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<Short Communication>

Antibacterial and therapeutic effects of a combination of *Coptidis rhizoma* and *Galla rhois* extracts in piglets challenged with *Campylobacter coli*

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Abstract : The antibacterial effects of a combination of *Coptidis rhizoma* and *Galla rhois* extracts (CGE) were evaluated in piglets. The minimum bactericidal concentration of CGE was 2.0 mg/mL. Thirty 5-week-old piglets were challenged with *Campylobacter* (*C.*) *coli* after allocation to three different groups, a control and two treatment groups fed with CGE at 2.0 or 4.0 g/kg feed for 7 days. On day 7, *C. coli* in the feces of the CGE-treated groups were significantly lower than in the control ($p < 0.01$). These results suggest that CGE can be used to control *C. coli* in piglets.

Keywords : *Campylobacter coli*, *Coptidis rhizoma*, *Galla rhois*, piglet

Campylobacter spp. are one of the most dominant zoonotic bacteria which cause human gastroenteritis in many developing and industrialized countries [11]. In the European Union, the number of confirmed-campylobacteriosis cases in human amounted to 220,209 in 2011. In addition, human campylobacteriosis in the United States was estimated to affect over 1.3 million persons every year [15].

Both *Campylobacter* (*C.*) *jejuni* and *C. coli* asymptomatically colonize in the intestinal tract of birds and mammals, including food production animals which pose an important risk for human *C.* infection from contamination of carcasses at slaughter and of milk [12]. Pigs are a natural reservoir of *Campylobacter* spp. with *C. coli* as the dominant species. The prevalence of *C. coli* infection in pigs is known to be between 50 and 100%, and excretion levels of this pathogen ranged from 10^2 to 10^7 colony forming units (CFU)/g feces [6].

Recent studies have shown a rapid increase in the prevalence of antibiotic resistance to *Campylobacter* spp. due to the use of antimicrobial agent for the prevention and treatment of bacterial infection in farming livestock [10]. Ultimately, the rise of antibiotic-resistant *C.* on livestock could impact on human health.

Recently, medicinal herbs have received more attention to resolve the problem of antibiotic resistance after the ban on antibiotics as growth promoters in animal feed. Bioactive components in medical herbs have been applied in clinical and therapeutic areas [3, 7]. *Coptidis rhizoma* has been used in oriental medicine as an antibacterial and anti-inflammatory agent from long time ago [14]. The extract of *Coptidis rhizoma* contains a high level of berberine, an alkaloid possessing various antimicrobial activities in a variety of pathogenic microorganisms [8]. In addition, *Galla* (*G.*) *rhois* has long been used in traditional Asian medicine and is a harmless natural material that contains a number of tannin-derived components, including methyl gallate and gallic acid which have an antibacterial activity [1].

Although many previous studies [9, 12, 13] investigated the antimicrobial effects of medicinal herbs against *Campylobacter* spp., few studies exist to investigate the antibacterial and therapeutic effects for the combination of herbs on livestock infected by the bacteria. The present study evaluated the antibacterial and remedial potentials of an herbal combination containing *Coptidis rhizome* and *G. rhois* on piglets challenged with *C. coli*.

C. coli (ATCC 33559) kept at −80°C was recovered on Mueller-Hinton (MH) agar with 5% sheep blood (Oxoid, UK) for 48 h at 37°C under microaerobic conditions (6% O_2, 7% CO_2, 80% N_2, 7% H_2). Liquid cultures were obtained by inoculation of colonies in Brucella broth (Becton, Dickinson and Company, USA) and cultivation under the same conditions for 24 h. For pig inoculation, colonies of *C. coli* were cultivated in Brucella broth (Becton, Dickinson and Com-

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pany) and incubated for 16 h under microaerobic conditions. After incubation, 0.5 mL of the culture with an optical density of approximate 0.3 at 600 nm were inoculated in 20 mL Brucella broth (Becton, Dickinson and Company) and cultivated for 4 h in order to obtain a solution of about 5.0×10^7 CFU/mL. Cell numbers were determined by performing standard plate counts according to ISO 10272-2 [5].

Coptidis rhizoma and *G. rhois* were purchased from the Korea National Animal Bio Resource Bank (Jinju, Korea) and ground to a powder after air-dry in a dark room. Each 100 g of *Coptidis rhizome* and *G. rhois* powder was soaked in 400 mL of 70% aqueous methanol (v/v) for 24 h under mantle-reflux. The solvent was removed under reduced pressure using a rotary vacuum evaporator (EYELA N-1000 S; Tokyo Rikakikai, Japan). The extracts were filtered using a Whatman No.1 filter paper, and the filtrates were freeze-dried with a vacuum freeze dryer (MCFD 8508; Ilshin Lab, Korea) and blended into powder using a mill (Kinematica, Switzerland) with 90 standard mesh. The extract powders were mixed in the inverse ratio of the minimum bactericidal concentration (MBC) of each herbal extract against *C. coli*, and the combination was designated as CGE.

To determine the minimum inhibitory concentration (MIC) and MBC of the herbal extracts, a modified microdilution method was used to determine the MIC of the methanol extracts from *Coptidis rhizoma* and *G. rhois* against *C. coli.* The extract (20 μL) was diluted to final concentrations ranging from 0.1 to 2.0 mg/mL in a 96-well microtiter plate and 80 μL of MH broth. One hundred microliters of bacterial suspension (10^6 CFU/mL) were inoculated and incubated at 37°C for 48 h under microaerophilic conditions. Controls without the extract were set up under the same conditions. MICs were observed at least in duplicate as the lowest concentration of the extract that produced a complete suppression of colony growth. MBCs were determined by streaking the contents of the microtiter wells that gave significant MIC on fresh MH agar supplemented with 5% sheep blood (Oxoid) and incubating at 37°C for 48 h under microaerophilic conditions. The concentration at which no bacterial growth was visible after 48 h of incubation was regarded as the MBC.

Thirty 4-week-old Landrace-Duroc-Yorkshire-cross piglets were randomly divided into three equal groups. After acclimation for 1 week, all animals (body weight, 5.8 ± 0.47 kg) were inoculated with a single dosage of about 5×10^7 CFU of *C. coli* by intragastric application using a stomach feeding tube under azaperone (1.5 mg/kg) according to the method of a previous study [2]. All animals were administered CGE at a dose of 0 (Control), 2.0 (Group A) and 4.0 (Group B) g/kg feed for 7 consecutive days. During the experimental period, the ambient temperature and the relative humidity were maintained at 25 ± 1°C and 65 ± 3%, respectively. The piglets were offered the commercial feed (Cargil Agri Purina, Korea) and distilled water *ad libitum*. All experimental procedures were reviewed and approved by the Animal Ethical Committee of Gyeongsang National University Institutional Animal Care and Use Committee (GNU-150117-0013).

Table 1. MIC and MBC of *Coptidis rhizoma* extract, *Galla rhois* extract and CGE on *Campylobacter coli*

Herbal extract	MIC (mg/mL)	MBC (mg/mL)
Coptidis rhizoma	0.15	2.5
Galla rhois	0.25	4.0
CGE	0.10	2.0

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; CGE, a combination of *Coptidis rhizoma* and *Galla rhois* extract prepared in a ratio of 8 : 5 (w/w).

Fecal samples were collected from each group on the 1st, 3rd, 5th, and 7th day after treatment of CGE, and the number of fecal *C. coli* were analyzed. To determine the *C. coli* counts, semi-quantification was performed according to the method of the previous study [4]. Briefly, 1 g of fecal sample was diluted 1 : 10 in Bolton broth with Bolton selective antibiotic supplement and 5% lysed horse blood (Oxoid). Samples were homogenized in BagMixer 400 (Interscience, France) at the maximum speed for 2 min. Serial 10-fold dilutions of up to 10^8 of the initial homogenate were made in selective enrichment (Bolton broth) and incubated for 48 h at 37°C in a microaerobic atmosphere. For quantification, 10 μL of each enrichment dilution was streaked on modified charcoal cefoperazonedeoxycholate agar plates (mCCDA; Oxoid) with and without the addition of 30 μg/mL erythromycin and 100 μg/mL neomycin (Sigma-Aldrich, Germany). After plates were incubated for 48 h under conditions mentioned above, colonies of *C. coli* were counted and expressed as log CFU/g fecal samples. Statistical analyses were carried out using a one-way analysis of variance (ANOVA) and Student's *t*-test. All data were expressed as the mean ± SD. The values at $p < 0.05$ were considered to be statistically significant.

As shown in Table 1, the MIC and MBC against *C. coli* were low in order of CGE, *Coptidis rhizoma* and *G. rhois* extract. As CGE was mixed with the inverse ratio of MBC of *Coptidis rhizoma* and *G. rhois* extract, the herbal mixture consisted of *Coptidis rhizoma* and *G. rhois* extract at a rate of 8 : 5 (w/w). In a previous study [9], the MIC of aqueous Chinese soft leek extracts against *C. coli* was 2.0 mg/mL. In another previous study [12], the MBC of *Eleutherine americana* extract against *Campylobacter* spp. ranged from 31.25 to 1,000 μg/mL. In preceding study on the anti-*Campylobacter* effects of herbal extracts [13], the MIC and MBC of the extract from *Drypetes gosseweileri* were 0.78 and 3.125 mg/mL, correspondingly, and those of the extract from *Parkia biglobosa* was both 1.56 mg/mL. Considering the herbal extract method and bacteria species, the MIC and/or MBC of CGE in this study were lower than that of the above herbal extracts, except for the *Parkia biglobosa* extract.

Figure 1 shows the change in *C. coli* numbers in feces of piglets administered with different concentrations of CGE during the experimental period. At the 1st day after treat-

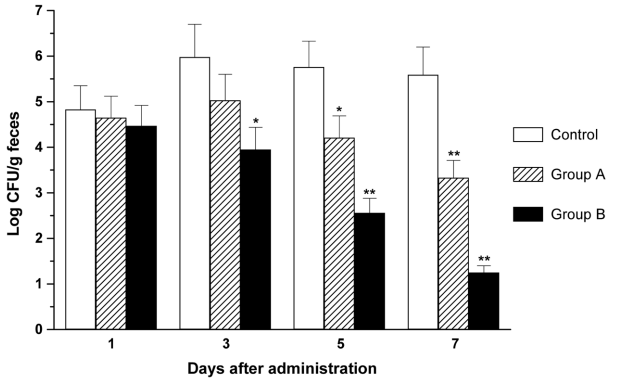

Fig. 1. *Campylobacter coli* counts in fecal contents of piglets. □, Control treated with normal feed (n = 10); ▨, Group A treated with 2.0 g/kg feed CGE [a mixture of *Coptidis rhizoma* extract and *Galla rhois* extract (8 : 5, w/w)] (n = 10); ■, Group B treated with 4.0 g/kg feed CGE (n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$, compared to control.

ment, *C. coli* numbers in group A and group B were not meaningfully reduced compared with those in control. However, *C. coli* counts in group A were significantly decreased compared with those in control at the 3rd day post-treatment ($p < 0.05$). On day 5 after treatment, the number of *C. coli* in group A ($p < 0.05$) and group B ($p < 0.01$) was significantly reduced compared with that in control, and *C. coli* counts in groups treated with CGE were significantly reduced compared with those in control at the 7th day post-treatment ($p < 0.01$).

Based on the results of this study, it appears that CGE, which contains *Coptidis rhizoma* and *G. rhois* extracts, was successful in decreasing the fecal *C. coli* counts in piglets. According to the previous study [3], the pig group fed with the basal diet added 0.35% organic acids containing 0.19% tannin extracted from the chestnut tree (*Castanea sativa* Mill.) for 59 days, showed significant reduction of *Campylobacter* spp. counts in fecal contents compared to the control group ($p < 0.05$). In addition, in another previous study [7], finisher pig group treated with the blue lupine seed in their feed at the concentration of 25% for 7 days, showed a significant decrease of *Campylobacter* spp. counts in the rectal fecal samples compared to the control group ($p < 0.01$). However, no reduction in *C. coli* counts was observed in the fecal contents of weaned piglets supplemented with probiotics (*Enterococcus faecium* NCIMB 10415, 10^9 CFU/kg feed) for 28 days [2]. Considering the extract solvents, dosage of administration and treatment period, the efficacy of CGE in reducing fecal *C. coli* counts was higher than that of all the above-mentioned substances.

In conclusion, the results in this study demonstrate that CGE at a concentration of 2.0 g/kg in feed may be used to reduce *C. coli* counts in feces of piglets. Therefore, CGE could be an effective candidate for the treatment of *C. coli* infection in piglets.

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