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The Clinical Effects of an Herbal Antimicrobial Feed Additive in Growing Chickens

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Abstract : The natural herbal antimicrobial additive, Flavo-SK™, was developed by Zoonosis Research Center of Wonkwang University. The purpose of this study was to evaluate the effects of Flavo-SK™ on the health status and performance of growing chickens. This study was conducted on the growing chickens (n=20,000) for 31 days in a growing chickens husbandry. The animals were divided with two groups; Flavo-SK™ treated group (n=10,000) and commercial diet feeding group (n=10,000). The Flavo-SK™ treated animals had provided with commercial diet adding the Flavo-SK™ as 0.29%. During the study period, we compared clinical signs, weight increase rate, diet consumption amount, gross finding, necropsy findings and histopathological findings between the treated group and non treated group. As the results of this clinical trial, the natural herbal antimicrobial additive, Flavo-SK™, showed the effects on disease reduction. It is suggested that Flavo-SK™ has the antimicrobial effects.

Key words **:** antimicrobials, antibacterial, herb, feed additive, chicken.

Introduction

Antibiotics are fed to production animals to prevent disease and promote growth (3). However, the use of antibiotics in animal feeds has been linked to antibiotic-resistant bacteria (9,14). The antibiotic chemotherapy produces sometimes side effects and fails to eliminate bacterial infection (2). Consequently, many countries have banned the use of subtherapeutic levels of antibiotics in production animal rations. One hope is herbal material for the alternative antimicrobial agents able to control these infections. Since natural herbs tend to be a source of greater structural and chemical diversity than are combinatorial chemistry libraries (17,23,24), natural products offer a potentially rewarding route for the identification of novel antimicrobial agents. Under the field conditions on the growing pigs, in-feed strategic medication program remain a common policy for the control of persistent infectious diseases (6), along with the use of antibiotics for performance enhancement. The consistent effectiveness of antimicrobial feed additives has led to extensive use in the poultry feeding industry. Despite their effectiveness, continued use of antimicrobial growth promoters faces a very uncertain future. The United Kingdom banned the use of penicillin and tetracycline for growth promotion in the 1970s (8). The United States banned the use of enrofloxacin in 2005 (27). Sweden and Denmark banned all growth-promoting antibiotics in 1986 and 1999, respectively (8). As a result of the ban, weaning pigs in Denmark were reported to encounter severe health problems requiring treatment with antibiotics at levels exceeding those required for subtherapeutic use (12). One concern associated with use of antimicrobial feed additives is the potential for antimicrobial residues in meat products of treated animals. We have given oversupply of antibiotics in domestic animals and suffered public health problem induced by antibiotic-resistance bacteria. Therefore, there are increasing concerns about the meat safety and raising requests on the alternative materials instead of antibiotics (28).

Recently, the natural herbal antimicrobial additive, Flavo-SK™, was developed by Zoonosis Research Center of Wonkwang University (15,21). In this work, we are aim to evaluate the effects of Flavo-SK™ on the health status and performance of growing chickens.

Materials and Methods

Experimental materials

In order to develop a natural herbal antimicrobial additive, Flavo-SK™, over 1000 kinds of herbal materials were tested by Zoonosis Research Center of Wonkwang University (15,21). The herbal specimens were purchased at the University Oriental Drugstore, Iksan, Korea. Voucher specimens were deposited at the Herbarium of the College of Pharmacy, Wonkwang University. Plant material (50 g) was extracted with ethanol

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under ultrasonic conditions for 3 h, followed by paper filtration. The filtrates were evaporated in vacuum to yield water soluble extracts. We employed *in vitro* antibacterial and *in vitro* antiviral screening tests to evaluate the effect of the medicinal herbal extracts on the pathogenic bacteria and viruses. We found Flavo-SK™ which has revealed very strong antibacterial and antiviral effects during the screening studies (15,21). In this study, we used Flavo-SK™ for experimental materials.

Animals and treatments

The investigation was carried out on a growing chicken unit with a capacity of 800,000 chickens in Iksan, Korea. 20,000 clinically healthy 1 day-old chickens of good physical condition were distributed in two experimental groups, depending on the inclusion or not of Flavo-SK™ in their feed, as follows: 1) Control group (10,000 chickens): basic on-farm mixed feed: feed without Flavo-SK™, 2) Treatment group (10,000 chickens): basic on-farm mixed feed containing Flavo-SK™ at the inclusion rate of 0.29%: feed supplemented with Flavo-SK™. All chickens were of the same genetic background (Cornish) and had received feed free of any antimicrobial or medical antibiotics.

Management and feeding of the experimental animals

20,000 chickens were randomly allotted to 4 pens that allowed heat and ventilation by electrical automated system. Pens were a cross-ventilated negative-pressure house with forced-air brooders. Prior to the allocation, the designated pens were thoroughly cleaned and disinfected. The first and second pens contained the Flavo-SK™ treatments and the third and fourth pens contained the feed without Flavo-SK™ treatments. Broilers were provided with feed and water, supplied through feed pans and nipple drinkers, for *ad libitum* consumption. Temperature of the rooms was maintained at 32.2°C and decreased to 21.1°C during the 0- to 3-wk period. For the 3- to 5-wk period, the temperature remained at 21.1°C. Following the standard management policy of the farm, all studied chickens were managed at same conditions. Considering the 5-week interval for the introduction of all participating animals in the unit and the monitoring period for each chickens (from growing age to slaughter age), the trial lasted over a total period of 31 days. Management of the animals and data recording during the study were carried out under the management of the animals and data recording during the study were carried out under the ethical guidelines of Wonkwang University IACUC.

Clinical evaluation

The experimental animals were daily observed for signs of disease throughout the monitoring period and any disease problems were recorded. Special attention was given to the effects of Flavo-SK™. The following parameters were considered: the average daily diarrhea score and clinical signs throughout the monitoring period. For each chickens showing diarrhea, the severity was assessed visually and characterized according to the following scale: 0, normal feces; 1, pasty; 2, liquid; 3, with mucus; and 4, with blood (18). The assessor was an experienced person, who was unaware of the treatment each pen was assigned to. For all chickens that revealed other clinical signs, the symptoms and severity was recorded. After the initial weighing at entrance, the experimental animals were further weighed on the 10(±1), 20(±1) and 30(±1) days of age (study participating time, end of growing periods, respectively). Feed consumption per group was recorded over the same periods. During the study period, we compared clinical signs, weight increase rate, diet consumption amount and fecal scores.

Postmortem evaluation

When the studied chickens were 31 days old, each 10 chickens per group were randomly selected. They were autopsied at the necropsy room in Center for Animal Resources Development, Wonkwang University, Iksan city, Korea. During the necropsy, we were conducted the gross observation on the pathological lesions. Tissue specimens were collected from lungs, kidneys, livers, small intestines and large intestines. For histopathological observation, collected tissues were fixed in 10% neutral buffered formalin. After fixation, tissues were embedded in the paraffin in the usual manner. Sections were cut 4 um thick, floated on a water bath, mounted on the slides and stained with hematoxylin & eosin. After staining, the microscopic observation was conducted and histopathological severity was assessed visually and characterized according to the following scale; 0, normal; 30, mild; 60, moderate; 100, severe. For bacteria isolation, tissues were aseptically homogenized and submitted to bacteria isolation as described previously (20). Nucleic acids were extracted from the collected samples. Tissues were homogenized and submitted to nucleic acids extraction as described previously (16,19). PCR assays were conducted for detection of the *Salmonella gallinarum* as described previously (26).

As described above, during postmortem evaluation, we performed gross examination, necropsy observation, histopathological observations, bacterial isolation and PCR analysis between the treatment group and the control group.

Data analysis

Values for all parameters under study were recorded for each experimental unit and statistical analysis was performed by the use of the general linear model. When appropriate, values are reported as average ± standard deviation. Student's *t*-test was used for pair-wise comparisons. The significance was attained if the *P*-value was <0.05.

Results

During the study period, we compared clinical signs, weight increase rate, diet consumption amount and fecal scores. In case of weight increase rate, the body weight gains of treatment group were average 0.07 kg more than those of control

chickens. The breeding efficacy percent was 1.1 % more than those of control chickens. When the feed consumption required was calculated from initiating to ending time, the feed consumption required of treatment group was revealed approximate 0.09 less than one of control group. In case of outcome index, the index of treatment group was 34 more than those of control chickens. In case of fecal status, the chickens of treatment group had showed normal appearances. The chickens of control group had revealed gross lesions more frequently than those of treatment group (Table 1). As the results of the gross observation during the necropsy of studied animals, the chickens of control group had revealed pulmonary pathological lesions more frequently than those of treatment group (Fig 1). The pneumonia cases were detected in 6 chickens of the control group, but 0 heads of the treatment group. We calculated the percentage of pneumonia (Pneumonia lesion %) as following method; Pneumonia lesion % = 100 x pneumonia lesion / total lung area. As compare with Pneumonia lesion %, the treatment group was revealed significantly less than one of control group (Table 2). As the other gross lesions during the necropsy of studied animals, the chickens of control group had revealed severe pathological lesions more frequently than those of treatment group (Table 1). The control animals showed breast muscle pale, thoracic fluid, thoracic organs adhesion, epicarditis (Fig 1), peritonitis (Fig 2), ascites, abdominal organs adhesion, liver swelling and liver pale. Also, on the histopathological scores, the treatment group was revealed significantly less than one of control group (Table 3). The chickens of control groups showed frequently severe pneumonia lesions (Fig 3). As the results of histopathological examinations, the chickens of treatment group which were fed Flavo-SK^{TM} for experimental period (31 days) had not significant pathological changes in liver, kidney, small intestine, large intestine and lung. However, the control chickens without feeding Flavo-SK^{TM} showed pathological lesions in liver, kidney, intestine and lung (Table 3). As the results of bacteria isolation analysis, the chickens of control group (3.5%) had revealed *Salmonella spp.* more fre-

Table 1. The gross lesions of the experimental chickens at the necropsy

Organ	Control (n=10)	Treatment (n=10)
Breast Muscle	Pale (n=4)	No gross lesion (n=10)
Thoracic cavity	Thoracic fluid (n=5) Adhesion (n=2)	No gross lesion (n=10)
Heart	Epicarditis (n=7)	No gross lesion (n=10)
Lung	Pneumonia (n=6)	No gross lesion (n=10)
Abdominal cavity	Peritonitis (n=4) Ascites (n=1) Adhesion (n=4)	No gross lesion (n=10)
Liver	Swelling (n=1) Pale (n=3)	No gross lesion (n=10)

Table 2. The percentages of pneumonia lesion of studied animal

Group	n	Pneumonia lesion %	Pneumonia Case
Control	10	22.0 ± 19.47	6
Treatment	10	0**	0

**$p < 0.01$ is the significance level compare to the control group.

Table 3. Histopathological findings of studied animals

Tissue	Control (n=10)	Treatment (n=10)
Liver	24.0 ± 30.98 (n=4)	0 ± 0*
Lung	44.0 ± 40.88 (n=6)	0 ± 0*
Kidney	15.0 ± 25.50 (n=3)	0 ± 0*
Intestine	21.0 ± 28.46 (n=4)	0 ± 0*

0, normal; 30, mild; 60, moderate; 100, severe
*$p < 0.05$ is the significance level compare to the control group.

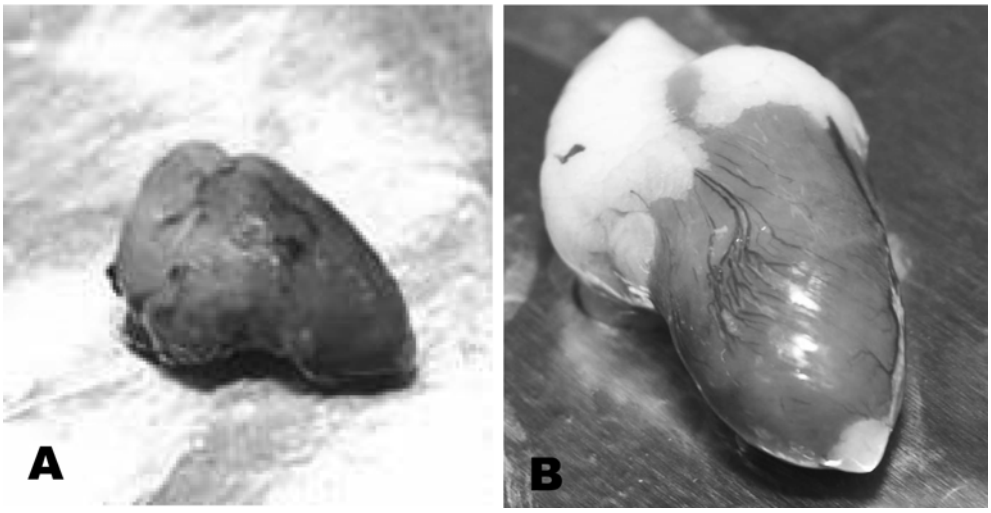

Fig 1. Gross appearances of the heart. A. Control group. The macroscopic observation is revealed severe epicarditis changes. B. Treatment group. The heart is showed normal appearance.

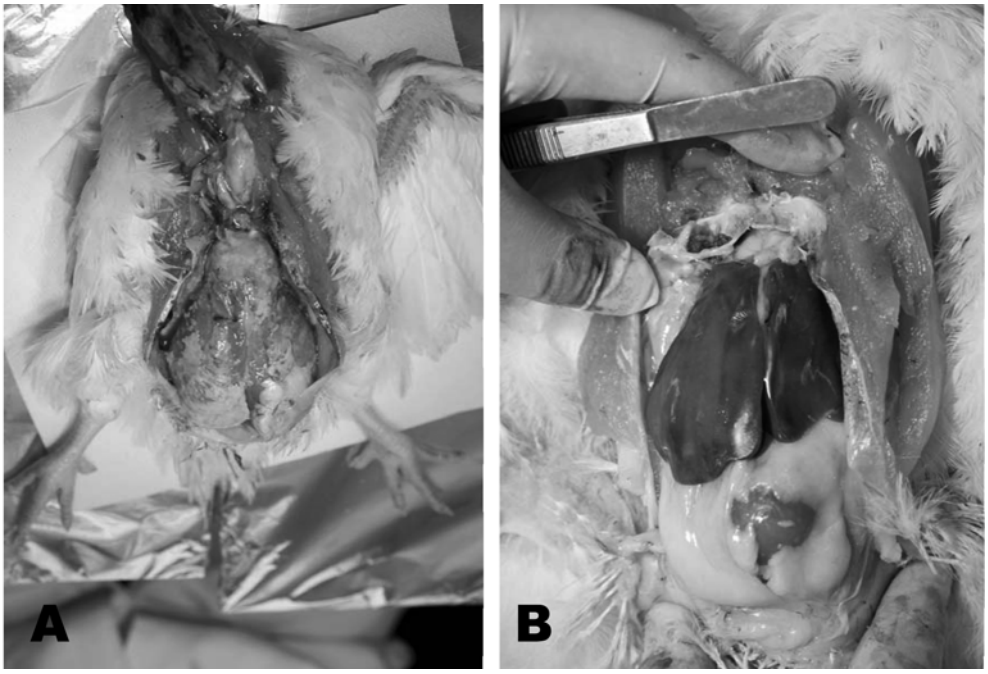

Fig 2. Marcroscopic appearances of abdominal cavity. A. Control group. The gross observation is revealed severe peritonitis changes. B. Treatment group. The abdominal organ is showed normal appearance.

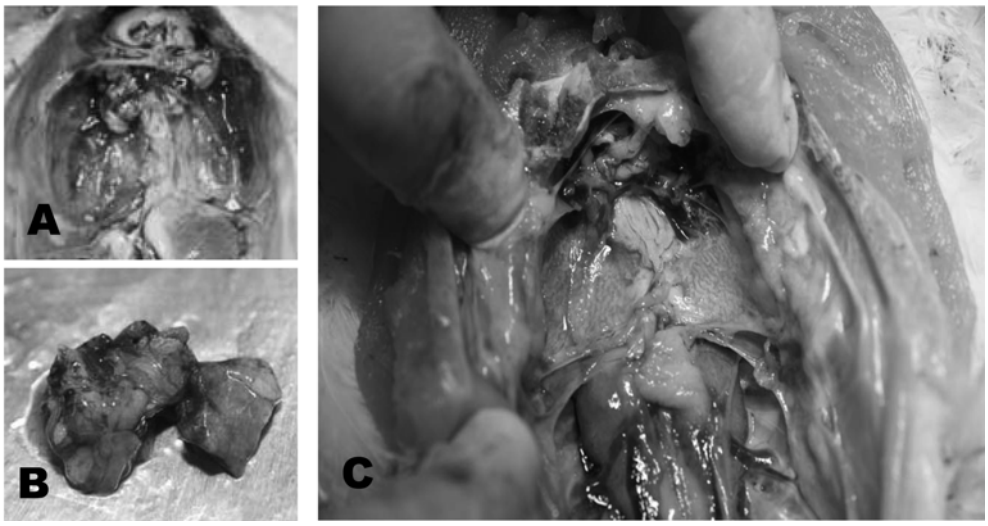

Fig 3. Gross appearances of the thoracic cavity. A. Control group. The macroscopic observation is revealed severe pneumonia changes in the lung and thoracic fluids. B. The dissected lung of control animal showed severe pneumonic lesion. C. Treatment group. The lung is showed normal appearance.

quently than those of treatment group (0.5%). PCR assays were conducted for detection of *Salmonella gallinarum*. Amplification of target nucleic acids from PCR products was electrophoresed on 1.2% agarose gel. As the results of PCR analysis, the chickens of control group (2.5%) had revealed positive reactions against *Salmonella gallinarum* more frequently than those of treatment group (1.25%). According to these results, Flavo-SK™, an herbal antimicrobial feed additive, was proved a very effective material preventing poultry pathogens.

Discussion

An even greater concern among regulatory agencies and consumers is the potential that regular use of antimicrobial feed additives may lead to the development of resistant microbes that may compromise the effectiveness of antibiotics in treating animal and human disease (5). The impetus for banning antimicrobial feed additives in food animal production is very strong (25). Such forces as consumer acceptance, export markets and medical community directives are likely

to force loss of some, if not all antimicrobial feed additives for performance enhancement in food animals (7,25). The issue has stimulated interest in alternatives to antimicrobial feed additives. In the future, regulatory action and consumer preferences may force swine producers to alter or discontinue antimicrobial feed additive use (28). A variety of substances are used in conjunction with, or as alternatives to, antibiotics in poultry diets. Probiotics, prebiotics, organic acids, and plant extracts have all shown promising results for use in organic poultry production (10). These alternatives are necessary because federal regulations prohibit the use of conventional antibiotics and growth promoters in organic production (1). Formic, acetic, and propionic acids reduce the prevalence of Salmonella and Campylobacter bacteria found in the intestines of broilers (4,13). Moreover, *Clostridium perfringens*, *Salmonella Typhimurium*, and *Escherichia coli* may be controlled through the use of essential oils derived from plant extracts (22). In order to develop a natural herbal antimicrobial additive, over 1,000 kinds of herbal materials were tested by Zoonosis Research Center of Wonkwang University. Previously, we employed *in vitro* antibacterial and *in vitro* antiviral screening tests to evaluate the effect of the medicinal herbal extracts on the pathogenic bacteria and viruses. We found Flavo-SK™, an herbal antimicrobial compound, which has revealed very strong antibacterial and antiviral effects during the screening studies (15,21). In this study, the natural herbal antimicrobial additive which was originated from the herb to treat diarrhea and respiratory disease was assessed on the health status and performance of growing chickens. As the results of this clinical trial, Flavo-SK™ showed the effects on respiratory disease and inflammation. The diet consumption efficiency was revealed that the treatment group was better than the control group. Also, on the disease occurrences like as pneumonia, the treatment group was revealed significantly less than one of control group. As the results of pathogens analysis, the chickens of control group had revealed positive reactions against several pathogenic agents like as *Salmonella gallinarum* more frequently than those of treatment group. *Salmonella gallinarum* induce fowl typhoid and result to economic losses in chickens industry (11).

According to these results, Flavo-SK™, an herbal antimicrobial compound, was proved a very effective material preventing chicken pathogens and a very safe material for feeding. The concern about antimicrobial additives has stimulated a renewed interest in potential alternatives (5). Natural herbal antimicrobial feed additives will potentially see increased interest if use of antimicrobials is banned or reduced. However, the same principles of safe, economical and effective use of feed additives will apply to these products as with antimicrobials.

Conclusion

The natural herbal antimicrobial additive, Flavo-SK™, was developed by Zoonosis Research Center of Wonkwang University. In this study, we are aim to evaluate the effects of Flavo-SK™ on the health status and performance of growing chickens. As the results of this clinical trial, the natural herbal antimicrobial additive, Flavo-SK™, showed the effects on disease reduction. It is suggested that Flavo-SK™ could be used as the effective alternative for antibiotics.

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References

1. Agricultural Marketing Service. National Organic Program: Amendments to the National List of Allowed and Prohibited Substances. Final Rule. 7CFR Part 205. Fed Regist 2003; 68: 61987-61993.
2. Buenz EJ, Bauer BA, Schnepple DJ, Wahner-Roedler DL, Vandell AG, Howe CL. A randomized Phase I study of *Atuna racemosa*: a potential new anti-MRSA natural product extract. J Ethnopharmacol 2007; 114: 371-376.
3. Centers for Disease Control and Prevention, National Antimicrobial Resistance Monitoring System. National Antimicrobial Resistance Monitoring System (NARMS) frequently asked questions (FAQ) about antibiotic resistance - Why are antibiotics used in food-producing animals? http://www.cdc.gov/narms/faq_pages/7.htm Accessed March 2007.
4. Chaveerach P, Lipman LJA, van Knapen F. Antagonistic activities of several bacteria on in vitro growth of 10 strains of Campylobacter jejuni/coli. Int J Food Microbiol 2004: 90: 43-50.
5. Cromwell GL. Antimicrobial and promicrobial agents. In: Swine Nutrition (Lewis A, Southern L. ed.), 2nd ed. Boca Raton, FL: CRC Press. 2001; 401.
6. Deen J, Dee S, Morrison RB, Radostits M. Health and production management in swine herds. In: Herd Health: Food Animal Production Medicine (Radostits OM. ed.), 3rd ed. Philadelphia, Pensylvania: WB Saunders. 2001; 635-764.
7. Elliott I. EU plans phase-out of antibiotic use. Feedstuffs 2001; 74: 1.
8. Food Marketing Institute. Low-level use of antibiotics in livestock and poultry. http://www.fmi.org/media/bg/antibiotics.pdf Accessed Aug. 2007.
9. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype *Typhimurium* DT104 infections in the United States. N Engl J Med 1998; 338: 1333-1339.
10. Griggs JP, Jacob JP. Alternatives to antibiotics in organic poultry production. J Appl Poult Res 2005; 14: 750-756.
11. Gupta S, Jindal N, Khokhar RS, Asrani RK, Ledoux DR, Rottinghaus GE. Individual and combined effects of ochratoxin A and *Salmonella enterica* serovar *Gallinarum* infection on pathological changes in broiler chickens. Avian Pathol 2008; 37: 265-272.
12. Hayes DJ, Jenson HH. Lessons from the Danish ban on feed-grade antibiotics. in Briefing Paper 03-BP 41. Ames, Center for Agric. Rural Dev., Iowa State Univ. 2003. Pages 1-12
13. Hinton M, Linton AH. Control of Salmonella infections in

broiler chickens by the acid treatment of their feed. Vet Rec 1988; 123: 416-421.
14. Holmberg SD, Osterholm MT, Senger KA, Cohen ML. Drug-resistant Salmonella from animals fed antimicrobials. N Engl J Med 1984; 311: 617-622.
15. Kim HY, Shin HS, Park H, Kim YC, Yun YG, Park S, Shin HJ, Kim K. *In vitro* inhibition of coronavirus replications by the traditionally used medicinal herbal extracts, *Cimicifuga rhizoma*, *Meliae cortex, Coptidis rhizoma*, and *Phellodendron cortex*. J Clin Virol 2008; 41: 122-128.
16. Kim O, Chae C. Experimental infection of piglets with a Korean strain of porcine epidemic diarrhea virus. J Comp Pathol 2003; 129: 55-60.
17. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005; 4: 206-220.
18. Kyriakis SC, Tsiloyiannis VK, Vlemmas J, Sarris K, Tsinas AC, Lexopoulos C, Jansegers L. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. Res Vet Sci 1999; 67: 223-228.
19. Lee JU, Kim O. Natural maternal transmission of Helicobacter pylori in Mongolian gerbils. J World Gastroenterol 2006; 12: 5663-5667.
20. Lee JU, Kim SK, Jung K, Kim O. Absence of natural maternal transmission of *Helicobacter pylori* in an animal model. J Vet Sci 2006; 7: 225-228.
21. Lee H, Hong S, Bok N, Kim B, Kim M, Park H, Kim Y, Kim O. Antiviral effect of natural herb extract WK01 and WK02 against porcine epidemic diarrhea virus. J Life Sci & Nat Res 2008; 30: 61-71.
22. Mitsch P, Zitterl-Eglseer K, Kohler B, Gabler C, Losa R, Zimpernik I. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult Sci 2004; 83: 669-675.
23. Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J Ethnopharmacol 2000; 73: 299-305.
24. Qu H, Zhang Y, Wang Y, Li B, Sun W. Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from *Forsythia suspensa*. J Pharm Pharmacol 2008; 60: 261-266.
25. Smith R. Antibiotic bans, regulations may stop development of drugs. Feedstuffs 1999; 71: 1.
26. Tuchili LM, Kodama H, Izumoto Y, Mukamoto M, Fukata T, Baba T. Detection of *Salmonella gallinarum* and *S. typhimurium* DNA in experimentally infected chicks by polymerase chain reaction. J Vet Med Sci 1995; 57: 59-63.
27. United States Food and Drug Administration, Department of Health and Human Services. Withdraw of approval of the new animal drug application for enrofloxacin in poultry. In:Docket No. 2000N-1571. United States Food and Drug Admin., Dept. Health and Human Serv., Washington, DC. 2005.
28. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J. Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. Emerg Infect Dis 2007; 13: 1834-1839.

천연물 유래 항생제 대체 사료첨가제의 육계 임상 효능에 대한 연구

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요 약 : 천연물 유래 항생제 대체 사료첨가제 Flavo-SK™은 원광대학교 인수공통감염병연구센터에서 1,000종 이상의 천연물로부터 효능평가를 거쳐 개발되었다. 본 연구는 육계 농장에서 대규모 임상 효능평가를 통한 Flavo-SK™의 항균 효과 규명과 항생제 대체 효과를 알아보고자 수행되었다. 20,000 마리의 병아리를 Flavo-SK™ 투여군과 비투여군 2 그룹으로 나누어 31일 동안 육계농장에서 연구가 수행되었다. Flavo-SK™ 투여군에는 항생제가 없는 사료에 Flavo-SK™를 0.29% 배합하여 급여하였고 비투여군은 항생제 포함된 일반 사료를 급여하였다. 연구기간 동안 사료섭취량, 체중, 임상증상을 분석하고 시험종료일에 부검하여 육안검사와 미생물학적 검사 및 병리조직학적 검사를 수행하였다. 연구결과 천연물 유래 항생제 대체 사료첨가제 Flavo-SK™은 육계에 항균효과를 가진 항생제 대체 사료첨가제로서 효과적인 것을 확인할 수 있었다.

주요어 : 항생제, 항균, 천연물, 사료첨가제, 닭, 양계.