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Hot-Water Extract from Mycelia of *Cordyceps sinensis* as a Substitute for Antibiotic Growth Promoters

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Introduction

The use of antibiotics for promoting the growth of food-producing animals has recently received increasing attention as a contributory factor to the international emergence of antibiotic-resistant bacteria in humans through the spreading of antibiotic resistance from animals to humans (JETACAR 1999). These types of antibiotics including antibiotic growth promoters (AGPs), are used for promoting growth and improving feed efficiency in situations where animals are intensively reared. AGPs, in low doses, act to reduce the population of, or suppress the growth of Gram-positive bacteria, especially pathogenic bacteria, in the gut flora. Their action appears to be by the control of sub-clinical, enteric challenge and the occasional reduction in obvious clinical disease (JETACAR 1999, Schwarz *et al.* 2001).

The ban on AGPs is encouraging livestock producers to seek alternative strategies for enhancing production of food-producing animals to the same or higher level obtained with AGPs. Most strategies aim at either altering the microbial population in the animal's gastro-intestinal tract or strengthening the animal's immune system. The latter is the best strategy, allowing the animals to defend themselves from diseases naturally. Fungi and some plants offer the possibility of an additive that may strengthen the animal's immune system (Kamel 2000). For example, *Cordyceps sinensis* can promote vitality, enhance the immune system and afford diverse physiological activity (James & Zeitz 1994). Also, extracts of *C. sinensis* mycelia have physiologically active substances that enhance the immune systems *in vitro* (Koh *et al.* 2001)

This experiment deals with the effect of CS-HW (hot-water extract of mycelia from cultured *Cordyceps sinensis*), as a substitute for AGPs on growth performance including body weight gain and survivability, and on health indices including antibody response to Newcastle disease virus vaccine and microflora in the small intestine.

Materials and methods

Microorganism and materials

Salmonella choleraesuis 40089, *Escherichia coli* 11234 and *Lactobacillus acidophilus* 32820 were used from Korea Culture Center of Microorganisms, Seoul, Korea. The media (Difco) for counting were Salmonella and Shigella agar for *Salmonella choleraesuis,* MacConkey agar for *Escherichia coli* and Rogosa SL agar for *Lactobacillus acidophilus.*

Preparation of CS-HW

Cordyceps sinensis was grown in a jar fermenter containing 3 l of potato dextrose medium (potato 200 g, dextrose 20 g and yeast extract 0.5 g per liter), at 150 rpm, 25°C and pH 5 with the aeration of 1.0 vvm. After harvesting, the *C. sinensis* mycelia mycelia (500 g) were boiled with 4.5 l of water at 100°C for hot-water extraction. The insoluble materials were removed by centrifugation, and the resulting supernatants were dried to give CS-HW (Koh *et al* 2001).

Animals

Forty-five commercial 1 day-old broiler chicks (Cobb strain, male) were held at 23 ± 2°C for five weeks with lighting 24 h a day. They had free access to food and water during the experimental periods The chicks were divided into one control and two test groups, and each group consisted of three treatments; three replicates per treatment and five chicks per replicate. Corn-soybean meal basal diet for broiler chicks was set to the chemical composition of the standard nutritional requirement (KFIC 1985). One control group was fed on only the basal diet, and the two test groups were fed on the above diets including 20 mg Avilamycin (Watkins *et al.* 1997) and 600 mg CS-HW per kg, respectively.

To evaluate growth performance in the three groups, body weight changes and surviving numbers of chicks were recorded weekly. The increasing in the body weight was expressed as live body weight gain and the numbers of chicks were counted every day to evaluate survival.

Estimation of microorganisms in the small intestine and in vitro experiments

In vitro experiments were carried out for estimating the direct effects of CS-HW on growth or inhibition of *Salmonella* sp., *Escherichia coli* and *Lactobacillus* sp. in the small intestines of chicks. To investigate the microflora of the content of the small intestine of each chick, more than one gram of content was collected in sterilized tubes, as soon as the chicks were sacrificed by cervical dislocation and their small intestines were exposed on sterilized sheets. One gram of wet content was diluted with saline buffer, and the properly diluted samples were inoculated on suitable media at 37°C for 24-48 h and then the colony forming units were counted. The bacteria that grew on Salmonella and Shigella agar and regrew on Bismus agar were regarded as *Salmonella* sp., those that grew on MacConkey agar as *Escherichia coli* and those that grew on Rogosa SL agar as *Lactobacillus* sp.

Antibody response to Newcastle disease virus (NDV) vaccine

To evaluate antibody response to inactivated NDV B1 vaccine from Daesung Microbiological Labs. Co., Ltd (Kyonggi, Korea), the first vaccination was enforced on 2 week-old chicks by injection into muscle with 2.5 ml of the NDV vaccine, and the second vaccination was done on 4 week-old chicks with the same

quantity of material. For the 5 week-old chicks, 5 ml of the blood of each chick was collected from a vein. Each blood serum sample was separated by centrifugation at 1,100 × g for 20 min at 4°C and treated for 30 min at 56°C to inactivate anti-complementary factors of the serum. Antibody (AB) titer was measured by the Hemagglutination Inhibition (HI) test of Beard (1975)

Statistical analysis

Data were expressed as the mean ± S.D. The difference between control and test groups in these experiments was investigated for statistical significance by Duncan's multiple range tests (SAS program). A value of $p<0.05$ was considered to indicate statistical significance.

Results

Microflora in the small intestine

Salmonella sp. and *E coli* are well known as harmful bacteria, but *Lactobacillus* sp. are utilized as helpful bacteria in the intestinal tracts of human and animals (Kwon *et al.* 2001). Increasing the population ratio of *Lactobacillus* sp. brings good conditions in the intestinal tracts (Kwon *et al.* 2001).

In vitro experiments for the direct effect of CS-HW and Avilamycin on *Salmonella choleraesuis* KCCM40089, *Escherichia coli* KCCM11234 and *Lactobacillus acidophilus* KCCM32820 tests were made on standard microorganisms; both CS-HW and Avilamycin did not have any effect on microbial populations (Table 1). This suggests that CS-HW has no direct effect on growth or inhibition of *Salmonella* sp., *E. coli* and *Lactocbacillus* sp. in the small intestine of chicks.

Table 1. Some bacterial populations in liquid media by addition of Avilamycin and hot-water extract from cultured mycelia of *Cordyceps sinensis* (CS-HW) (10^7 cfu/ml)

Additives[1)]	*S. choleraesuis*[a]	*E coli*[b]	*L. acidophilus*[c]
Control	43	31	28
CS-HW[2)]	44	32	29
Avilamycin[3)]	43	36	27

[1)]Initial bacterial cells in each liquid media were adjusted to 1.0×10^3; [2)]containing 0.1% of hot-water extract from cultured mycelia of *Cordyceps sinensis*; [3)]containing 40 mg of Avilamycin per liter, [a]*Salmonella choleraesuis* KCCM40089, [b]*E coli* KCCM11234, [c]*Lactobacillus acidophilus* KCCM32820.

The populations of *Salmonella* sp. in the content of the small intestine for the CS-HW and Avilamycin fed test groups were significantly lower than those for the control group, and the numbers of *E coli* in the content of the small intestine for the CS-HW fed group were significantly lower than those for the control and the Avilamycin fed groups, but the numbers of *E. coli* for the Avilamycin fed group were significantly higher than those for the control ($p<0.05$) as shown in Table 2. Values for populations of *Lactobacillus* sp. in the content of the small intestine for each group were 8.0×10^7 cfu/g for control group, 3.3×10^9 cfu/g

for CS-HW fed group and 2.5×10^8 cfu/g for Avilamycin fed group, and there were significantly different in populations of *Lactobacillus* sp. among these three groups. The populations of harmful bacteria-helpful bacteria were expressed as the ratio of *Salmonella* sp. to *Lactobacillus* sp. (S/L) and *E. coli* to *Lactobacillus* sp. (E/L) in Table 2. Both ratios, S/L and E/L, of each group were 0.14 and 0.25 for the control, 0.0003 and 0.002 for the CS-HW fed group, and 1.46 and 1.62 for the Avilamycin, respectively. The S/L and E/L of the CS-HW fed group were for lower than those of both the control and the Avilamycin fed groups, because of high populations of *Lactobacillus* sp. in the CS-HW fed group. There were significant differences in the value of S/L and E/L between the CS-HW fed group and the other two groups.

Table 2. Some bacterial populations of the content of the small intestine in broiler chicks after feeding Avilamycin and hot-water extract from cultured mycelia of *Cordyceps sinensis* (CS-HW)

(10^7 cfu/g of content)

Treatment[1)]	*Salmonella* sp. (S)	*E. coli* (E)	*Lactobacillus* sp. (L)	S/L[d]	E/L[d]
Control	1.2^a	2.0^a	8.0^a	0.14	0.25
CS-HW[2)]	0.1^b	0.7^b	325.0^b	0.0003	0.002
Avilamycin[3)]	36.5^c	40.5^c	2.5^c	1.46	1.62

[1)]Among 15, 5 chicks were used to count the populations of intestinal microbes; [2)]containing hot-water extract from cultured mycelia of *Cordyceps sinensis* in diets; [3)]containing Avilamycin in diets; [a, b, c]values with different superscripts within the same column of a sub-part are significantly different at $p<0.05$; [d]ratios *Salmonella* sp. and *E. coli* to *Lactobacillus* sp.

Antibody response to Newcastle disease virus vaccine

When NDV AB titers were checked for 5 weeks-old chicks, the AB titers of the CS-HW fed group were far higher than those of the control and the Avilamycin fed groups as shown in Table 3. The values of the AB titers of the CS-HW fed group were 9.1 ± 0.3, those of the control and the Avilamycin fed groups were 8.0 ± 0.4 and 7.5 ± 0.1, respectively. The AB titers of the CS-HW fed group were increased significantly more than those of the other two groups ($p<0.05$). Thus it can be predicted that the CS-HW fed group will have a stronger defensive system against a virulent field strain of NDV than the control and the Avilamycin fed groups.

Table 3. Changes of antibody titer against Newcastle disease virus vaccine in broiler chicks after feeding diets containing Avilamycin and hot-water extract from cultured mycelia of *Cordyceps sinensis* (CS-HW)

(Unit : $\log_2$)

	Control	CS-HW	Avilamycin
NDV AV titer	8.0 ± 0.4^a	9.1 ± 0.3^b	7.5 ± 0.1^c

After both the 1st vaccination at 2 weeks old and the 2nd vaccination at 4 weeks old were injected into muscle with NDV inactivated B1 vaccine, the NDV AB titer was detected from blood serum obtained at 5-wk old; a value of $p<0.05$ indicates statistical significance between control and tests; $^{a, b, c}$values with different superscripts are significantly different at $p<0.05$

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