-borne illness (Korsak *et al.*, 2003).

Generally, antimicrobial agents are used both therapeutically and prophylactically in *E. coli* and *Salmonella* infection. However, the increased resistance to these drugs has produced an unavoidable side effect of antibiotic use, and recent studies have shown a rapid increase in the prevalence of antibiotic resistance to *E. coli* and Salmonella (O'Brien, 2002; Alali *et al.*, 2009).

To mediate the problem of antibiotic resistance, medicinal herbs and clay minerals are gathering more attention. Bioactive components in medical herbs have been applied in clinical and therapeutic areas (Muragami *et al.*, 1996). *Coptidis rhizoma* (*C. rhizoma*) is used in oriental medicine as an antibacterial and anti-inflammatory agent (Tang *et al.*, 2009). The extract of *C. rhizoma* contains a high level of berberine and antibacterial activities in a variety of pathogenic microorganisms, including *E. coli*, *Salmonella*, *Pneumococcus*, *Mycobacterium tuberculosis*, *Staphylococcus* (Kwon *et al.*, 2008). *Lonicerae flos*

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(*L.flos*) is a widely used herb prescribed in many Korean formulas. It has antibacterial activity, antipyretic, detoxicant, and anti-inflammatory actions (Yun *et al.*, 2007). The aqueous extract of *Paeonia japonica* (*P. japonica*) has been used in oriental medicine to treat various illnesses because it possesses various pharmacological properties such as sedative, analgesic, anti-inflammatory, antimicrobial, immunoaugmentative, and anti-stress action (Muragami *et al.*, 1996; Jeong *et al.*, 2003).

The intentional consumption of clay materials by humans and animals is known as cosmetics, dietary or nutritional needs and medicinal benefits (Abrahams, 2002; Haydel *et al.*, 2008). Dioctahedral smectite clay mineral has been reported to have anti-diarrheal and antibacterial activity (Szajewska *et al.*, 2006; Chang *et al.*, 2007).

Although previous studies (Muragami *et al.*, 1996; Bae, 2005) investigated the antibacterial and antidiarrheal effects for each of Korean traditional herbs, few studies exist to investigate the effect of the combination of herbs and clay minerals on livestock. The present study evaluated the therapeutic potentials of the combination of dioctahedral smectite and methanol extracts from the medicinal herbs, *C. rhizoma, L. Flos,* and *P. japonica* on piglet diarrhea caused by *E. coli* and *S. typhimurium*.

MATERIALS AND METHODS

Preparation of a combined Herbal Extract

C. rhizoma, L. flos, and *P. japonica* were purchased from the Korea National Animal Bio Resource Bank (Gyeongnam, Korea) and dioctahedral smectite was purchased from Zhejiang Sanding Technology Co. (Shaoxing, China).

C. rhizoma, L. flos, and *P. japonica* were air-dried in a dark room and ground to a powder. Approximately 100 g of the combination of *C. rhizoma, L. flos,* and *P. japonica* (1:1:1, v/v/v) powder was soaked in 400 ml of methanol for 24 h under mantle-reflux. The solvent was removed under reduced pressure in a rotary evaporator (N-1000 S, EYELA Co., Tokyo, Japan). The extracts were filtered using Whatman No.1 filter paper, and the filtrates were freeze-dried with a vacuum freeze dryer (MCFD 8508, IlShinLab Co., Seoul, Korea) and blended into powder using a mill (KINEMATICA AG, Lucerne, Switzerland) with 90 standard mesh. The combination of the extract powder and dioctaheral smectite (1:1, v/v) was designated CLPD.

Animals and experimental diets

All experimental procedures were reviewed and approved by the Animal Ethical Committee of Gyeongsang National University Institutional Animal Care and Use Committee (GNU-LA-20).

A total of 30 piglets (Large White × Duroc × Landrace) post-weaned at 5 weeks of age with initial body weight of 6.1 ± 0.51 kg were used. Thirty pigs infected with *E. coli* and *S. typhimurium* were obtained from a commercial supplier (Kayainte Co., Ltd., Sacheon, Korea) and transported to an animal facility at Gyeongsang National University, Korea. Pens contained plastic-slatted flooring with a nipple bowl drinker and a feeder. A round feeding bowl was placed in each pen to encourage feed consumption. The ambient temperature was maintained at 25 ± 1°C during the experimental period. The piglets were offered the commercial feed (Purina Feed, Seongnam, Korea) and distilled water *ad libitum*.

Pigs were allocated to their experimental treatments based on initial body weight and block within room in the animal facility. The 10 pigs (control group) were housed in 1 room that contained 2 pens, with 5 pigs allocated to each pen (2 × 3 m), and were given the commercial feed (Purina Feed, Seongnam, Korea) without CLPD. The 10 pigs (group 1) were housed in another 2 rooms that contained 2 pens, with 5 pigs allocated to each pen, and were administered 0.5% CLPD mixed with feed for 7 days. The other 10 pigs (group 2) were housed in another 3 rooms that contained 2 pens, with 5 pigs allocated to each pen, and were administered 1% CLPD mixed with feed for 7 days.

Feces Score

The severity of diarrhea in control, group 1 and group 2 was noted by visually scoring the consistency of the feces on a standardized scale of 0–3 as described by Cox *et al*. (1987).

Enumeration of *E. coli* and *S. typhimurium* in diarrheal feces

Fecal samples were collected from each group on the 1st, 3rd, 5th, and 7th day after treatment of CLPD, and the number of *E. coli* and *S. typhimurium* were analyzed. To analyze the number of *E. coli*, fecal samples (1 g) were suspended in 0.1% bacto-peptone (Difco, MI, USA) solution containing 0.1% Tween 80 (Merck, Darmstadt, Germany). The initial suspensions and their tenfold serial dilution (0.1 ml) were cultured in duplicate on MacConkey agar (Difco, MI, USA). After incubation for 24 h at 37˚C, *E. coli* colonies were counted and represented as CFU/g. To analyze the number of *S. typhimurium*, fecal samples (1 g) were suspended in 0.1% phosphate buffered saline (PBS). The initial suspensions and their tenfold serial dilution (0.1 ml) were cultured in duplicate on Salmonella-Shigella agar (SS agar) (Oxoid Ltd., England, UK). After incubation for 24 h at 37˚C, *S. typhimurium* colonies were counted and represented as CFU/g.

Statistical analysis

The data were analyzed by a one-way analysis of variance (ANOVA) (SAS, 1999), followed by the results expressed as mean ± standard deviation (SD). The means were compared for significance by Student's *t*-test at $P<0.05$.

RESULTS

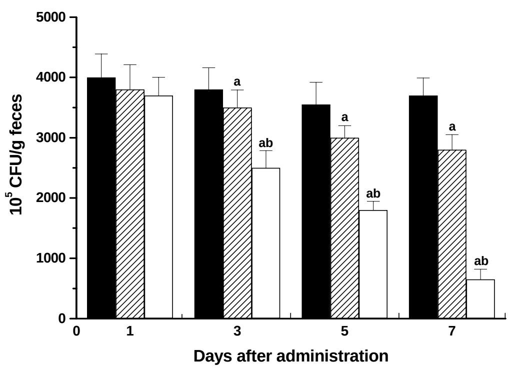
Diarrhea scores in group 1 were decreased depending on time, but there was no significant difference with the control group. In group 2, diarrhea scores were significantly decreased depending on time compared with control and group 1 ($P<0.05$), and was decreased 77.8%, compared to that of control on the 7th day as given in Table 1.

Table 1: Effect of CLPD on diarrhea index in piglets with diarrhea

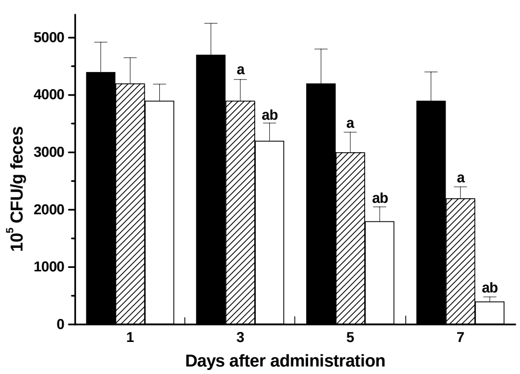
Group	No. of pig	Diarrhea Index[a]				
		0 day	1 day	3 day	5 day	7 day
Control	10	2.7±0.21	2.8±0.25	2.6±0.28	2.8±0.21	2.7±0.20
Group 1[b]	10	2.7±0.37	2.7±0.26	2.6±0.25	2.5±0.23	2.5±0.27
Group 2[c]	10	2.8±0.18	2.4±0.22*	1.7±0.19*	1.2±0.17*	0.6±0.24*

[a]0=firm, not remarkable feces (normal feces); 1=slightly loose diarrhea (pasty feces); 2=moderately loose diarrhea (semi-liquid feces); 3=profuse watery diarrhea; [b]Group 1 was administered with 0.5% CLPD; [c]Group 2 was administered with 1.0% CLPD; *P<0.05, compared to control and group 1.

The number of *E. coli* and *S. typhimurium* in group 1 was significantly decreased compared to control from the 3rd day to the 7th day after administration (P<0.05). In group 2, the number of *E. coli* and *S. typhimurium* was significantly decreased compared to control and group 1 during overall experimental periods (P<0.05) as shown in Fig. 1.



(A)



(B)

Fig. 1: Fecal *E. coli* (A) and *Salmonella* (B) counts in groups during the experimental period. Control group (■) administered with the normal feed (n=10); group 1 (▨) administered with the feed contained 0.5% CLPD (n=10) and group 2 (□) administered with the feed contained 1% CLPD (n=10). [a]Significantly different from the control group (P<0.05); [b]Significantly different from group 1 (P<0.05).

DISCUSSION

The antibacterial effect of herbal extracts has been well reported in many studies (Kim, 2002; Bae, 2005; Lin *et al.*, 2006; Kwon *et al.*, 2008), but few studies exist to investigate the effect of the combination of herb extracts and clay minerals on piglets. The present study was carried out to investigate the anti-diarrheal effect of CLPD in piglets infected with *E. coli* and *S. typhimurium*.

At 3 days after administration in the present study, fecal score of group 1 piglets treated with 1% CLPD was reduced by 34.6% compared with the control group. Bhandari *et al.* (2008) reported that the reduction of lesion score in diarrhea piglets challenged with *E. coli* K88$^+$ was 18.7% compared to control at 3 days treated with a complex of antibiotics (chlortetracycline 110 mg/kg feed, sulfamethazine 110 mg/kg feed, and penicillin G 55 mg/kg feed). Compared with the results by Bhandari *et al.* (2008), the effect of CLPD on pig diarrhea was evident in piglets where the reduction in the fecal score was higher than that produced by a complex of antibiotics composited of chlortetracycline, sulfamethazine, and penicillin G.

On the other hand, Jacks *et al.* (1980) reported that cephamycin C was used at a concentration of 10.4 mg/kg body weight orally twice a day for 4 days to treat diarrhea in piglets infected with *E. coli* and the reduction of diarrhea score was 63.3% compared to control at 3 days after medication. As cephamycin C was well-known highly effective antibiotics in restoring the piglets to good health by eliminating diarrhea, the effect of cephamycin C for the reduction of the fecal score was 1.8 times higher than that of CLPD used in the present study. However, cephamycin C-resistant bacteria including *E. coli* and *S. typhimurium* have emerged in recent years (Winokur *et al*., 2000; Clarke *et al*., 2003). But, CLPD composite of natural antibacterial herbs and clay mineral has the advantage of being an effective anti-diarrhea and does not appear to produce bacterial resistance.

Casey *et al.* (2007) reported that the administration of a five-strain probiotic combination in piglet infected with *S. typhimurium* for 9 days showed a 77% reduction in the fecal score compared with the control group. In our study, fecal score of group 2 treated with 1% CLPD at 7 days after administration was reduced 77.8% compared with the control group. With the consideration of the administration period, it indicated that the anti-diarrheal effect of 1% CLPD against *S. typhimurium* was higher than that of the study by Casey *et al.* (2007).

Jung *et al.* (2010) reported that the oral treatment of 1ml 20% dioctahedral smectite suspension three times a day in piglet infected with *E. coli* and *S. typhimurium* for 3 days showed a 77.7% reduction in the fecal score compared with the control group. Dioctahedral smectite is a natural hydrated alumino-magnesium silicate that strongly binds to digestive mucus and has the ability to absorb endotoxins and exotoxins, bacteria and rotavirus (Szajewska *et al*., 2006; Safaeikatouli *et al*., 2010). The effect of dioctahedral smectite for the reduction of the fecal score was more than 2 times higher than that of CLPD used in the present study, because there are differences in the dose of administration and absorption rate noted between oral administration and administration in feed.

In the study by Liu *et al.* (2008), throughout administration of chito-oligosaccharide supplement 200 mg/kg feed and chlortetracycline 80 mg/kg feed for 21 days, the number of *E. coli* was reduced 42.6 and 60.1%, respectively, compared with that before its administration. Jung *et al.* (2010) reported that administration of 1ml 20% dioctahedral smectite three times a day in piglet infected with *E. coli* and *S. typhimurium* reduced the number of *E. coli* and *S. typhimurium* in piglet feces by 93.7 and 97.4%, respectively, compared with that of the control group at 3 day after administration. Also, Casey *et al.* (2007) reported that administration of the fermented five-strain probiotic combination in piglet challenged with *S. typhimurium* for 7 days reduced by 56.5% the number of *S. typhimurium* in piglet feces compared with that at 1 day after administration. In the present study, the number of *E. coli* and *S. typhimurium* in group 2 at 7 days after treatment was reduced by 83.7 and 91.0%, respectively, compared with that of the control group. As the results of the above, the antibacterial activity of CLPD against *E. coli* and *S. typhimurium* was higher than that of the study by Liu *et al.* (2008) and Casey *et al.* (2007), respectively. As the use of probiotics and oligosaccharides reduced colonization levels and decreased the severity of diarrhea in *E. coli* and *S. typhimurium*-infected animals (Genovese *et al*., 2000; Paton *et al*., 2006), there was no the direct effect of antibiotics and absorption of toxin produced by pathogenic bacteria. However, CLPD had the antibiotic effects and the effects of absorption of bacterial toxins, which may be rapidly decreased the severity of diarrhea caused by *E. coli* and *S. typhimurim*. And the antibacterial activity of CLPD against *E. coli* and *S. typhimurium* was lower than that of the study by Jung *et al*. (2010) because of differences in dose and route of administration.

In conclusion, our results demonstrate that CLPD may be used to control diarrhea in piglets infected with *E. coli* and *S. typhimurium*. Then, CLPD could be an effective candidate for the treatment of enteric bacterial infections in piglets.

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