Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response to sheep red blood cells

Seyed A. Hosseini,¹ Amir Meimandipour,² Fatemeh Alami,³ Ali Mahdavi,³ Hozier Mohiti-Asli,⁴ Houshang Lotfollahian,¹ Deborah Cross⁵

¹Animal Science Research Institute of Iran, Karaj, Iran
²Animal and Marine Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
³Faculty of Veterinary Medicine and Animal Husbandry, University of Semnan, Iran
⁴Department of Animal Science, University of Guilan, Rasht, Iran
⁵Independent Consultant, Belfast, Northern Ireland

Abstract

A trial was conducted to study the effects of the aromatic plant thyme, a commercial probiotic (Protexin) and avilamycin on broiler performance, blood biochemical parameters and also the antibody response to sheep red blood cells. A total of 750 broilers were assigned into five replicate groups for each of five dietary treatments, namely; control (C), 2.5 mg/kg avilamycin (AB), 0.1 g/kg commercial probiotic (P), 5 g/kg ground thyme (T1), and 7.5 g/kg ground thyme (T2). In general, body weight, feed consumption and feed conversion ratio were not affected by dietary treatments compared to the control birds (P>0.05). Birds fed the P supplemented treatment had the greatest feed efficiency. Moreover, active components of herbs may improve digestion and absorption of nutrients.

Introduction

For many years, several antibiotic growth promoters (AGP) have been effectively used as additives in poultry production. However, the overuse of these compounds has led to the emergence of antibiotic-resistant bacteria. Pressures are increasing from consumers, in their requirement for safe, healthy and nutritious foods which are produced naturally. From January 2006, all sub-therapeutic AGP have been banned in the European Union (European Commission, 1998). Removal of AGP from diets requires the industry to look for various alternatives to maintain or improve the health and performance of broilers. However, most of the alternatives can exert growth-promoting effects and some of the effects are comparable to those of AGP, but these growth-promoting effects are very variable; under certain circumstances the alternatives can even negatively affect the performance (Yang et al., 2009).

Plant-based additives are perceived to be safer and healthier than synthetic AGP. Herbs and probiotics are incorporated into poultry diets to stimulate or promote a more effective use of feed nutrients, which may subsequently result in a more rapid gain in body weight and improved feed conversion efficiency. Moreover, active components of herbs may improve digestion and stimulate broiler immune function (Ghazal and Ali, 2008). Thyme (Thymus vulgaris) is known in Iran as shirazian thyme, and is a popular medicinal plant in the region. The active components of thyme are thymol and carvacrol, which are known for their antioxidant and antimicrobial properties and are the health and performance of broilers.

Materials and methods

Dietary treatments

A total of 750 one-day-old male Cobb broiler chicks were obtained from a local hatchery and used in the experiment. The chicks were randomly allocated in groups by weight to one of five replicates for a total of five dietary treatments in a completely randomized experimental design. Each replicate consisted of 30 chicks housed in a floor pen (3x1 m). The basal diet consisted of a maize-soybean meal control ration (C), was supplemented with; 2.5 mg/kg of avilamycin (AB); 0.1 g/kg of a commercial probiotic (Protexin, consisting Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. rhamnosus, Bifidobacterium bifidum, Candida pittolopesi, Enterococcus faecium, Aspergillus oryzae and Streptococcus thermophilus with minimum 2×10⁹ CFU g⁻¹ powder, supplied by Nikotech Inc., Tehran, Iran); 5 g/kg ground thyme (T1; Thymus vulgaris supplied by Animal Science Research Institute, Karaj, Iran); 7.5 g/kg ground thyme (T2), to form the four test diets. The Protexin was stored at 4°C and incorporated into the feed each day to ensure viable bacterial cells in the feed throughout the experimental period. The viability of the bacterial cells was checked every two weeks to ensure the concentration of these viable bacterial cells
remained at \(10^6\) CFU/kg of feed. The test diets were prepared in mash form and formulated to be adequate in all nutrients, according to the National Research Council (1994) (Table 1). Food and water were provided ad libitum and the chicks were provided with artificial fluorescent illumination for 23 hours/day. The experimental diets were offered from day 1 to the end of the experiment at 42 days of age.

### Traits

#### Body weight and feed consumption

All chicks were weighed individually, and their feed consumption recorded weekly during the experiment.

#### Serum biochemical studies

At 21 days of age, 5 mL of blood was collected from the brachial vein of 2 birds from each pen. The concentrations of total protein, calcium, phosphorus, albumin, globulin, triglyceride and cholesterol in serum samples were subsequently analyzed using colorimetric methods on an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY), following manufacturer instructions for the corresponding reagent kit (Zhongsheng Biochemical Co., Ltd., Beijing, China).

#### SRBC response

At 28 days of age, 3 birds from each pen were inoculated with a volume of 0.2 mL from a 7% suspension of sheep red blood cell (SRBC). At 7 days post-inoculation (35 days of age); SRBC-inoculated birds were bled through the brachial vein, then the antibody titers (log 2) were measured by the microtiter method. The reciprocal of the highest titers (log 2) were measured by the microtiter method. The reciprocal of the highest titers (log 2) were measured by the microtiter method.

#### Statistical analysis

Data was analyzed using SPSS version 11.0 (SPSS GmbH, München, Germany). The Kolmogorov Smirnov test was used to test the normal distribution of the data. One-way ANOVA and Duncan’s multiple range test were used to compare treatments for each parameter. Statements of statistical significance were based on \(P<0.05\).

### Results and discussion

Previous studies reported varying effects of herbal plants or their extracts, probiotics and organic acids on broiler performance (Yakhkeshi et al., 2011). In the present study, feed intake tended \((P=0.086)\) to be reduced by increasing the concentration of thyme in diets for broilers at 14 days (Table 2). According to the literature, the effects of plant extract on feed intake are inconsistent (Cross et al., 2007; Bolubkasi et al., 2006; Calislar et al., 2009; Kirkpinar et al., 2011). It has been shown that when relatively high amounts (5 g/kg) of thyme essential oil were added into diet, early feed consumption (8-14 days) was decreased in broilers, but adaptation by the birds lessened the negative effects of thyme on feed intake by birds as they got older (Cross et al., 2003). Similarly, results in the present study confirmed the negative influence of high concentrations of ground thyme herb (7.5 g/kg) on feed intake by 21 days of age (Table 2).

### Table 1. Ingredients and basal diet composition.

| Ingredients, % | 1-14 | 15-28 | 29-42 |
|----------------|------|-------|-------|
| Corn           | 56.85| 59.50 | 63.58 |
| Soybean meal, 44% CP | 39.00| 34.30 | 30.00 |
| Dicalcium phosphate | 1.95| 1.70  | 1.60  |
| Oyster shell, 38% Ca | 1.40| 1.20  | 1.30  |
| DL-Methionine   | 0.30 | 0.35  | 0.30  |
| L-lysine        | 0.12 | 0.12  | 0.20  |
| Common salt     | 0.33 | 0.30  | 0.30  |
| NaHCO3          | 0.15 | 0.15  | 0.15  |
| Micro ingredients premix* | 0.50| 0.50  | 0.50  |
| Sum             | 100  | 100   | 100   |

*This premix supplied the following per kilogram of diet: vitamin A, 12,000 U; vitamin D₃, 5000 U; vitamin E, 75 U; vitamin K₃, 3 mg; thiamin, 3 mg; riboflavin, 8 mg; pantothenic acid, 13 mg; niacin, 55 mg; pyridoxine, 5 mg; folate acid, 2 mg; biotin, 0.2 mg; coline, 1600 mg; manganese, 120 mg; iron, 40 mg; copper, 15 mg; iodine, 1.25 mg; zinc, 100 mg; selenium, 0.3 mg.

### Table 2. Effects of antibiotic, probiotic and thyme on broiler performance.

| Age, days | C, control treatment; AB, diet supplemented with 2.5 mg/kg avilamycin; P, diet supplemented with 0.1 g/kg commercial probiotic; T1, diet supplemented with 5 g/kg ground thyme; T2, diet supplemented with 7.5 g/kg ground thyme. |
|-----------|------------------------------------------------------------------------------------------------|
| Feed intake, g/bird | 408.6 | 411.4 | 410.6 | 425.7 | 393.0 | 4.087 | 0.089 |
| 0-21 d | 948.2 | 890.9 | 1007.1 | 1058.8 | 930.4 | 23.27 | 0.214 |
| 0-28 d | 1971 | 1811 | 2054 | 1975 | 2106 | 79.80 | 0.273 |
| 0-35 d | 3450 | 3094 | 3513 | 3292 | 3345 | 137.89 | 0.543 |
| 0-42 d | 5084 | 4887 | 5418 | 4977 | 5590 | 257.87 | 0.370 |
| Body weight, g | 75.14 | 74.80 | 75.14 | 74.43 | 75.14 | 0.26 | 0.899 |
| 4 d | 760 | 638 | 374 | 366 | 356 | 5.67 | 0.971 |
| 14 d | 663 | 623 | 646 | 646 | 601 | 8.86 | 0.132 |
| 21 d | 1256 | 1228 | 1231 | 1243 | 1185 | 11.63 | 0.566 |
| 28 d | 1869 | 1873 | 1852 | 1882 | 1752 | 28 | 0.614 |
| 35 d | 2222 | 2452 | 2405 | 2428 | 2421 | 45.93 | 0.556 |
| 42 d | 1135 | 1118 | 1098 | 1163 | 1104 | 0.019 | 0.435 |
| Feed conversion ratio | 1.148 | 1.430 | 1.559 | 1.639 | 1.549 | 0.028 | 0.569 |
| 0-28 d | 1.505 | 1.475 | 1.669 | 1.589 | 1.777 | 0.054 | 0.535 |
| 0-35 d | 1.846 | 1.652 | 1.897 | 1.749 | 1.909 | 0.087 | 0.340 |
| 0-42 d | 2.558 | 1.993 | 2.253 | 2.050 | 2.309 | 0.126 | 0.087 |

Statistical analysis

Data was analysed using SPSS version 11.0 (SPSS GmbH, München, Germany). The Kolmogorov Smirnov test was used to test the normal distribution of the data. One-way ANOVA and Duncan’s multiple range test were used to compare treatments for each parameter. Statements of statistical significance were based on \(P<0.05\).
feed intake at early stages in the life of the chick (Table 2). It can be hypothesized that this negative effect may be due to low diet palatability when thyme is included.

Body weight was not affected by any of the dietary treatments over the course of the study (Table 2). Several factors such as genotype, housing, hygienic conditions, management, feeding system and diet affect BW gain. Previous studies have reported the beneficial effects of antibiotic, probiotic (Meimandipour et al., 2010) and phytogenics (Çiftçi et al., 2005; Simsek et al., 2007) on broiler performance. As alternatives to AGP, natural feed additives can help to promote better intestinal health, increase the production of digestive enzymes, and can improve dietary digestion and absorption to significantly impact chick performance, particularly when subjected to stressful environmental condition (disease, high temperature, poor feeding and management) (Meimandipour et al., 2010; Lee et al., 2003b; Zulkifi et al., 2000; Mountzouris et al., 2007). In the present study, good quality chicks were selected and reared in a controlled environment system, resulting in final chick mortality rates below 5%. There was no effect of dietary treatment on chick mortality. As shown in Table 2, birds fed antibiotic tended (P=0.087) to lower FCR compared to control group at 42 days of age.

Serum protein is the total amount of protein in the blood in which albumin is the most abundant. Albumin helps keep the blood from leaking out of blood vessels as well as to carry some medicines and other substances through the blood that is important for tissue growth and healing. Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. In the present study, dietary probiotic and thyme supplementation adversely influenced serum protein and albumin concentrations, with highest serum protein and albumin concentrations in the P-fed group compared to those birds fed the AB and C treatments (Table 3). Several authors have identified that probiotic supplementation in diets increased serum protein and albumin concentrations, in broilers (Agawane and Lonkar, 2004, Capcarova et al., 2011), piglets (Harding et al., 2008) and fish (Zhou et al., 2010). Harding et al., (2008) reported that probiotics did not stimulate gastrointestinal protein synthesis or reduce the severity of intestinal colitis, but reported an unidentified signaling mechanism between the gut and liver which could be responsible for the probiotic-induced increase in liver protein and plasma protein synthesis. Furthermore, dietary probiotic supplementation may increase the serum protein and albumin concentrations by increasing iron absorption in the lower intestine (Eizaguire et al., 2002). Alternatively, the lowest decrease in serum protein and albumin, which was dependent on the level of dietary supplementation, was observed in the broilers fed different levels of dietary thyme supplements, in the present experiment. This finding supports the results of previous research which investigated the bioavailability of iron from local plants in Sprague-Dawley rats, where the researchers concluded that thyme supplementation with high iron content was absorbed most efficiently but utilized least efficiently in the rats, resulting in a decreased serum hemoglobin concentration (Abo-Jadayil et al., 1999).

In this study, birds fed the dietary probiotic supplements had lowest cholesterol and triglyceride concentrations (P<0.05), which was followed by T2, T1 and antibiotic groups (Table 3). Nutritional therapies such as probiotics have been suggested to be helpful in the management of elevated cholesterol. Some probiotic strains such as Lactobacillus acidophilus can possess the ability to hydrolyze bile salts (BSH), enabling the cholesterol levels to be reduced. Nguyen et al. (2007) fed 10^6 CFU/g Lactobacillus plantarum on a daily basis to hypercholesterolemic mice, demonstrating that probiotic treatment lowered the serum cholesterol and triglycerides by 7% and 10% compared to the controls, respectively, potentially due to a direct breakdown of cholesterol and the deconjugation of bile salts. Thyme supplementation has also reduced blood cholesterol and triglyceride concentrations (Table 3). Abdolkarimi et al. (2011) added thyme extract in the drinking water of broilers, and reported that this significantly decreased plasma low density lipoprotein (LDL) cholesterol, total cholesterol and also triglyceride. These authors reported the effect in animals fed diet or water containing thyme could have been due to a suppressing effect of thymol or carvacrol on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis and/or responsible for the formation of insoluble saponin-cholesterol complexes in the gastrointestinal tract of the broiler (Case et al., 1995; Lee et al., 2003a). Thyme contains saponins, which form insoluble complexes with cholesterol in the digesta and also inhibits the intestinal absorption of endogenous and exogenous cholesterol (Oakenfull et al., 1990).

The birds fed diets containing probiotic had the highest concentrations of blood calcium and phosphorus, while those receiving the control diet were lowest and the birds fed thyme supplements were intermediate (Table 3). This finding corroborates the ideas of Eizaguire et al. (2002), who suggested that the probiotic acted to lower the pH in the gut, subsequently improving the absorption of minerals such as calcium, iron and magnesium, due to an increased mineral solubility.

The SRBC, a nonpathogenic antigen, is classified as a thymus-dependent antigen that obviously needs the help of T lymphocytes to produce antibodies. Herbs that are rich in flavonoids such as thyme (Thymus vulgaris) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune response.

Table 3. Effects of antibiotic, probiotic and thyme on blood parameters and SRBC responses at 21 and 35 days of age, respectively.

| Treatment | Total protein, g/dL | Albumin, g/dL | Cholesterol, mg/dL | Triglyceride, mg/dL | Calcium, mg/dL | Phosphorus, mg/dL | SRBC |
|-----------|---------------------|--------------|-------------------|--------------------|----------------|------------------|------|
| C         | 4.89<sup>b</sup>    | 2.23<sup>b</sup> | 146.21<sup>a</sup> | 80.85<sup>a</sup>   | 7.67<sup>a</sup> | 3.93<sup>a</sup>  | 5.50 |
| AB        | 5.27<sup>b</sup>    | 2.52<sup>b</sup> | 133.51<sup>b</sup> | 70.66<sup>b</sup>   | 8.30<sup>b</sup> | 4.81<sup>b</sup>  | 5.11 |
| P         | 6.52<sup>b</sup>    | 3.14<sup>c</sup> | 102.70<sup>c</sup> | 45.41<sup>c</sup>   | 13.98<sup>c</sup> | 7.29<sup>c</sup>  | 5.11 |
| T1        | 4.15<sup>c</sup>    | 1.45<sup>c</sup> | 121.00<sup>c</sup> | 61.45<sup>c</sup>   | 10.74<sup>c</sup> | 5.31<sup>c</sup>  | 5.83 |
| T2        | 2.58<sup>c</sup>    | 1.09<sup>d</sup> | 114.25<sup>d</sup> | 52.81<sup>d</sup>   | 9.92<sup>d</sup>  | 6.14<sup>d</sup>  | 5.67 |
| SE        | 0.221               | 0.129        | 2.619             | 2.132              | 0.353          | 0.188            | 0.215|
| P value   | 0.001               | 0.001        | 0.001             | 0.001              | 0.001          | 0.001            | 0.565|

C, control treatment; AB, diet supplemented with 2.5 mg/kg astilamycin; P, diet supplemented with 0.1 g/kg commercial probiotic; T1, diet supplemented with 5 g/kg ground thyme; T2, diet supplemented with 7.5 g/kg ground thyme. <sup>a,b</sup>Means with different superscript letter within a column are significantly different (P<0.05).
function (Manach et al., 1996; Cook and Samman, 1996). In the present study, dietary treatments failed to have any significant (P>0.05) influence on antibody response to SRBC (Table 3). Similar results were obtained by previous researcher using thyme and antibiotic (Toghyani et al., 2010). Logambal et al. (2000) reported that a dose of 20 g leaf extract of Ocimum sanctum did not produce any significant change in the primary antibody response while evoked a significant (P<0.01) increase in the secondary antibody response. Thus, the lack of response to SRBC was probably due to the low level of thyme supplementation which failed to evoke the primary immune response.

Conclusions

A clear finding to emerge from this study is that probiotic and thyme supplementation at low levels (5 g/kg) in diets produced birds that were equivalent in performance to those fed supplementary avilamycin. Additionally, dietary supplementation with probiotics in particular, and to intermediate extent ground thyme, had beneficial effects on blood biochemistry and mineral utilization in broilers. However, these theoretical beneficial effects should be further studied with large samples in the condition of the commercial farms.

References

Abdulkarimi, R., Daneshyar, M., Aghazadeh, A., 2011. Thyme (Thymus vulgaris) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. Ital. J. Anim. Sci. 10:e20.

Abu-Jadayil, S., Tukan, S.K.H., Takruri, H.R., 1999. Bioavailability of iron from four different local food plants in Jordan. Plant Food. Hum. Nutr. 54:285-294.

Agawane, S.B., Lonkar, P.S., 2004. Effect of probiotic containing Saccharomyces boulardii on experimental ochratoxicosis in broilers: Hematobiochemical studies. J. Vet. Sci. 5:359-367.

Bolubksi, S.C., Erhan, M.K., Ozkan, A., 2006. Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. S. Afr. J. Anim. Sci. 36:189-196.

Calislar, S., Gemci, I., Kamalak, A., 2009. Effects of Orego-Stim® on broiler chick performance and some blood parameters. J. Anim. Vet. Adv. 8:2617-2620.

Capcarova, M., Hascik, P., Kolesarova, A., Kacaniova, M., Mihok, M., Pal, G., 2011. The effect of selected microbial strains on internal milieu of broiler chickens after peroral administration. Res. Vet. Sci. 91:132-137.

Case, G.L., He, L., Ho, M., Elson, C.E., 1995. Induction of geranyl pyrophosphate pyrophosphatase activity by cholesterol-suppressive isoprenoids. Lipids 30:357-359.

Çiftçi, M., Guler, T., Dalkılıç, B., Ertas, O.N., 2005. The effect of anise oil (Pimpinella anisum L.) on broiler performance. Int. Poultry Sci. 4:851-855.

Cook, N.C., Samman, S., 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem. 7:66-76.

Cross, D.E., McDevitt, R.M., Hillman, K., Acamovic, T., 2007. The effects of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poultry Sci. 48:496-506.

Cross, D.E., Svoboda, K., McDevitt, R.M., Acamovic, T., 2003. The performance of chickens fed diets with or without thyme oil and enzymes. Brit. Poultry Sci. 44:18-19.

Eizaguire, I., Urkia, N.G., Asensio, A.B., Zubillaga, I., Zubillaga, P., Vidasina, C., Garcia-renanza, J.M., Adzaban, P., 2002. Probiotic supplementation reduces the risk of bacterial translocation in experimental short bowel syndrome. J. Pediatr. Surg. 37:699-702.

European Commission, 1998. Council Regulation (EC) No. 2821/98 of 17 December 1998 amending, as regards withdrawal of the authorisation of certain antibiotics, Council Directive 70/524/EC concerning additives in feeding stuffs. In: Official Journal, L 351/4, 29/12/1998, pp 1-5.

Ghazalah, A.A., Ali, A.M., 2008. Rosemary leaves as a dietary supplement for growth in broiler chickens. Int. Poultry Sci. 7:234-239.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J.R., Shafir, S., 2005. Modulation of antibody-mediated immune response by probiotics in chickens. Clin. Diagn. Lab. Immun. 12:1387-1392.

Harding, S.V., Fraser, K.G., Wykes, L.J., 2008. Probiotics stimulate liver and plasma protein synthesis in piglets with dextran sulfate-induced colitis and macronutrient restriction. J. Nutr. 138:2129-2135.

Kirkpinar, F., Unlu, H.B., Ozdemir, G., 2011. Effects of oregano and garlic essential oils on performance, carcass, organ and blood characteristics and intestinal microflora of broilers. Livest. Sci. 137:219-225.

Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., Beynen, A.C., 2003a. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res. 12:394-399.

Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., Beynen, A.C., 2002b. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Brit. Poultry Sci. 44:450-457.

Logambal, S.M., Venkatalakshmi, S., Dinakaran, R.M., 2000. Immunostimulatory effect of leaf extract of Ocimum sanctum Linn. in Oreocharis mossambicus (Peters). Hydrobiologia 430:13-120.

Manach, F., Regerat, F., Texier, O., 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr. Res. 16:517-544.

Meimandipour, A., Hoir-Bejo, M., Shahumaim, M., Aghar, K., Soleimani, A.F., Rasti, B., Yazid, A.M., 2010. Gastrointestinal tract morphological alteration by unpleasant physical contact and modulating role of Lactobacillus in broilers. Brit. Poultry Sci. 51:52-59.

Mountzouri, K.C., Tsirtsisoks, P., Kalamara, E., Nitach, S., Saitzmayr, G., Fegeros, K., 2007. Evaluation of the efficacy of a probiotic containing lactobacillus, bifidobacterium, enterococcus, and pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Sci. 86:309-317.

National Research Council, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC, USA.

Nguyen, T.D.T., Kang, J.H., Lee, M.S., 2007. Characterization of Lactobacillus plantarum PH04, a potential probiotic bacterium with cholesterol-lowering effects. J. Food Microbial. 51:358-361.

Oakenfull, D.G., Siddhu, G.S., 1990. Could saponins be a useful treatment for hypercholesterolemia? Eur. J. Clin. Nutr. 44:79-88.

Ouwehand, A.C., Tilhonen, K., Kettunen, H., Peuranen, S., Schulze, H., Rautonen, N., 2010. In vitro effects of essential oils on [Ital J Anim Sci vol.12:e19, 2013] [page 119]
potential pathogens and beneficial members of the normal microbiota. Vet. Med. Czech 55:71-78.

Simsek, U.G., Çiftçi, M., Dalkılıç, B., Guler, T., Ertas, O.N., 2007. The effects of dietary antibiotic and anise oil supplementation on body weight, carcass characteristics and organoleptic analysis of meat in broilers. Rev. Med. Vet. 158:514-518.

Toghyani, M., Tohidi, M., Gheisari, A.A., Tabeidian, S.A., 2010. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr. J. Biotechnol. 9:6819-6825.

Wegmann, T.G., Smithies, O.A., 1966. Simple haemagglutination system requiring small amounts of red cells and antibodies. Transfusion 6:67-73.

Yakhkeshi, S., Rahimi, S., Naseri, K.G., 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. J. Med. Plants 10:80-95.

Yang, Y., Iji, P.A., Choct, M., 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to infeed antibiotics. World Poultry Sci. J. 65:97-114.

Zhou, X., Wang, Y., Yao, J., Li, W., 2010. Inhibition ability of probiotic, Lactococcus lactis, against A. hydrophila and study of its immunostimulatory effect in tilapia (Oreochromis niloticus). Int. J. Eng. Sci. Technol. 2:73-80.

Zulkifli, I., Abdullah, N., Azrin, N.M., HO, Y.W., 2000. Growth performance and immune response of two commercial broiler strain fed diets containing lactobacillus cultures and oxytetracycline under heat stress conditions. Brit. Poultry Sci. 41:593-597.