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Effects of pitamin on growth performance, carcass characteristics and cecal microflora of broiler chicken

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Abstract

The aim of this study is to determine the effect of dietary pitamin as an antibiotic replacement in broiler chicken. The treated groups were as follows: 1) the control, 2) the antibiotics (8 mg of avilamycin kg^{-1} of diet) and 3) the pitamin (70 mg of pitamin kg^{-1} of diet) groups. Body weight gain, feed intake, and feed efficiency were significantly higher in the pitamin group than in the antibiotics and control groups ($p<0.05$). Carcass weight, dressing percentage, and the weight of breast and thigh muscle recorded significantly higher levels in the pitamin group as compared to the other groups ($p< 0.05$). The addition of pitamin to the diets for broilers reduced abdominal fat by 23.35% and stimulated the growth of the thymus, the spleen, and the bursa of Fabricius. TAG levels of the pitamin group declined by 12.03 and 10.45% as compared to the control and antibiotics groups, and their TC levels were reduced by 15.17 and 14.39%, and LDL-C levels were reduced by 10.56 and 11.24%, respectively. Serum IgG was increased significantly by 137.43 and 36.80% in the pitamin group as compared to the control and antibiotics groups, respectively ($p< 0.05$). The numbers of *Bifidobacterium* and *Lactobacillus* on the cecum digesta were significantly higher in the pitamin group than in the antibiotics and control groups and the numbers of *Escherichia coli* and Salmonella tended to be reduced ($p<0.05$). In conclusion, when Korean red pine bark extract, pitamin, was added to the broiler diets at a concentration of 70 mg of pitamin kg^{-1} of diet, it resulted in better growth performance as compared to the antibiotics by improving immunity and the cecal beneficial microfloral population.

Key words

Pine bark extract, Pitamin, Broiler chicken, Immunoglobulin, Cecal microorganisms

Introduction

Pine bark extract harbors a variety of beneficial bioactive substances including proanthocyanidin, bioflavonoid as a polyphenol, and organic acids, and the majority of proanthosyanidins are catechin and epicatechin subunits (Rohdewald, 2002) and has a profound antioxidant capacity related to the inhibition of the action of free radicals and activated oxygen molecules as compared to vitamins C and E (Silliman *et al.,* 2003). Pine bark extract has been demonstrated to prevent cancer and cardiovascular diseases (Ozlem *et al.,* 2009), to suppress inflammation, to increase immunity (Grimm *et al.,* 2006). Pine bark extract has been shown to evidence antimicrobial activity in pathogenic prokaryotic (gram-positive and gram-negative) and eukaryotic (yeast and fungi) microorganisms (Torras *et al.,* 2005). Antioxidant materials in pine bark extract have been reported to reduce the numbers of *Escherichia coli O157:H7* and *Salmonella Typhimurium* in cooked ground beef during the storage period (Ahn *et al.,* 2007).

These days the emergence of antibiotic-resistant bacteria has become a serious social issue. The horizontal transfer of antibiotic resistance genes in the environment (Shakibaie *et al.*, 2009), the discovery of resistant bacteria in livestock farm products (Toroglu *et al*., 2009) have become medical and social issues of great concern. With the increasing popularity of antibiotic-free organic livestock farming in the past decade, the European Union ruled that feeds used for the production of organic livestock products be completely organic and that the use of antibiotics be no longer permitted (Commission Regulation EC 2277, 2003). Following this lead, regulations concerning the use of antibiotics in environmentally-sensitive settings and in organic livestock farming are being implemented in countries around the globe, including Korea. Therefore, it is imperative to develop new alternative substances to replace antibiotics to minimize the damage or loss that plant-intensive livestock farming industry may suffer by the exclusion of antibiotics from animal feed, and to insure continuous improvement of livestock productivity (Sang *et al.,* 2010).

The usage and current situation of antimicrobial agents for application to livestock have been already fairly thoroughly reviewed (Dibner and Richards, 2005) and antimicrobial phytochemicals from plant extract have been identified as substitutes for them (Cowan, 1999). Pitamin is a standardized Korean red pine bark extract manufactured by Nutrapharm Co. Ltd. (Seoul Korea), and contains bioflavonoids, members of the polyphenol family, which are commonly observed in French maritime pine bark extract (pycnogenol), grape seed extract, green tea extract, and gingko leaf extract (Lee *et al.,* 2008). In a previous work (Choi *et al.,* 2007) it was suggested that pitamin might be utilized as a anti-wrinkle agent based on its rate of catechin absorption. The possibility of using it in laying hens (Hong *et al.,* 2008) and broilers was studied (Kim *et al.,* 2009). Against this background, the hypothesis was formed that pine bark extract, pitamin, can be used as a replacement for an antibiotics in broiler diets. In summary, the positive effects of pitamin reported in previous studies can be applied to antimicrobial agents for broilers, and more research will be necessary to determine the appropriate level of addition for the use of pitamin as an antibiotic replacement agent in the diet.

The objectives of this study was to evaluate the growth performance, carcass characteristics, blood lipids, serum immunoglobulin, and cecum microorganisms in broiler chickens.

Materials and Methods

Experimental design: All experimental procedures, including those dealing with animals, were conducted in accordance with the scientific and ethical regulations in the Scot PIL training manual (1994), and this study was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Korea.

A total of 270, one-day-old male broiler chicks (Ross 308) were purchased from a commercial hatchery. After attained the body weight of 44 ± 1.30 g the chicks were discretionarily arranged into three treated groups with three repetitions and 30 chicks for each repetition, and the experimental diet was administered for 35 days. The treated groups were as follows: 1) control, 2) antibiotics (8 mg of avilamycin kg^{-1} of diet) and 3) pitamin (70 mg kg^{-1} of diet). The level of pitamin was determined by the results of succession study over many repetitions on the basis of the report of Kim *et al.* (2009). The antibiotic and pitamin were added at the expense of corn and all nutrient levels including crude protein and metabolic energy were the same as isocalorific and isonitrogenous diets.

Broilers were maintained under standard conditions (density: 10 chicks m^{-2}) with free access to diets and water. The bottom of each pen was covered with chaff to a height of 10 cm. The temperature of the feeding room was to be set at 33°C from the first day to the third day, then reduced by 2-3°C per week and maintained at 25°C from the 22nd day. Its relative humidity was 70% and 24 hr continuous light was utilized.

Body weight and Feed intake: Feed was supplied for ad libitum consumption and feed intake of 30 animals per each repetition was measured for 7 consecutive days. Feed intake (FI), body weight gain (BW), and feed efficiency (FE) of each stage according to broiler growth were calculated when the experimental chicks were 21 and 35 days old. FE was calculated by dividing BW during a specific period by FI. Feed and water were withdrawn 12 hr prior to slaughter. 15 chickens (five from each pen) were sacrificed via cervical dislocation (Close *et al*., 1997). The carotid arteries were cut and then after 90 seconds of bleeding the chickens were placed in hot water (58-60°C) for around 4 min. Their feathers were removed by passing a defeathering machine for the elimination for 2 min. After the blood, feather, head (cut at the first cervical vertebra) and shank were isolated and evisceration was conducted, the carcass was weighed. The dressing percentage, breast and thigh muscle, abdominal fat, bursa of Fabricius, spleen and thymus weight were measured.

Blood lipid and immunoglobulin analysis: When the experiment was ended on 35th days, 1 ml of blood was obtained from the wing veins of the chickens and stored at room temperature for 30 min. Serum was isolated via the centrifugation of blood at 2,200 ×*g* for 15 min at 4°C and stored at -80°C until biochemical analysis. Triacylglycerol (TAG), total cholesterol (TC), high density lipoprotein cholesterol (HDL·C) and low density lipoprotein (LDL·C) were analyzed using commercial enzyme kits (Sigma Co. Ltd., USA) with an automatic analyzer (Autoanalyzer 7150, Hitachi, Tokyo, Japan) and immunoglobulin levels were assessed via the double-antibody sandwich ELISA method using a commercial kit (Bethyl Laboratories, Inc., Montgomery, TX, USA). IgG (chicken IgG ELISA quantitation set, E30-104), IgA (chicken IgA ELISA quantitation set, E30-103) and IgM (chicken IgM ELISA quantitation set, E30-102) were assessed by response and via measurements of their optical density at 450 nm with a precision microplate reader (Molecular Devices, Inc., New York, USA).

Microbiological analysis: The cecum isolated was kept in sealed anaerobic jars (Oxoid, Basingstoke, UK) equipped with AnaeroGen sachets (Oxoid, Hampshire, UK). Immediately, the cecal digesta were diluted by 10 fold (1:9, w/v) by blending them with anaerobically sterilized phosphorus buffered saline (PBS, 0.1 M, pH 7.0). For enumeration, dilution procedures were conducted in an anaerobic chamber (5% hydrogen, 5% CO_2, balanced nitrogen). After a 0.1 ml sample diluted by 10^{-2}~10^{-7} was spread onto sterilized flat selective media - *Lactobacillus* SPP. (MRS agar, Oxoid, Basingstoke, UK), *Bifidobacterium* SPP. (bifidobacterium selective agar, BIM-25 medium), *Salmonella* (SS agar Difco, CM0099) and *Escherichia coli* (McConkey Purple agar) for culture. *Salmonella* and *Escherichia coli* were aerobically cultivated for 24 hr at 37°C and *Lactobacillus* SPP. and *Bifidobacterium* SPP were subjected to stationary cultivation for 48 hr. The numbers of colonies on each flat medium was measured using a microorganism counter and presented as a common logarithm of colony-forming units (CFU) g^{-1} of wet cecal digesta.

Statistical analysis: All data were analyzed via ANOVA using the Generalized Linear Model procedure of SAS software (SAS, 2004)

and the statistical significance was confirmed using Duncan's multiple range test ($p<0.05$).

Results and Discussion

Growth performance: For overall period, BW ($p<0.001$), FI ($p=0.009$) and FE ($p=0.002$) in broiler chickens fed on diets containing pitamin were significantly higher than in the antibiotics group and the control group ($p<0.05$) (Table 1). According to the results of this study broiler performance parameter; BW, FI and FE could be considerably improved via the addition of pitamin as compared to the antibiotics and control groups. These results differed from the findings of a previous study (Kim *et al*., 2009) which was reported that the body weight gain in broiler chickens fed on diets with 50 mg of pitamin kg^{-1} of diet and antibiotics was similar to each other, but the body weight gain of the group fed on diet with 100 mg of pitamin kg^{-1} of diet was lower than in the antibiotics group.

This better growth performance of the pitamin group was considered to be attributable to the fact that antioxidant capacity, increased immunity, and antimicrobial substances in pitamin worked to improve the health of animals (Torras *et al*., 2005; Cheshier *et al*., 1996). Torras *et al*. (2005) determined that the antioxidant capacity of pycnogenol from French maritime pine bark extract was significantly higher than that of vitamin E and C and grape seed extract, and pycnogenol strongly suppressed the growth of harmful bacteria and was quite effective in stimulating the growth of beneficial microorganisms. When these data were combined with this present results, the role of pitamin in the stimulation of growth performance did seem to be very important in broiler chicken.

Carcass characteristic: Carcass characteristics and weight of immune organs in the birds fed pitamin were presented in Table 2. Carcass weight ($p<0.001$), dressing percentage ($p<0.001$) and the weight of breast muscle ($p<0.001$) and thigh muscle ($p<0.001$) were larger in the birds receiving pitamin than those taking antibiotics and those in the control group; this difference was statistically significant ($p<0.05$). The antibiotics group evidenced levels significantly higher than the control group ($p<0.05$). Abdominal fat levels in the pitamin

Table - 1: Effect of dietary pitamin on body weight, feed intake and feed efficiency of broiler chickens

Period of measurement (day)	Groups				Significance
	1	2	3	SE	(P value)
		(BW) g			
1 to 21	721^{c}	748^{b}	771^{a}	7.704	0.001
22 to 35	1,049^{b}	1,065^{ab}	1,085^{a}	5.968	0.012
1 to 35	1,770^{c}	1,813^{b}	1,856^{a}	12.743	<0.001
		(FI) g			
1 to 21	938^{b}	952^{b}	975^{a}	6.382	0.018
22 to 35	1,999	1,989	1,995	5.388	NS
1 to 35	2,937^{b}	2,941^{b}	2,950^{a}	4.044	0.009
		(FE)			
1 to 21	0.75^{c}	0.77^{b}	0.79^{a}	0.006	<0.001
22 to 35	0.52^{b}	0.52^{ab}	0.53^{a}	0.002	0.068
1 to 35	0.60^{b}	0.60^{b}	0.62^{a}	0.004	0.005

1) Control = no pitamin, 2) Antibiotics = added with 8 mg of avilamycin kg^{-1} of diet, 3) Pitamin = added with 70 mg of pitamin kg^{-1} of diet. ^{a,b}Means within a row without a common superscripts are significantly different ($p<0.05$)

Table - 2: Effects of dietary pitamin on weight of immune organs and carcass characteristics of broiler chickens

Items	Groups				Significance
	1	2	3	SE	(P value)
Carcass weight, g	1,287^{c}	1,328^{b}	1,367^{a}	11.743	<0.001
Dressing percentage	72.71^{c}	73.29^{b}	73.64^{a}	0.107	0.011
Breast muscle weight (%)	22.71^{c}	23.15^{b}	23.47^{a}	0.111	<0.001
Thigh muscle weight (%)	18.20^{c}	18.51^{b}	18.83^{a}	0.096	<0.001
Abdominal fat weight (%)	1.67^{a}	1.60^{a}	1.28^{b}	0.021	0.005
Bursa weight (%)	0.12^{c}	0.16^{b}	0.20^{a}	0.011	<0.001
Spleen weight (%)	0.04^{c}	0.09^{b}	0.18^{a}	0.020	<0.001
Thymus weight (%)	0.12^{c}	0.16^{b}	0.20^{a}	0.012	0.001

1) Control = no pitamin, 2) Antibiotics = added with 8 mg of avilamycin kg^{-1} of diet, 3) Pitamin = added with 70 mg of pitamin kg^{-1} of diet. [1]% of breast and thigh muscle weight to carcass weight, % of abdominal fat, bursa, spleen and thymus weight to live weight. ^{a,b}Means within a row without a common superscripts are significantly different ($p<0.05$).

Table - 3: Effects of dietary pitamin on immunoglobulins and blood lipids of broiler chickens

Parameters	Groups				Significance
	1	2	3	SE	(P value)
Triacylglyceride (mg dl^{-1})	136.3[a]	133.9[a]	119.9[b]	2.496	<0.001
Total cholesterol (mg dl^{-1})	88.76[a]	87.95[a]	75.29[b]	0.201	<0.001
LDL cholesterol (mg dl^{-1})	32.66[a]	32.91[a]	29.21[b]	0.595	<0.001
HDL cholesterol (mg dl^{-1})	19.55[b]	19.71[b]	21.30[a]	0.288	<0.001
IgG ($\mu g\ ml^{-1}$)	45.76[c]	79.42[b]	108.65[a]	9.086	<0.001
IgM ($\mu g\ ml^{-1}$)	43.97[b]	44.21[b]	66.05[a]	3.657	<0.001
IgA ($\mu g\ ml^{-1}$)	30.50[b]	30.31[b]	45.83[a]	2.568	<0.001

1) Control = no pitamin, 2) Antibiotics = added with 8 mg of avilamycin kg^{-1} of diet, 3) Pitamin = added with 70 mg of pitamin kg^{-1} of diet. [a,b]Means within a row without a common superscripts are significantly different ($p<0.05$).

Table - 4: Effects of dietary pitamin on cecal bacterial populations in broiler chickens (log_{10} cfu g^{-1})

Bacterial population	Groups				Significance
	1	2	3	SE	(P value)
Bifidobacteria	7.04[c]	8.13[b]	9.57[a]	0.367	<0.001
Lactobacillus	7.12[c]	8.07[b]	9.13[a]	0.290	<0.001
E. coli	9.47[a]	6.74[b]	5.06[c]	0.643	<0.001
Salmonella	8.85[a]	7.04[b]	4.53[c]	0.622	<0.001

1) Control = no pitamin, 2) Antibiotics = added with 8 mg of avilamycin kg^{-1} of diet, 3) Pitamin = added with 70 mg of pitamin kg^{-1} of diet. [a,b]Means within a row without a common superscripts are significantly different ($p<0.05$)

group ($p=0.005$) were significantly lower than those of the antibiotics group and the control group ($p<0.05$). The abdominal fat was reduced by 23.35% as compared to the control group. The decreasing rates of abdominal fat between the antibiotics group and the control group did not differ significantly. With regard to the weight of the immune organs of the broilers, the pitamin group evidenced significantly heavier weights of the thymus ($p<0.001$), the bursa of Fabricius ($p<0.001$), and the spleen ($p<0.001$) as compared to the antibiotics group and the control group ($p<0.05$). The weights of the immune organs of the antibiotics group were significantly higher than the weight of the control group ($p<0.05$). The lower level of abdominal fat after feeding with pitamin was considered to be related to the reduced blood lipid level (Table 3) and the phenol components contained in pitamin can contribute to this change. Polyphenolic compounds from pine bark extract were reported to be quite effective in inhibiting lipid synthesis and stimulating lipid degradation (Ikeguchi *et al*., 2006). The pine bark extract was also shown to suppress the accumulation of lipid droplets on fat tissues (Hasegawa, 2000) and to have a strong effect of breaking down lipids via the stimulation of β-receptor mediated activity (Mochizuki and Hasegawa, 2004; Hasegawa, 1999).

The addition of pitamin to the broiler diets was observed to significantly stimulate the growth of the thymus, the spleen, and the bursa of Fabricius. The heavier weight of immune organs resulted in a growing serum immunoglobulin level (Table 3). The thymus, spleen, and bursa of Fabricius are important antibody-producing organs in birds. For the increased weight of the thymus, pitamin taken up by the broilers was thought to enhance the growth of thymocytes and could maintain a consistent increase in serum IgG production. The growth of immune tissues is the basis of immune system functionality and the immune organs of birds differ slightly from those of rats and mice. The bursa of Fabricius tends to be consistent in birds, and is thus often utilized for studies of the development and functional maturity of β-lymphocytes. The thymus and the bursa of broiler wither as the birds mature, and then the immune responses of birds is dependent on the spleen and the peripheral lymph nodes (Wang *et al*., 2000). According to Grimm (2006) pine bark extract prevented the harmful inflammation caused by immune system response and the increased weight of immune organs observed in this study was considered to promote the growth ability of broilers by suppressing inflammation.

Blood lipids and immunoglobulin analysis: TAG ($p<0.001$), TC ($p<0.001$) and LDL·C ($p<0.001$) levels in the blood of broilers were lower in the pitamin group than in the control group and the antibiotic group, and their difference was significant ($p<0.05$). The HDL·C ($p<0.001$) levels in the pitamin group were significantly higher than those of the control and the antibiotics group ($p<0.05$) (Table 3). For the decreasing rate of TAG, the level of the pitamin group was reduced by 12.03 and 10.45% as compared to the control and the antibiotics groups and TC declined by 15.17 and 14.39%, and LDL·C did by 10.56 and 11.24%, respectively. Although this study did not investigate the cholesterol contents in chicken meat, its levels could be assumed to be lower in birds fed on pitamin. Blood cholesterol in animals is well known to be accumulated *in vivo* after moving to muscle tissues and eggs. Hong *et al.* (2008) reported that when different levels of pitamin were added to the diet for laying hens, the total cholesterol and LDL·C contents in blood were reduced significantly, and this is similar to the findings of this study. They said

that the reduced levels of blood cholesterol ultimately contributed to lower cholesterol levels of eggs. The reason for the decreased blood lipid levels following the intake of pitamin was considered to be polyphenol in the pine bark extract, and the results of this study were similar to those of previous studies (Ikeguchi *et al.*, 2006; Devaraj *et al.*, 2002). According to Ikeguchi *et al.* (2006), when the cholesterol and the bile acids eliminated through the feces of rats fed on a combination diet of high-cholesterol and pine bark extract increased, the TC levels in blood and the liver were reduced and the HDL·C levels grew. Devaraj *et al.* (2002) reported that after subjects took pycnogenol or French maritime pine bark extract the LDL·C levels were reduced and the HDL·C levels were significantly increased. They determined that there were no significant differences in LDL oxidizability or plasma lipid peroxides following pycnogenol intake, and concluded that pycnogenol significantly increases the antioxidant capacity of plasma and exerts favorable effects on lipid profiles.

Blood immunoglobulins were presented in Table 5. The IgG levels ($p<0.001$) of the pitamin group were significantly higher than those of the antibiotics group and the control group ($p<0.05$). Their IgG levels increased by 137.43 and 36.80% as compared to the control group and antibiotics group, respectively. The level of the antibiotics group was significantly higher than that of the control group ($p<0.05$). Although the IgA ($p<0.001$) and IgM ($p<0.001$) levels of the pitamin group and the antibiotic group were higher than those of the control group, no significant differences were noted between the pitamin group and the antibiotic group. The higher blood IgG in broilers fed pitamin was considered to be attributable to the fact that the broilers of pitamin group resulted in more proliferation the cell of immune organs as compared to antibiotics group (Table 2).

Microbiological measurement: The numbers of *Bifidobacteria* ($p<0.001$) and *Lactobacilli* ($p<0.001$) were larger in the pitamin group than in the antibiotics and control groups, and the differences were significant ($p<0.05$). The numbers of *Escherichia coli* ($p<0.001$), *Salmonella* ($p<0.001$) of the pitamin group were significantly smaller than those of the control group and the antibiotics group and the numbers in the antibiotics group were significantly lower than those of the control group ($p<0.05$) (Table 4). In a novel finding of this study, the numbers of *Bifidobacteria* and *Lactobacilli*, which were known to be beneficial microorganisms, of the pitamin group were higher compared to the antibiotics and control group, whereas the numbers of harmful microorganisms-*Escherichia coli* and *Salmonella*-were lower as compared to those of the antibiotics or control groups. That was considered to be caused by polyphenols, strong antimicrobial materials contained in pine bark extract (Ahn *et al.*, 2007). Torras *et al*. (2005) reported that pycnogenol, an extract of maritime pine bark, considerably inhibited the growth of harmful microorganisms and was effective in stimulating the growth of beneficial ones. The decreased colony counts of *Escherichia coli* and *Salmonella* found in the broilers fed on pitamin were related to the increased colony counts of *Bifidobacteria* and *Lactobacilli* on the cecum. As the total intestinal *Bifidobacterium* and *Lactobacillus* species compete with potential pathogens for nutrients and intestinal adhesion areas, they decline the intestinal pathogens. Additionally, *Bifidobacterium* and *Lactobacillus* species secrete bacteriocin, one of the materials active to *Escherichia coli,* and *Bifidobacterium* species generate organic acids and substrates to other microorganisms. The majority of organic acids formed by the fermentation of *Lactobacillus* species are lactic acid and acetic acid, and all of these substrates can suppress intestinal pathogenic colonization (Gibson and Wang, 1994). The significantly reduced numbers of the cecal *Escherichia coli* and *Salmonella* noted in the pitamin group can be considered to be a component of this mechanism. According to the results of this study, the broilers fed on pitamin specifically stimulate the growth of *Bifidobacterium* and *Lactobacillus* species on the cecum, which enhances health and suppresses the growth of *Escherichia coli* and *Salmonella*, which are not beneficial or are harmful.

In conclusion, the addition of 70 ppm of pitamin in the diets for broiler chickens increased the growth of *Bifidobacterium* and *Lactobacillus* species and reduced the numbers of *Escherichia coli* and *Salmonella* among cecal microorganisms, improved the growth of the cells of major immune organs; the spleen, the thymus and the bursa of Fabricius, and boosted the immunoglobulin IgG content. Therefore, pitamin can be utilized as a natural antimicrobial agent for organic livestock farming to reduce the risk of bacterial resistance to antibiotics and to produce safer poultry.

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References

Ahn, J., I.U. Grun and A. Mustapha: Effects of plant extracts on microbial growth, color change and lipid oxidation in cooked beef. *Food Microbiol.,* **24**, 7-14 (2007).

Cheshier, J.E., S. Ardestani-Kaboudanian, B. Liang, M. Araghiniknam, S. Chung, L. Lane, A. Castro and R.R. Watson: Immunomodulation by pycnogenol in retrovirus-infected or ethanol fed mice. *Life Sci.,* **58**, 87-96 (1996).

Choi, J.H., M.K. Choi, O.T. Han, S.J. Han, S.J. Chung, C.K. Shim and D.D. Kim: Evaluation of skin absorption of catechin from topical formulations containing Korean pine bark extract (pinexol®). *J. Kor. Pharm. Sci.,* **37**, 359-364 (2007).

Close, B., K. Banister, V. Baumans, E.M. Bernoth, N. Bromage, J. Bunyan, W. Erhardt, P. Flecknell, N. Gregory, H. Hackbarth, D. Morton and C. Warwick: Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals,* **31**, 1-32 (1997)

Commission Regulation EC 2277: Amending annexes I and II to council regulation (EEC) 2092/91 on organic production of agricultural products and indications referring there to agricultural products and foodstuffs. *Off. J. Eur. Union. L336.,* **46**, 68-74 (2003).

Cowan, M.M.: Plant products as antimicrobial agents. *Clin. Microbiol. Rev.,* **12**, 564-582 (1999).

Devaraj, S., S. Vega-Lopez, N. Kaul, F. Schonlau, P. Rohdewald and I. Jialal: Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids,* **37**, 931-934 (2002).

Dibner, J.J. and J.D. Richards: Antibiotic growth promoters in agriculture : History and mode of action. *Pollut. Sci.,* **84**, 634-643 (2005).

Gibson, G.R. and X. Wang: Bifidogenic properties of different types of fructooligosaccharides. *Food Microbiol.,* **11**, 491-498 (1994).

Grimm, T., Z. Chovanova, J. Muchova, K. Sumegova, A. Liptákova, Z. Ïuraèkova and P. Högger: Inhibition of NF-ºB activation and MMP-9 secretion by plasma of human volunteers after ingestion of maritime pine bark extract (Pycnogenol). *J. Inflammation.* **3,** 1-8 (2006).

Hasegawa, N.: Stimulation of lipolysis by pycnogenol. *Phytotherapy Res.*, **13**, 19-620 (1999).

Hasegawa, N.: Inhibition of lipogenesis by pycnogenol. *Phytotherapy Res*., **14**, 472-473 (2000).

Hong, B.J., J.S. Oh., B.W. Kim and B.S. Park: Effect of feeding dietary pitamin as a organic livestock feed additives in laying hens. *Kor. J. Organic Agric.,* **16**, 205-218 (2008).

Ikeguchi, M., T. Masahito, T. Atsushi and T. Kinya: Effects of pine bark extract on lipid metabolism in rats. *J. Japanese Soc. Nutr. Food Sci.,* **59**, 89-95 (2006).

Kim, B.W., J.S. Oh, O.T. Han, S.O. Park and B.S. Park: Effect of pitamin as an antibiotics replacement for organic livestock feed additives in broiler chickens. *Kor. J. Organic Agric.,* **17**, 111-125 (2009).

Lee. O.H., K.Y. Kim, M.K. Jang, K.H. Yu, S.G. Kim, M.H. Kim and S.H. Lee: Evaluation of proanthocyanidin contents in total polyphenolic compounds of pine (*pinus densiflora*) needle extracts and their antioxidative activities. *J. Life Sci.,* **18**, 213-219 (2008).

Mochizuki, M. and N. Hasegawa: Pycnogenol stimulates lipolysis in 3t3-L1 cells via stimulation of β-receptor mediated activity. *Phytotherapy Res.,* **18**, 1029-1030 (2004).

Ozlem, Y.C., M. Ganzera, I. Akgun, C. Sevimli, K. Korkmaz and E. Bedir: Determination of polyphenolic constituents and biological activities of bark extracts from different *Pinus* species. *J. Sci. Food Agric.,* **89**, 1339-1345 (2009).

Rohdewald, P.A.: A review of the French maritime pine bark extract (pycnogenol), an herbal medication with a diverse clinical pharmacology. *Int. J. Clin. Pharmacol. Ther.,* **40**, 158-168 (2002).

Sang O., Park, Jae Hong Shin, Won Keun Choi, Byung Sung Park, Jin Seok Oh: Antibacterial activity of house fly-maggot extracts against MRSA (Methicillin-resistant *Staphylococcus aureus*) and VRE (Vancomycin-resistant *enterococci*). *J. Environ. Biol*., **31**, 865-871 (2010).

SAS: SAS/STAT User's guide: Statistics. SAS Inst. Inc., Cary, NC. (2004). Scot PIL training manual: Glasgow Univ. UK. (1994).

Shakibaie, M.R., K.A. Jalilzadeh and S.M. Yamakanamardi: Horizontal transfer of antibiotic resistance gene among gram negative bacteria in sewage and lake waterand influence of some physico-chemical parameters of water on conjugation process. *J. Environ. Biol*., **30**, 45-49 (2009).

Silliman, K., J. Parry, L.L. Kirk and R.L. Prior: Pycnogenol does not impact the antioxidant or vitamin C status of healthy young adults. *J. Am. Diet Assoc.,* **103**, 67-72 (2003).

Torras, M.A., C.A. Faura., F. Schonlau and P. Rohdewald: Antimicrobial activity of pycnogenol. *Phytother Res.,* **19**, 647-648 (2005).

Toroglu, E and S. Toroglu: Micobial pollution of water in Golbasi Lake in Adiyaman, Turkey. *J. Environ. Biol*., **30**, 33-38 (2009).

Wang, Y.W., C.J. Field and J.S. Sim: Dietary polyunsaturated fatty acids alter lymphocyte subset proportion and proliferation, serum immunoglobulin G concentration, and immune tissue development in chicks. *Poult. Sci.,* **79**, 1741-1748 (2000).