The Thai Journal of Veterinary Medicine

Volume 45
Issue 3 *September, 2015*

Article 9

9-1-2015

Study of Free Radical Scavenging Activity of Antibiotic Growth Promoters Flavophospholipol and Avilamycin

Krittika Kabploy

Nuntavan Bunyapraphatsara

Noppawan Phumala Morales

Nuanchan Paraksa

Original Article

Study of Free Radical Scavenging Activity of Antibiotic Growth Promoters Flavophospholipol and Avilamycin

Krittika Kabploy[1,2*] Nuntavan Bunyapraphatsara[3] Noppawan Phumala Morales[4]

Nuanchan Paraksa[5]

Abstract

Non-antibiotic feed additives have been developed to be used as alternatives, but none of them is effective as antibiotic growth promoters (AGPs). Understanding other mechanisms related to growth promoter of AGPs may be beneficial to the search for effective feed additives for animal production. *In vitro* and *in vivo* studies were conducted to evaluate the free radical scavenging activities of flavophospholipol and avilamycin. The *in vitro* study showed that flavophospholipol had a potent scavenging activity for DPPH, hydroxyl and nitric oxide radicals with the exception of superoxide anion radicals and their IC_{50} were 155.1, 62.6 and 105.6 mg/l, respectively. The scavenging capacity of one milligram flavophospholipol was about 197.5 nmoles of DPPH, 132.0 nmoles of hydroxyl and 5.8 nmoles of nitric oxide radicals. In contrast, avilamycin was able to scavenge only the hydroxyl radical with 150.6 mg/l as IC_{50} and one milligram of avilamycin scavenged approximately 52.6 nmoles. The *in vivo* study demonstrated that DPPH radical scavenging activity of broiler serum at 21 and 42 DOA was significantly higher in AGPs supplemented groups. In conclusion, the anti-oxidative property of AGPs may be one of the mechanisms to promote animal health and productivity.

Keywords: avilamycin, ESR, flavophospholipol, free radical scavenger

[1]*Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[2]*Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand*
[4]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[5]*Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen campus , Nakhon Pathom, 73140, Thailand*
***Correspondence**: krittika_k@yahoo.com*

Introduction

Free radicals are produced by oxidative reaction in physiological and pathological processes, especially in oxidative stress condition. Reactive oxygen species (ROS) including hydroxyl ($OH^{\bullet}$) and superoxide anion ($O_2^{\bullet}$) and reactive nitrogen species (RNS) including nitric oxide ($NO^{\bullet}$) are major free radicals produced in biological system (Orrenius et al., 2007). The excessive productions of free radicals induce oxidation reaction of DNA, proteins and lipids, in both polar and non-polar compartments (Meo et al., 2013). The deleterious effects of damaged-tissue impair the development, growth and reproduction (Hochachka and Somero, 2002).

Antimicrobial growth promoters (AGPs) have been used in livestock animal to improve growth performance, feed efficiency and gut health (Perez et al., 2007). Nowadays, the restriction of antibiotics is used in animal feed and focuses on the control of residues in animal tissue. This restriction involves normal flora bacteria of human and consequently leads to a problem of increasing strains of pathogen bacteria that could potentially impact on human health (Barton, 2000). Thus, the European Union has proposed phasing out of antibiotics as a growth promoter. However, the US allows the using of flavophospholipol in cattle, swine and poultry at sub-therapeutic level (Perez et al., 2007; Barros et al., 2012). Nevertheless, many countries still use avilamycin as sub-therapeutic doses in poultry, including Thailand (Delsol et al., 2005; La-ongkhum et al., 2011). Flavophospholipol is also known as bambermycin or moenomycin and is mainly against gram-positive bacteria such as staphylococci and streptococci, but does not kill beneficial bacteria in part of the normal intestinal microbiota (Pfaller, 2006). A previous report showed that supplementation with 16 mg/kg flavophospholipol in broiler diet significantly improved body weight, FCR from 1 to 45 days of age and feed intake from 1 to 21 days of age (Barros et al., 2012). Avilamycin is an oligosaccharide antibiotic substance. Its acts through binding with the 30S subunit of the ribosome and interferes with the polypeptide-synthesis function (Chauvin et al., 2005). Feeding with 10 mg/kg avilamycin improved broiler performance, carcass weight and dressing percentage (Wellenreiter et al., 2000).

The theoretical mechanisms for controlling the proliferation of intestinal microbes are still questionable due to the lower usage dose than their MIC (Niewold, 2007). The subtherapeutic doses of flavophospholipol and avilamycin for broiler are 1 to 20 mg/kg and 2.5 to 10 mg/kg, respectively (Bolder et al., 1999; Barros et al., 2012), while their MIC values against *Escherichia coli* and *Salmonella* spp. are >128 mg/l (Delsol et al., 2005; Pfaller, 2006). Furthermore, use of antibiotics can contribute to antibiotic resistance in pathogens and other bacteria (Niewold, 2007). Likewise, several researches reported the growth-mediated ability without significant effect on the intestinal microbes and intestinal morphology of AGPs in broiler chicken (La-ongkhum et al., 2012; Barros et al., 2012). Knowledge about the mechanisms of AGP-mediated growth enhancement will be beneficial to the search for effective alternatives for AGP with similar properties. Regarding the incidental result in our previous study, which the lipid-peroxidation in terms of TBARs concentration in serum was significantly decreased by avilamycin supplementation (Paraksa et al., 2011), other mechanisms of the AGPs especially anti-oxidative abilities should be proven and proposed. Therefore, the present study examined the scavenging activity of flavophospholipol and avilamycin on 1,1 diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide anion and nitric oxide radicals by using highly sensitive equipment, the spin trapping technique with electron spin resonance (ESR) spectroscopy, *in vitro* study as well as their *in vivo* effects on DPPH radical scavenging activity in broiler blood serum.

Materials and Methods

Two types of AGP, avilamycin (Maxus®, Eli Lilly, Dept. Elanco, Germany) and flavophospholipol (Flavomycin® 80, Huvepharma, Sofia, Bulgaria) were used in this experiment. Maxus® contained the active substance in the concentration of 10% avilamycin, whereas flavomycin® 80 contained the active substance in the concentration of 8% flavophospholipol.

Evaluation of anti-oxidative activity of avilamycin and flavophospholipol *in vitro* study

DPPH radical scavenging assay: DPPH scavenging assay is commonly used to study the radical-scavenging ability of antioxidants (Huang et al., 2005). Scavenging activity of the tested AGPs was measured with DPPH using the modified methods of Ancerawicz et al. (1998) and Papariello and Janish (1966). Flavophospholipol and avilamycin were extracted in methanol and diluted to give various concentrations of flavophospholipol and avilamycin. The solutions were reacted with 0.25 mM DPPH in methanol (Sigma, St. Louis, Mo, U.S.A.) in a total volume of 200 µl. The final concentrations of flavophospholipol and avilamycin were 50, 100, 150, 200, 250 mg/l and 200, 400, 600, 800, 1,000 mg/l, respectively. ESR signals were recorded at 1 min after adding antibiotic by x-band ESR spectrophotometer (ELEXSYS, Bruker Germany) under conditions as follows: central field 3505±50 G, modulation frequency 100 KHz, modulation amplitude 2.0 G, microwave power 5.07 mW, receiver gain 67 dB, and time constant 40.96 msec. Trolox (Sigma, St. Louis, Mo, U.S.A.) was used as a standard antioxidant.

Hydroxyl radical scavenging assay: Hydroxyl radicals were generated by Fenton reaction using ferrous sulfate and hydrogen peroxide. 5, 5-dimethyl-l-pyroline-*N*-oxide (DMPO) was used as spin trapping (Togashi et al., 1999). Various concentrations of flavophospholipol (25, 50, 100, 150 and 200 mg/l) and avilamycin (50, 100, 150, 200 and 300 mg/l) in deionized water were added in reaction mixtures composed of 0.1 mM Fe_2SO_4 (Sigma, St. Louis, Mo, U.S.A.), 1 M DMPO (Sigma, St. Louis, Mo, U.S.A.) and 1 mM H_2O_2 (Sigma, St. Louis, Mo, U.S.A.) in a total volume of 100 µl. ESR signals were recorded at 2 min after adding H_2O_2 by x-band ESR spectroscopy

(ELEXSYS, Bruker Germany) under conditions as follows: central field 3520±100 G, modulation frequency 100 KHz, modulation amplitude 1.25 G, microwave power 10.06 mW, receiver gain 78 dB, attenuation 13 dW, and time constant 1.28 msec. L-ascorbic acid (Sigma, St. Louis, Mo, U.S.A.) was used as a standard antioxidant.

Superoxide anion radical scavenging assay: Superoxide anion radicals were generated by hypoxanthine/xanthine oxidase system with 5, 5-dimethyl-l-pyroline-*N*-oxide (DMPO) as spin trap (Togashi et al., 1999). Five concentrations of flavophospholipol (25, 50, 100, 150 and 300 mg/l) and avilamycin (50, 100, 150, 200 and 300 mg/l) in deionized water were added in reaction mixtures composed of 4 mM hypoxanthine (Sigma, St. Louis, Mo, U.S.A.), 1 M DMPO (Sigma, St. Louis, Mo, U.S.A.) and 1 U xanthine oxidase from bovine milk (No. X4376; EC. 1.17.3.2, Sigma, St. Louis, Mo, U.S.A.) in a total volume of 100 µl. ESR signals were recorded at 2 min after adding xanthine oxidase by x-band ESR spectroscopy (ELEXSYS, Bruker Germany) under conditions as follows: central field 3520±100 G, modulation frequency 100 KHz, modulation amplitude 1.25 G, microwave power 10.06 mW, receiver gain 78 dB, attenuation 13 dW, and time constant 1.28 msec. To confirm that the ESR signals of DMPO-OOH adduct were produced from hypoxanthine/xanthine oxidase system, superoxide dismutase (SOD; Sigma, St. Louis, Mo, U.S.A.) was used as a standard scavenger.

Nitric oxide radical assay: Nitric oxide radicals were generated by *S*-nitroso-*N*-acethylpenicillamine (SNAP) and were trapped by an iron-dithiocarbamate complex [$(MGD)_2$-Fe^{2+}] (Hogg, 2010). Various concentrations of flavophospholipol (50, 100, 150, 200 and 250 mg/l) and avilamycin (200, 400, 600, 800 and 1,000 mg/l) in methanol were added in reaction mixtures composed of 5 mM SNAP (Sigma, St. Louis, Mo, U.S.A.), 25 mM *N*-(Dithiocarboxy)-*N*-methyl-D-glucamine sodium salt monohydrate (MGD; Fluka, Switzerland), 5 mM Fe_2SO_4 (Sigma, St. Louis, Mo, U.S.A.) in a total volume of 100 µl. The reaction mixtures were incubated at 37°C in a water bath for 10 min before ESR measurement (ELEXSYS, Bruker Germany). The conditions of ESR measurement were as follows: central field 3510±50 G, modulation frequency 100 KHz, modulation amplitude 3.0 G, microwave power 1.75 mW, receiver gain 70 dB and time constant 1.28 msec. To confirm that the ESR signals of [$(MGD)_2$-Fe^{2+}-NO] adduct were produced from SNAP, hemoglobin (Sigma, St. Louis, Mo, U.S.A.) was used as a standard scavenger.

In vivo study of AGPs enhance scavenging activity in broiler serum using DPPH assay: A total of four hundred and fifty six, one-day-old broilers (ROSS 308) were divided into three groups with four replications that consisted of nineteen males and nineteen females per replication. Three experimental diets composed of a corn-soybean meal based diet supplemented with 0, 10 mg/kg avilamycin and 5 mg/kg flavophospholipol, respectively, were randomly fed to each group. Dietary nutrient components for each period of growth were calculated according to recommendation of NRC (1994). The animals were kept in a pen with 12 birds/m² density in an evaporative-cooling house where water and feed were available *ad libitum*. Eight broilers per treatment were randomized for blood collection at 21, 35 and 42 days old age (DOA). The blood samples were centrifuged at 3500×g for 15 min to obtain blood serum. The blood serum was reacted with 0.5 mM DPPH (Sigma, St. Louis, Mo, U.S.A.) and detected by spectrophotometer (PerkinElmer, USA) at 525 nm (Brand-Williams et al., 1995). A_{525} was shown by the absorbance difference of A_{525} (=ΔA_{525}), and its effect attained a maximum in 5 min. Concentration of reduced DPPH molecules per minute was calculated from calibration curve.

Data analysis: The free radical scavenging activities of AGPs were calculated as percentage of inhibition (% inhibition) by the following equation:

$$\% \text{ inhibition} = 100 - [(\text{ESR signal height (sample)} \times 100) / \text{ESR signal height (control)}]$$

The scavenging capacity values were estimated by calculating the amounts of radicals that were scavenged by one milligram of AGP. In case of hydroxyl, superoxide anion and nitric oxide radicals, Hydroxyl-TEMPO (Sigma, St. Louis, Mo, U.S.A.) was used as a standard radical for calculating the radical concentrations, whereas capacity value for DPPH was directly calculated due to its radical property (Gulcin et al., 2005). All determinations were performed in at least 3 independent experiments with triplicate.

$$\text{Capacity value} = \frac{\text{Radical concentration of control} - \text{Radical concentration of the sample}}{\text{Concentration of AGP}}$$

Furthermore, IC_{50}, a value representing the concentration of compounds that cause 50% inhibition, for each radical was calculated from the relation between concentration of AGP and % inhibition of free radicals.

Statistical analysis: Data obtained from *in vivo* study was analyzed as Completely Randomized Design (CRD). Mean values of treatment groups were compared by using the Duncan's New Multiple Range Test (DMRT) and differences were considered statistically significant at $p<0.05$.

Results

ESR spectrum of the DPPH radical is shown in Figure 1. Trolox was used as a positive control. Its IC_{50} value was 6.03 mg/l and one milligram of trolox was able to scavenge about 10.4 µmoles of the DPPH radical. The ESR spectrum of DPPH radical was rapidly decreased in a dose-dependent manner after the addition of tested AGPs. Flavophospholipol had a potent scavenging activity for the DPPH radical with an IC_{50} of 155.1 mg/l and one milligram of flavophospholipol was able to scavenge about 197.5 nmoles of the DPPH radical (Table 1). However, the

scavenging capacity of flavophospholipol was about 50 times less than the standard antioxidant, trolox. On the other hand, avilamycin expressed slight scavenging activity for the DPPH radical by less than 40% reduction in ESR intensity at 1,000 mg/l (Fig 2).

Hydroxyl radicals generated in Fenton reaction were trapped by the DMPO forming DMPO-OH adduct. The typical 1:2:2:1 quarter-line ESR spectra of the DMPO-OH adduct is showed in Figure 3. The height of the second signal of spectrum was measured. DMPO-OH adduct was reduced in a dose-dependent manner after adding both avilamycin and flavophospholipol. The relation between the percentage of inhibition and concentration demonstrated that avilamycin and flavophospholipol had potent scavenging activity for the hydroxyl radical (Fig 4) with 60% and 80% maximum of inhibition and their IC_{50} were 150.6 and 62.6 mg/l, respectively.

This result found that one milligram of avilamycin and one milligram of flavophospholipol were able to scavenge about 52.6 and 132.0 nmoles of the hydroxyl radical, respectively (Table1). L-ascorbic acid was used as a positive control. Its IC_{50} value was 3.31 mg/l and one milligram of ascorbic acid was able to scavenge about 2.3 µmoles of the hydroxyl radical. The scavenging capacity of avilamycin and flavophospholipol was about 50 and 20 times less than ascorbic acid, respectively.

Table 1 IC_{50} and capacity values of AGPs on free radical scavenging activity

Radicals	Avilamycin		Flavophospholipol	
	IC_{50} (mg/l)	capacity (nmole/mg)	IC_{50} (mg/l)	capacity (nmole/mg)
DPPH	ND[1]	17.1	155.1	197.5
Hydroxyl	150.6	52.6	62.6	132.0
Superoxide anion	ND	0.5	ND	0.6
Nitric oxide	ND	ND	105.6	5.8

[1]ND: not detectable

Table 2 Effect of avilamycin and flavophospholipol as growth promoters on free radical scavenging capacity in broiler serum

Treatment	Free radical scavenging capacity (mM DPPH/min)*		
	21 DOA	35 DOA	42 DOA
Control	5.93±0.65^{b}	6.03±0.56	2.95±0.64^{B}
Avilamycin	6.43±0.92^{ab}	6.29±0.75	4.94±0.73^{A}
Flavophospholipol	7.18±0.84^{a}	6.45±0.63	4.69±1.08^{A}
P-value	0.02	0.40	0.0002

^{A,B}Means within a column without a common superscript are highly significantly different (p<0.01).
^{a,b}Means within a column without a common superscript are significantly different (p<0.05).
*mean±SD

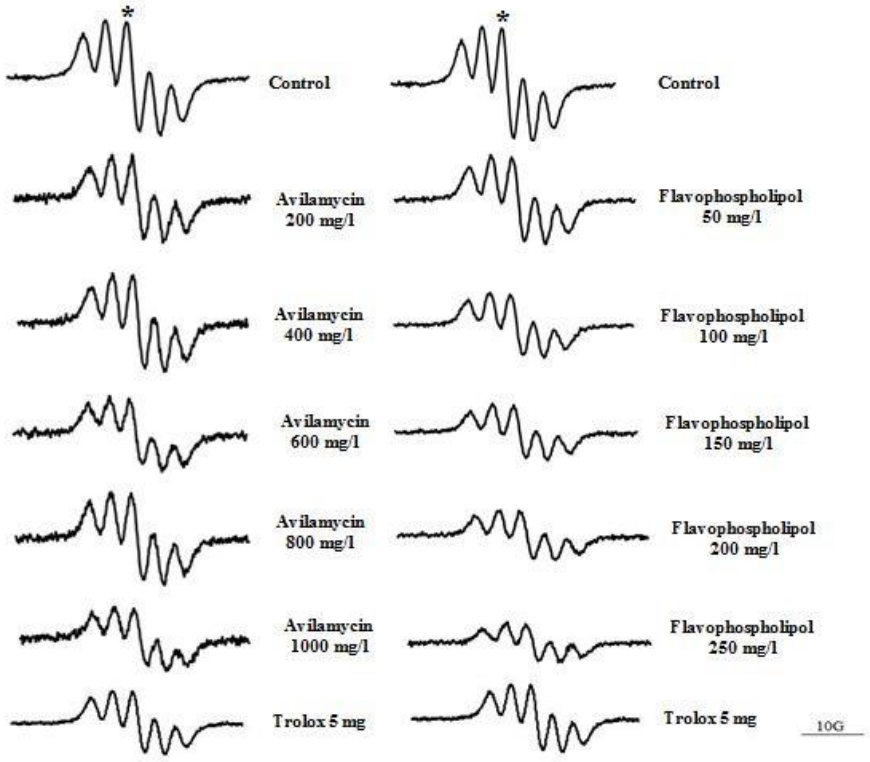

Figure 1 ESR spectrum of DPPH radical in the presence of antioxidants; avilamycin and flavophospholipol and a positive control, trolox. ESR intensity of DPPH was measured at indicated signal (*).

DMPO traps superoxide giving DMPO-OOH adduct, which distinguish ESR spectra from hydroxyl radical (Fig 5). Superoxide dismutase (SOD) was used as the reference antioxidant because it can convert the superoxide radical to hydrogen peroxide. Thus, the signal of DMPO-OOH adduct, which was inhibited by SOD, presented superoxide anion radical. The addition of avilamycin and flavophospholipol up to 300 mg/l slightly decreased ESR signal intensity with approximately 28.4% and 35.2% inhibition, respectively. This result showed that both avilamycin and flavophospholipol did not have an effective scavenging activity for the superoxide anion radical. Lastly, the nitric oxide radical, which was generated from SNAP and $[(MGD)_2\text{-}Fe^{2+}]$ complex, was used as the trapping agent. The triplet ESR signal of $[(MGD)_2\text{-}Fe^{2+}\text{-}NO]$ adduct is shown in Figure 6. Hemoglobin was used as nitric oxide scavenger for verifying the signal of $[(MGD)_2\text{-}Fe^{2+}\text{-}NO]$ adduct. Flavophospholipol expressed high potential scavenging activity for the nitric oxide radical with IC_{50} of 105.6 mg/l and one milligram of flavophospholipol was able to scavenge about 5.8 nmoles of the nitric oxide radical, while the nitric oxide radical was not scavenged by avilamycin (Table1).

The *in vivo* study evaluated the antioxidant capacity in serum using the DPPH radical scavenging assay. The scavenging capability of DPPH radical was determined by the decrease in its absorbance at 525 nm. The decrease in absorbance of DPPH radical solution is a reaction between antioxidant molecule and radical, which results in the scavenging of radical by hydrogen donation. Data from Table 2 show that flavophospholipol significantly scavenged the DPPH radical in the broiler blood serum at 21 (p<0.05) and 42 (p<0.01) DOA, while avilamycin significantly scavenged the DPPH radical in the broiler blood serum at 42 (p<0.01) DOA, compared with the control group.

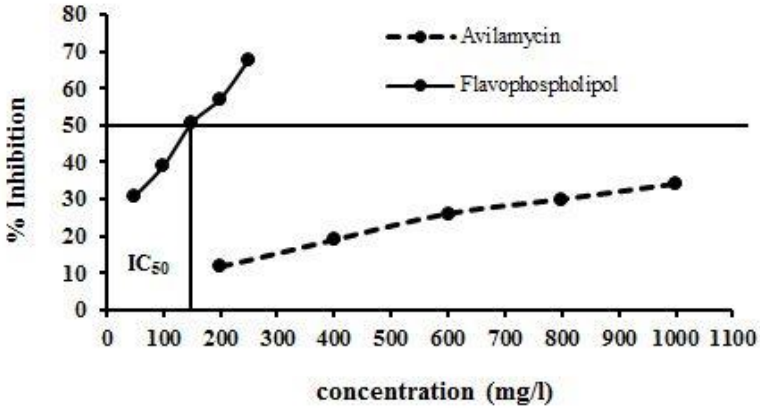

Figure 2 Relation between percentage of inhibition on DPPH radical and concentration of avilamycin and flavophospholipol

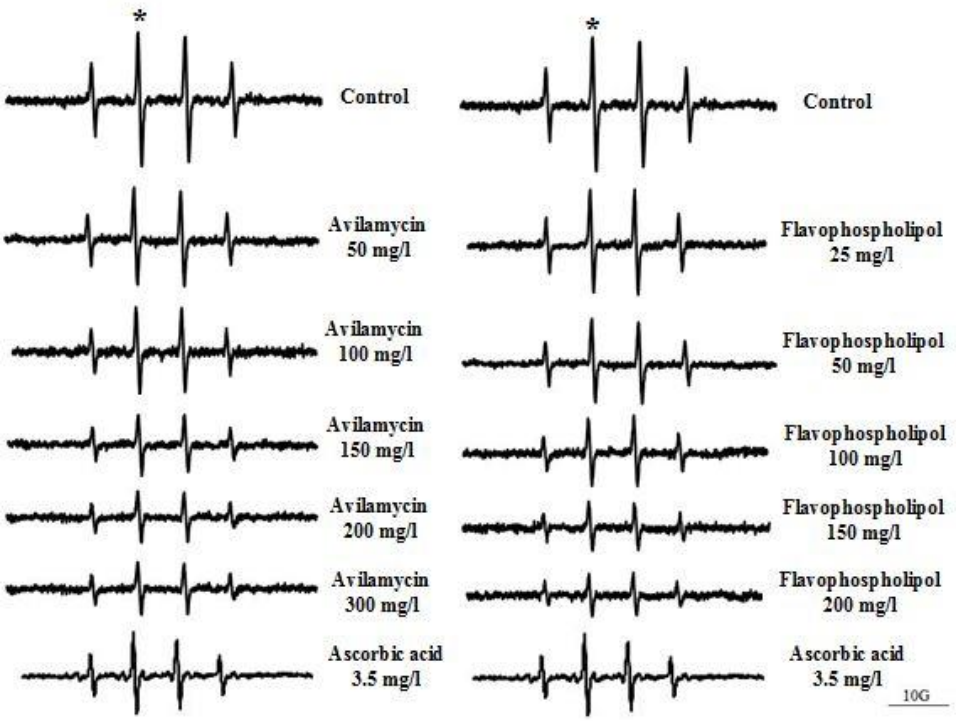

Figure 3 ESR spectrum of DMPO-OH adduct in the presence of antioxidants; avilamycin and flavophospholipol and a positive control, ascorbic acid. ESR intensity of DMPO-OH adduct was measured at indicated signal (*).

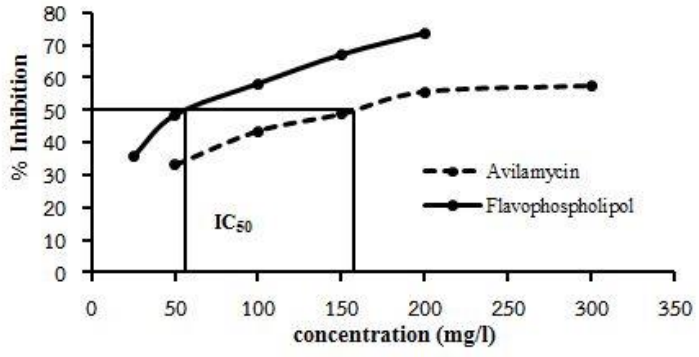

Figure 4 Relation between percentage of inhibition on hydroxyl radical and concentration of avilamycin and flavophospholipol

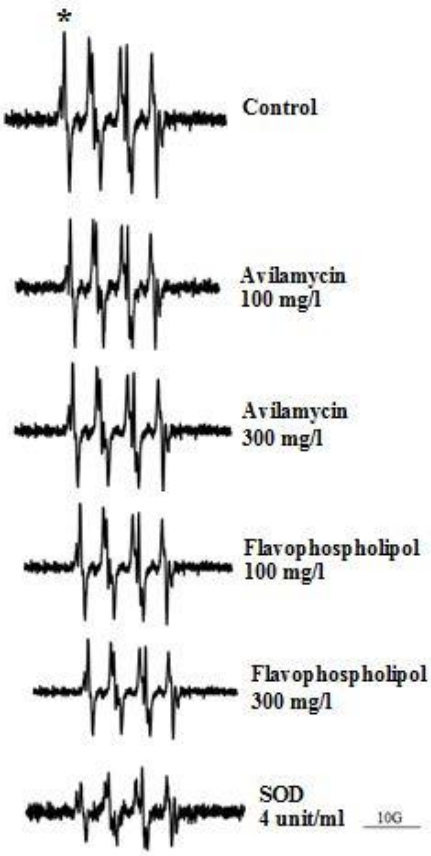

Figure 5 ESR spectrum of DMPO-OOH adduct in the presence of antioxidants; avilamycin and flavophospholipol and positive control, superoxide dismutase. ESR intensity of DMPO-OOH adduct was measured at indicated signal (*).

Discussion

ESR spectroscopy with spin trapping is a direct quantitative and qualitative method for radical scavenging assay (Kleschyov et al., 2007). Free radicals have extremely short half-lives which are difficult to be detected by ESR instrument. In addition, spin trapping agents are organic nitrone or nitroso compounds that trap the radical and give stable nitroxide radical adduct (Togashi et al., 1999), and signal intensity is directly proportional to the concentration of radicals. This experiment evaluated the scavenging activity of flavophospholipol and avilamycin on 1,1 diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide anion and nitric oxide radicals by using ESR spectroscopy. Comparison of the scavenging effectiveness between the two AGPs found that flavophospholipol had stronger antioxidant activity than avilamycin, because it could neutralize the free radicals and inhibit the hydroxyl and nitric oxide radicals, whereas avilamycin could scavenge effectively only the hydroxyl radicals. The degree of inhibition of free radicals may be related to their chemical structure. Flavophospholipol structure contains the C_{25} lipid alcohol, moenocinol that binds via an ether bridge to the C-2 hydroxyl group of 3-phosphoglyceric acid and the phosphate group is linked by phosphoacetal ester bond to a branched carbohydrate moiety (Fig 7a) (Bause and Legler, 1982). The hydroxyl groups in structure play an important role in scavenging mechanism of antioxidants by H-atom transfer from OH-group to the free radicals. For example, polyphenols (ArOH), the compound with numerous OH-group in their structure, are well-known as powerful free radical scavengers (Meo et al., 2013). Therefore, the numerous hydroxyl groups in flavophospholipol structure may contribute to the stronger antioxidant activity. On the other hand, the structure of avilamycin, which consists of six members of oligosaccharide, dichloroisoeverninic acid (DCIEA), and methyl eurekanate (Fig 7b) (Magnussen et al., 1991), has less hydroxyl groups, thus avilamycin exhibited lower scavenging activity compared to flavophospholipol.

DPPH is widely used for primarily the evaluation of free radical scavenging ability and antioxidant ability of phenolic compounds which consist of hydroxy group (-OH) bonded directly to an aromatic hydrocarbon (Gulcin et al., 2005). The reaction depends on the structural conformation of the antioxidant compound. Some compounds react very quickly with DPPH radical, reducing a number of DPPH radicals equal to their available hydroxy groups (Bondet et al., 1997). Any antioxidant that donates a hydrogen atom to the solution of DPPH could reduce the free radical (Milardovic et al., 2006). The *in vivo* study showed that avilamycin and flavophospholipol significantly scavenged the DPPH radicals in the broiler serum at 21 and 42 DOA. Intensive broiler breed has a high metabolic rate that elevates level of free radicals and increases depletion of anti-oxidative capacity (Mahmoud and Edens, 2003). Antioxidant can protect biological molecules of animal by removal of free radical initiators and propagators (Fellenberg and Speisky, 2006). Therefore, a compound with anti-oxidative activity could be used to eliminate and control free radicals, resulting in improvement in animal growth performance. Our results *in vivo* suggested the benefits of supplementing AGPs with

antioxidant properties. For the scavenging capacity of AGPs, although in the *in vitro* study they were slightly comparable with the standard antioxidant, avilamycin or flavophospholipol supplements in the broiler diet could improve and maintain the anti-oxidative property for all periods of growth. In addition, in agreement with the *in vitro* study, flavophospholipol showed higher antioxidant capacity than avilamycin. Although the scavenging activity of some antibiotics were previously reported, e.g. nitric oxide or superoxide anion inhibition of macrolide antibiotic such as erythromycin (Tenson et al., 2003; Pasquale and Tan, 2005; Perez et al., 2007), none were reported for avilamycin and flavophospholipol. The anti-oxidative activity of AGPs found in this research can be one of the mechanisms for AGP to promote the growth and health of animals and can be used for the rapidly identifying replacement of feed additives.

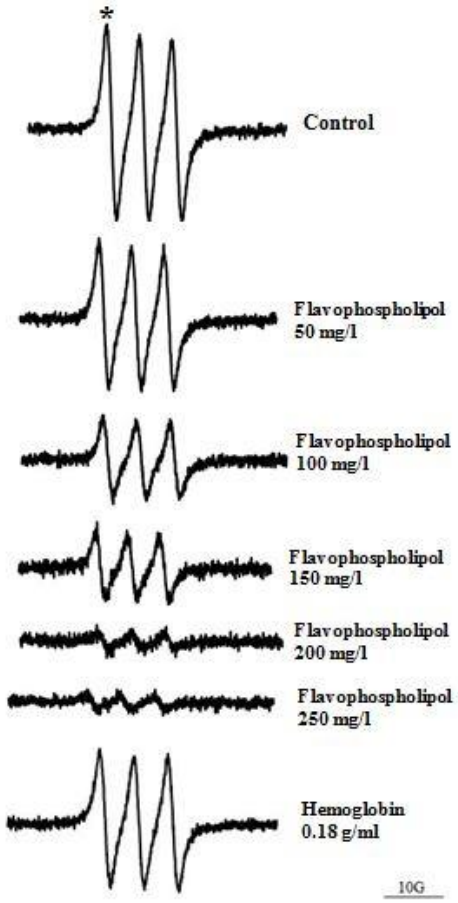

Figure 6 ESR spectrum of $[(MGD)_2\text{-}Fe^{2+}\text{-}NO]$ adduct in the presence of antioxidant; flavophospholipol and positive control, hemoglobin. ESR intensity of $[(MGD)_2\text{-}Fe^{2+}\text{-}NO]$ adduct was measured at indicated signal (*).

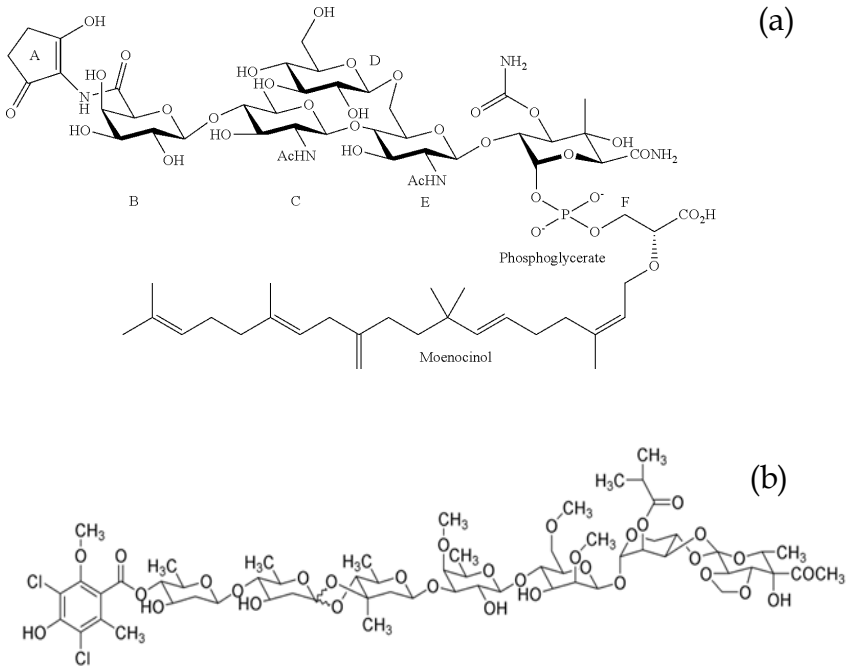

Figure 7 Chemical structure of flavophospholipol A (a) and avilamycin A (b)
Sources: Kakarla et al. (1999); Treede et al. (2003)

In conclusion, the increasing restriction on the use of antibiotic as growth promoters in animal production has led to a search for the development of alternatives. However, none of the alternatives has been as effective as AGPs due to the lack of knowledge about possible mechanisms other than the antimicrobial property. The results obtained from this study indicated that avilamycin and flavophospholipol had the anti-oxidative ability through the donation of hydrogen or electron atoms and scavenging free radicals. Therefore, feed utilization and growth performance of animal will be enhanced.

Acknowledgements

This research was partially funded by a grant from the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE) and The Natural Products for Animal Production Research and Testing Development Center, Kasetsart University. The authors greatly thank to the Department of Pharmacology, Faculty of Science, Mahidol University and Office of Atom for Peace for supporting electron spin resonance spectroscopy.

References

Ancerawicz J, Migliavacca E, Carrupt P, Testa B, Bree F, Zini R, Tillement J, Labidalle S, Guyot D, Chauvet-Monges A, Crevat A and Ridant A 1998. Structure-property relationships of trimetazidine derivatives and model compounds as potential antioxidants. Free Radic Biol Med. 25(1): 113-120

Barros R, Vieira SL, Favero A, Taschetto D, Mascarello NC and Cemin HS 2012. Reassessing flavophospholipol effects on broiler performance. R Bras Zootec. 41(12): 2458-2462.

Barton MD 2000. Antibiotic use in animal feed its impact on human health. Nutr Res Rev. 13: 279-299.

Bause E and Legler G 1982. The effect of flavomycin on the synthesis and transfer of lipid-linked saccharides in pig brain. Biochem J. 201: 481-487.

Bolder NM, Wagenaar JA, Putirulan FF, Veldman KT and Sommer M 1999. The effect of flavophospholipol (Flavomycin®) and salinomycin sodium (Sacox®) on the excretion of *Clostridium perfringens*, *Salmonella enteritidis*, and *Campylobacter jejuni* in broiler after experimental infection. Poult Sci. 78: 1681-1689.

Bondet V, Brand-williams W and Berset C 1997. Kinetics and mechanism of antioxidant activity using the DPPH• free radical method. Lebensm-Wiss U-Technol. 30: 609-615.

Brand-Williams W, Cuvelier ME and Berset C 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss U-Technol. 28: 25-30.

Chauvin C, Gicquel-Bruneau M, Perrin-Guyomard A, Humbert F, Salvat G, Guillemot D and Sanders P 2005. Use of avilamycin for growth promotion and avilamycin-resistance among *Enterococcus faecium* from broiler in a matched case-control study in France. Prev Vet Med. 70: 155-163.

Delsol AA, Randall L, Cooles S, Woodward MJ, Sunderland J and Roe JM 2005. Effect of the growth promoter avilamycin on emergence and persistence of antimicrobial resistance in enteric bacteria in the pig. J Appl Microbiol. 98: 564-571.

Fellenberg MA and Speisky H 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. World Poultry Sci J. 6(1): 53-70.

Gulcin I, Alici HA and Cesur M 2005. Determination of *in vitro* antioxidant and radical scavenging activity of propofol. Chem Pharm Bull. 53: 281-285.

Hochachka PW and Somero GN 2002. Biochemical Adaptation: Mechanisms and Process in Physiological Evolution. Oxford University Press, New York.

Hogg N 2010. Detection of nitric oxide by electron paramagnetic resonance spectroscopy. Free Radic Bio Med. 49: 122-129.

Huang D, Ou B and Prior RL 2005. The chemistry behind antioxidant capacity assays. J. Agric Food Chem. 53: 1841-1856.

Kakarla R, Ghosh M, Anderson JA, Dulina RG and Sofia MJ 1999. Expeditious route to F unit building block of moenomycin A. Tetraheddron Lett. 40: 5-8.

Kleschyov AL, Wenzel P and Munzel T 2007. Electron paramagnetic resonance (EPR) spin trapping of biological nitric oxide. J Chromatogr B. 851: 12-20.

La-ongkhum O, Pingsungvorn N, Amornthewaphat N and Nitisinprasert S 2012. Effect of the antibiotic avilamycin on the structure of the microbial community in the jejunal intestinal tract of broiler chickens. Poult Sci. 90: 1532-1538.

Magnussen JD, Daildowicz JE, Thomson TD and Donoho AL 1991. Tissue residues and metabolism of avilamycin in swine and rats. J Agric Food Chem. 39: 306-310.

Mahmoud KZ and Edens FW 2003. Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). Comp Biochem Physiol B. 136: 921-934.

Meo FD, Lemaur V, Cornil J, Lazzaroni R, Duroux JL, Olivier Y and Trouillas P 2013. Free radical scavenging by natural polyphenols: atom versus electron transfer. J Phys Chem. 117: 2082-2092.

Milardovic S, Ivekovic D and Grabaric BS 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry. 68: 175-180.

National Research Council (NRC) 1994. Nutrient Requirements of Poultry. 9thed, National Academy Press, Washington, DC.

Niewold TA 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? a hypothesis. Poult Sci. 53: 281-285.

Orrenius S, Gogvadze V and Zhivotovsky B 2007. Mitochondrial oxidative stress: implications for

cell death. Annu Rev Pharmacol Toxical. 47: 143-183.

Papariello G and Janish M 1966. Diphenylpicrazyl as an organic analytical reagent in the spectrophotometric analysis of phenols. Anal Chem. 38: 21-24.

Paraksa N, Junyaprasert V, Sribusarakum A and Bunyapraphatsara N 2011. Feed additive from Mangifera indica L. The 2nd International Forum on Screening Functional Componentsiculturral Residues and Mangiferi Research. Guangxi, China

Pasquale TR and Tan JS 2005. Nonantimicrobial effects of antibacterial agents. Clin Infect Dis. 40(1): 127-135.

Perez S, Mcjury BE, Eichhorn P and Aga DS 2007. Determination of the antimicrobial growth promoter moenomycin-A in chicken litter. J Chromatogr A. 1175: 234-241.

Pfaller MA 2006. Flavophospholipol use in animals: Positive implication for antimicrobial resistance based on its microbiologic properties. Diagn Microbiol Infect Dis. 56: 115-121.

Tenson T, Lovmar M and Ehrenberg M 2003. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J Mol Biol. 330: 1005-1014.

Togashi H, Shinzawa H, Maysuo T, Takeda Y, Takahashi T, Aoyama M, Oikawa K and Kamada H 1999. Analysis of hepatic oxidative stress status by electron spin resonance spectroscopy and imaging. Free Radic Bio Med. 28: 846-853.

Treede I, Jakobsen L, Kirpekar F, Vester B, Weitnauer G, Bechthold A and Douthwaite S 2003. The avilamycin resistance determinants AviRa and AviRb methylate 23S rRNA at the guanosine 2535 base and the uridine 2479 ribose. Mol Microbiol. 49(2): 309-318.

Wellenreiter RH, Mowrey DH, Stobbs LA and Assonville JA 2000. Effects of avilamycin on performance of broiler chickens. Vet Therapeutics. 1: 118-124.

บทคัดย่อ

การศึกษาฤทธิ์ต้านอนุมูลอิสระของยาปฏิชีวนะ Flavophospholipol และ Avilamycin ในระดับเร่งการเจริญเติบโต

กฤติกา กาบพลอย[1,2*] นันทวัน บุณยะประภัศร[3] นพวรรณ ภู่มาลา มอราเลส[4] นวลจันทร์ พารักษา[5]

ปัจจุบันมีการค้นคว้าและพัฒนาผลิตภัณฑ์สารเสริมชีวนะเพื่อเป็นสารทดแทนยาปฏิชีวนะในระดับเร่งการเจริญเติบโตของสัตว์อย่างกว้างขวาง แต่ยังไม่มีสารใดมีประสิทธิภาพเทียบเท่าการใช้ยาปฏิชีวนะในระดับเร่งการเจริญเติบโต เพื่อให้เกิดความเข้าใจถึงกลไกการทำงานของยาปฏิชีวนะที่มีผลต่อการเจริญเติบโตของสัตว์ และเพื่อเป็นแนวทางในการพัฒนาสารเสริมชีวนะให้มีประสิทธิภาพสำหรับการผลิตสัตว์ จึงทำการศึกษาฤทธิ์ต้านอนุมูลอิสระของยาปฏิชีวนะ flavophospholipol และ avilamycin ในระดับห้องปฏิบัติการและในสัตว์ทดลอง พบว่ายาปฏิชีวนะ flavophospholipol มีฤทธิ์ในการกำจัดอนุมูล DPPH ไฮดรอกซิล และไนตริกออกไซด์ ซึ่งมีค่า IC_{50} เท่ากับ 155.1, 62.6 และ 105.6 มก./ลิตร ตามลำดับ โดยยาปฏิชีวนะ flavophospholipol 1 มิลลิกรัมสามารถกำจัดอนุมูล DPPH ได้ 197.5 นาโนโมล อนุมูลไฮดรอกซิล 132.0 นาโนโมล และอนุมูลไนตริกออกไซด์ 5.8 นาโนโมล แต่ยาปฏิชีวนะ avilamycin สามารถกำจัดได้เฉพาะอนุมูลไฮดรอกซิล ซึ่งมีค่า IC_{50} เท่ากับ 150.6 มก./ลิตร โดยยาปฏิชีวนะ avilamycin 1 มิลลิกรัมสามารถกำจัดอนุมูลไฮดรอกซิลได้ 52.6 นาโนโมล และจากการศึกษาผลของยาปฏิชีวนะทั้งสองชนิดในขนาดที่ใช้เร่งการเจริญเติบโตต่อการกำจัดอนุมูล DPPH ในซีรั่มไก่เนื้อ พบว่ายาปฏิชีวนะ flavophospholipol และ avilamycin สามารถกำจัดอนุมูล DPPH ในซีรั่มของไก่เนื้ออายุ 21 วันและอายุ 42 วันอย่างมีนัยสำคัญทางสถิติ ดังนั้นคุณสมบัติในการเป็นสารต้านอนุมูลอิสระจึงน่าจะเป็นอีกหนึ่งกลไกของยาปฏิชีวนะที่ช่วยส่งเสริมทั้งทางด้านสุขภาพและผลผลิตของสัตว์

คำสำคัญ: avilamycin ESR flavophospholipol สารต้านอนุมูลอิสระ

[1]ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

[2]ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี สำนักงานคณะกรรมการอุดมศึกษา กรุงเทพฯ 10900

[3]ภาควิชาเภสัชวินิจฉัย คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล กรุงเทพฯ 10400

[4]ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล กรุงเทพฯ 10400

[5]ภาควิชาสัตวบาล คณะเกษตร กำแพงแสน มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

****ผู้รับผิดชอบบทความ** E-mail: krittika_k@yahoo.com*