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Immune Responsiveness and Performance of Broiler Chickens Fed a Diet Supplemented with High Levels of Chinese Star Anise Fruit (*Illicium verum* Hook. f)

M.S. Alhajj, M. Alhobaishi, A.R. Ger El Nabi and S.I. Al-Mufarrej
Department of Animal Production, College of Food and Agriculture Sciences,
King Saud University, P.O. Box 2460, 11451 Riyadh, Saudi Arabia

Abstract: The aim of this study was to evaluate the effect of Chinese star anise (*Illicium verum* Hook. f) on the performance and immune response of broiler chickens. A total of 288, 1 day old broiler chickens were grown over a 42 days period. The chickens were weighed individually and randomly assigned to seven treatments. The chickens in group 1 and 2 (positive and negative control groups, respectively) were fed starter and finisher diets without star anise. The chickens undergoing treatments 3-7 were fed the control starter and finisher diets supplemented with 1, 2, 4, 6 or 8 g of Chinese star anise per kg of diet, respectively. The weekly body weight, weight gain, feed intake and feed conversion ratios were measured and the immune responses against the Newcastle Disease Virus (NDV), the Infectious Bursal Disease Virus (IBDV) and the Infectious Bronchitis Virus (IBV) were evaluated. The relative weights of the lymphoid organs (thymus, bursa and spleen) were calculated as a percentage of the live body weight. The groups fed the diet supplemented with 1 or 2 g of star anise/kg of diet exhibited significant improvements ($p \leq 0.05$) in their body weight, daily weight gain and feed conversion ratio. However, there was no significant difference ($p > 0.05$) in their feed intake. The results showed that broiler chickens fed the diet supplemented with 6 g kg^{-1} had higher antibody titers against NDV and IBV whereas the chickens fed the diet containing 1 g kg^{-1} had the highest antibody titers against IBDV. No significant changes in the relative weights of the lymphoid organs were detected. These results indicate that Chinese star anise could be used as a natural additive to improve the immune responsiveness and performance of broiler chickens.

Key words: Broiler chickens, star anise, immune responsiveness, performance, lymphoid organs

INTRODUCTION

The administration of antibiotics at sub-therapeutic levels in poultry as growth promoters results in microbial resistance to such antibiotics and residues in the poultry products that can be harmful to consumers. The deleterious effects of the broad application of antibiotics in poultry and animals on humans have been well documented (Pelicano *et al*., 2004; Edens, 2003). These concerns have resulted in the restricted use of antibiotics as growth promoters in the European Union, the United States and other countries (Castanon, 2007). Following the ban on the use of antibiotics as growth promoters in animal feed, the search for natural and safe alternatives that could be used to improve growth or control diseases has increased. In this sense, aromatic plants and their extracts are interesting due to their antimicrobial, antiviral and antifungal effects. Herbal plants have been used in therapeutic medicine since ancient times and this has led to increased attention on the study of the chemical and medicinal properties of many plants (Mothana and Lindequist, 2005; Radulovic *et al*., 2007; Pavithra *et al*., 2010).

Chinese star anise (*Illicium verum*) is an aromatic evergreen plant with a star-shaped fruit that is native to China. Chinese star anise contains several compounds such as phenylpropanoids, neolignans, seco-prezizaane-type sesquiterpenes (Wang *et al*., 2011) and essential oils such as trans-anethole, estragole, limonene, linalool and cis-anethole (Dzamic *et al*., 2009). Chinese star anise extract has shown antioxidant activities (Singh *et al*., 2006), antibacterial effects (Iauk *et al*., 2003), antiviral activities (Song *et al*., 2007) and antifungal (Mugnaini *et al*., 2012), insecticidal (Park and Shin, 2005) and anticancer activities (Yadav and Bhatnagar, 2007). However, only a few studies have been carried out using star anise as a feed additive for poultry and its effects on poultry performance have shown inconsistent results in

Corresponding Author: M.S. Alhajj, Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, 11451 Riyadh, Saudi Arabia

the literature. It was reported that the use of anise seeds as a growth promoter in broilers resulted in positive effects on the live body weight, body weight gain, feed intake and feed conversion ratio (Al-Beitawi *et al*., 2010). Additionally, the body weight, digestibility, feed efficiency ratio and microbial content of the ceca showed improvements in broiler chickens supplemented with essential oils from oregano, anise and citrus (Mountzouris *et al*., 2011). However, Amad *et al*. (2011) found no significant effects of the essential oils of thyme and star anise on the weight gain, body weight or feed intake of broiler chickens or the average weights of the heart, liver, spleen and kidneys. Conversely, the digestibility of the crude protein, crude ash, crude fat, calcium, phosphorus and the feed conversion ratio were improved.

In addition, using anise extract in poultry resulted in an improvement in the antibody titers against NDV, IBDV and IBV (Al-Beitaw *et al*., 2009). Durrani found that treatment with 40 mL of aniseed extract/L of water led to a higher antibody titer against IBDV ($p \leq 0.05$) whereas no significant differences in the antibody titers against NDV and IBV were observed. However, Soltan *et al*. (2008) found that anise supplementation (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 g anise seed/kg diet) had an insignificant effect on the antibody titer against NDV using a hemagglutination inhibition test. The addition of anise at a level of 0.50 g kg^{-1} of diet significantly improved the lymphocyte levels compared with the control. In addition, the relative weight of the thymus was insignificantly increased in chickens fed a diet containing 0.50 g kg^{-1} and there was no effect on the relative weights of the bursa or spleen but the highest anise concentration (1.50 g kg^{-1}) showed a negative effect on the relative weights of the spleen, bursa and thymus. Furthermore, *Illicium verum* shows sedative and anti-inflammatory effects. It was reported (Jin *et al*., 2013) that *Illicium verum* possess potent anti-oxidative and anti-inflammatory activities and may be useful in the field of health products.

In this study, the influence of high level of Chinese star anise (*Illicium verum* Hook. f) on the performance and immune responses of broiler chickens was evaluated.

MATERIALS AND METHODS

Chickens and husbandry: A total of 288, 1 day old broiler chickens (Ross 308) obtained from a local hatchery were grown over a 42 days period. The chickens were weighed individually and randomly assigned to seven groups following a completely randomized design with seven replicates per treatment group. The birds were housed in electrically heated battery cages with continuous light during the experimental period. The chickens in group 1 and 2 were fed starter (days 1-21) and finisher (days 22-42) diets without star anise and were kept as control groups (the positive group was vaccinated and the negative group was not vaccinated). The chickens in group 3, 4, 5, 6 and 7 were fed the starter and finisher diets plus 1, 2, 4, 6 or 8 g of star anise/kg of diet, respectively. The chemical analysis of the commercial diet, obtained from ARASCO is presented in Table 1. The feed and water were provided *ad libitum*. This research was performed at the Laboratory of Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia.

Preparation of the chinese star anise (*Illicium verum*) fruits: The dried Chinese star anise (*Illicium verum*) fruits were obtained from a local herb store in Riyadh, Saudi Arabia and were added to the diets after being ground to a powder at 1, 2, 4, 6 or 8 g kg^{-1} diet.

Performance measurements: The performance of the broiler chickens including the body weight, daily weight gain, feed consumption and Feed Conversion Ratio (FCR) was determined over 6 weeks. All of the birds in each group were weighed individually at hatching and again at 7, 14, 21, 28, 35 and 42 days of age. The daily weight gain was then calculated for the following periods: hatch-7, 14, 21, 28, 35 and 42 days of age. At the beginning of each week, the feed offered to each replicate was weighed and at the end of each week, the remaining feed residue was weighed and subtracted from the offered amount to calculate the feed consumption during that week. The feed conversion ratios were calculated for the following time periods: hatch-7, 14, 21, 28, 35 and 42 days of age. The mortality rate was recorded daily and any dead birds were removed daily (in the morning) and weighed. Their feed consumption was estimated and removed from the group consumption.

Table 1: Chemical analysis of the basal diets

	Basal diet	
Ingredients	Starter (days 1-21)	Finisher (days 22-42)
Metabolic energy	2900 kcal kg^{-1}	3000 kcal kg^{-1}
Crude protein	21.5%	18.5%
Crude fat	2.5%	3.%
Calcium	1.0%	0.9%
Available phosphorus	0.42%	0.4%
Sodium	0.15%	0.15%
Lysine	1.20%	1.0%
Methionine	0.50%	0.45%
Meth+cysteine	0.85%	0.80%

Each kilogram of diet contains the following nutrients: vitamin A: 12000 IU; vitamin D: 5000 IU; vitamin E: 60 mg; vitamin C: 100 mg; vitamin K: 4 mg; vitamin B12: 0.03 mg; vitamin B6: 5 mg; vitamin B2: 8 mg; vitamin B1: 3 mg; zinc: 70 mg; selenium: 0.2 mg; manganese: 90 mg; iron: 35 mg; Iodine: 2 mg; copper: 8 mg; cobalt: 0.5 mg, choline: 900 mg; biotin: 0.2 mg; folic acid: 2 mg; pantothenic acid: 15 mg and niacin: 40 mg

Table 2: Body weights of the broiler chickens fed a diet supplemented with different levels of star anise (*Illicium verum* Hook. f)

Treatments	Age (days)			
(g kg^{-1} diet)	1	7	21	42
1	46.30	151.44	681.33	2358.0000^{a}
2	46.28	146.97	682.56	2383.6300^{a}
4	46.30	147.47	681.73	2246.2200^{bc}
6	46.30	143.26	680.34	2242.4500^{bc}
8	46.31	143.02	677.33	2163.8400^{c}
Control+ve	46.34	147.26	688.53	2226.5300^{bc}
Control-ve	46.33	147.07	704.67	2291.4800^{ab}
SEM	0.01	2.98	7.42	31.7800
Pr>F	0.21	0.49	0.19	0.0002

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Table 3: Daily weight gain of the broiler chickens fed a diet supplemented with different levels of star anise (*Illicium verum* Hook. f)

Treatments	Age (days)		
(g kg^{-1} diet)	1-21	22-42	1-42
1	32.45	77.3000^{b}	55.1200^{a}
2	32.55	81.0800^{a}	55.7300^{a}
4	32.42	75.2500^{b}	52.4600^{bc}
6	32.31	74.3900^{b}	52.3700^{bc}
8	32.01	69.8800^{c}	50.5000^{c}
Control+ve	32.76	74.4100^{b}	51.9900^{bc}
Control-ve	32.40	76.0900^{b}	53.5300^{ab}
SEM	0.31	1.2200	0.7600
Pr>F	0.78	<.0001	0.0002

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Vaccination of the chickens: The birds were vaccinated against ND and IB using live attenuated vaccines at 5 days of age and again at 28 days of age as a booster dose and against IBD at 18 day of age according to the manufacturer's directions (Fort Dodge Animal Health, USA). Blood samples were collected from 12 birds from each group at 7, 14, 21, 28, 35 and 42 days of age and then serum separated and stored in a deep freeze until required. The antibody titer to ND, IB and IBD were detected using an Enzyme Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions (IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine, 04092, United States).

Collection of the lymphoid organs: At 21 and 42 days of age, 5 birds from each group were slaughtered and their total body weights were recorded. Then, the birds were dissected and their lymphoid organs (thymus, bursa and spleen) were carefully removed and individually weighed. The relative weights of the different organs were calculated as a percentage of the live body weight (Table 2).

Statistical analysis: The data were subjected to a one-way analysis of variance using the general linear models procedure in SAS® (SAS, 1999).

RESULTS AND DISCUSSION

Broiler performance: The chickens were in good health and did not suffer from any health problems during the entire experimental period. The addition of the Chinese star anise (*Illicium verum* Hook. f.) fruits to the broiler chicken's feed led to an improvement in the broiler performance. The results showed that the groups fed the diet supplemented with 1 and 2 g kg^{-1} exhibited a significant improvement ($p \leq 0.05$) in their body weight, daily weight gain and feed conversion ratio compared with the other groups and the control. The broiler chickens fed the diet containing 2 g kg^{-1} had the highest daily weight gain during the finisher period with an improvement in their daily weight gain of approximately, 9% compared with the control, followed by the 1 g kg^{-1} treatment which had an improvement of approximately, 4% (Table 3). However, the lowest body weight was found in the groups fed the diet containing the highest level of star anise (8 g kg^{-1}) with a lower daily weight gain during the finisher stage as well as during the entire period. The groups fed the diet supplemented with 1 and 2 g of Chinese star anise/kg of diet showed a significant improvement in their body weights during the entire period (days 1-42) which were approximately, 6% (2358 g) and 7% (2383 g) higher than the control, respectively (Table 2). The results were consistent with previous studies that showed that anise has a positive effect on the live body weight, body weight gain and feed conversion ratio of broiler chickens (Al-Beitawi *et al*., 2010; Al-Kassie, 2008). The body weight, digestibility of organic matter, feed efficiency ratio and ceca microbial content of broiler chickens supplemented with essential oils from oregano, anise and citrus were also found to show improvements (Mountzouris *et al*., 2011). The improvement of the broiler performance in this study could be due the active component in star anise, anethol which may lead to better digestion through the induction of endogenous secretion enzymes, better absorption and an enhanced microbial balance in the gut. This is supported by the findings by Amad *et al*. (2011) who found that the inclusion of essential oils from thyme and star anise resulted in an improvement of the digestibility of the crude protein, crude ash, crude fat, calcium and phosphorus. This enhancement in the digestibility also leads to an increased surface area for absorption in the intestine and improved nutrient absorption. Additionally, it was found that essential oils have a stimulatory effect on pancreatic enzymes (Rao *et al*., 2003) and Ghalib and Mayada reported that anise can be used as an antimicrobial balance in the intestinal tract of broiler chickens.

There were no significant differences ($p \leq 0.05$) among the treatments during the starter phase (days 1-21); instead, the effects of the star anise on the broiler performance appeared during the finisher period (days 22-42) which indicates that the efficiency of Chinese star anise on broiler performance is cumulative. By contrast, Simsek *et al*. (2005) reveled that there was a significant difference in the body weight and body weight gain of broiler chickens fed a diet supplemented with a blend of essential oils extracted from cloves, thyme and anise at 20 days but no difference was found at 40 days ($p \leq 0.05$).

The Feed Conversion Ratio (FCR) differed significantly between the treatment groups (Table 4). The broiler chickens fed the diet containing 1 or 2 g of star anise/kg of diet showed a better feed conversion ratio compared with the other treatments as well as the control. However, there was no significant difference in the feed intake among all of the groups (Table 5). The groups fed the diet containing 8 g kg^{-1} of star anise exhibited no significant differences in their feed consumption compared with the other groups. The reduction in the feed intake, body weight and daily weight gain of the group fed the diet supplemented with 8 g of star anise/kg of diet may be due to its negative effect on the tastiness of the feed and the digestive system. The results agreed with Soltan *et al*. (2008) who found that the body weight gain and relative growth rate were improved in groups a fed diet supplemented with 0.50 or 0.75 g of anise/kg of diet. However, there was no significant difference among the treatments in regards to the feed intake and feed conversion ratio. In addition, the highest level of anise supplementation (1.50 g kg^{-1}) exhibited a negative effect on the growth performance. Additionally, the addition of anise to broiler diets at levels of 0.5 and 1% was found to improve the FCR (Al-Kassie, 2008). Another study that used a mixture of essential oils (extracted from oregano, anise and citrus) resulted in a significant improvement in the FCR ($p \leq 0.05$) of broiler chickens during the grower and finisher stages (Eckert *et al*., 2010).

Table 4: Feed conversion ratio of the broiler chickens fed a diet supplemented with different levels of star anise (*Illicium verum* Hook. f)

Treatments	Age (days)		
(g kg^{-1} diet)	1-21	22-42	1-42
1	1.41	1.78	1.640^{b}
2	1.47	1.70	1.640^{b}
4	1.41	1.87	1.740^{a}
6	1.42	1.86	1.740^{a}
8	1.48	1.78	1.760^{a}
Control +ve	1.43	1.86	1.750^{a}
Control -ve	1.46	1.92	1.750^{a}
SEM	0.04	0.04	0.030
Pr>F	0.25	0.08	0.002

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Table 5: Feed intake of the broiler chickens fed a diet supplemented with different levels of star anise (*Illicium verum* Hook. f)

Treatments	Age (days)		
(g kg^{-1} diet)	1-21	22-42	1-42
1	852.58	2930.09	3782.67
2	907.34	2929.65	3836.99
4	870.67	2948.29	3818.96
6	855.52	2962.47	3817.99
8	890.22	2771.02	3661.24
Control +ve	892.83	2912.16	3804.99
Control -ve	929.87	2991.50	3921.37
SEM	21.65	52.55	63.52
Pr>F	0.15	0.12	0.20

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Immune response: The effect of the Chinese star anise on the immune response in the poultry was limited. The results of the experiment showed that the broiler chickens fed the diet inoculated with 6 g of Chinese star anise/kg of diet exhibited higher antibody titers against NDV compared with the positive control group (Table 6) at 6 weeks of age, although, no significant differences were observed between the groups fed 1, 2, 4 or 8 g kg^{-1} compared with the positive control group. This indicates that the addition of 6 g of star anise/kg of diet to the diet of broiler chickens has a positive effect on the immune response against the Newcastle disease vaccine. However, the results showed that the star anise did not affect the immune response during the 1st 5 weeks of age while a significant difference between treatments appeared at 6 weeks of age.

The results of the antibody titers to the IBDV vaccine in the broiler chickens fed a diet supplemented with star anise resulted in an improvement in the immune response against IBDV; all of the treatments that received a diet supplemented with anise (1, 2, 4, 6 and 8 g kg^{-1} of diet) showed a higher antibody titer against IBDV than the control group. The broiler chickens that were fed the diet

Table 6: Antibody titers against NDV of the broiler chickens fed a diet supplemented with star anise (*Illicium verum* Hook. f)

Treatments	Age (weaks)					
(g kg^{-1} diet)	1	2	3	4	5	6
1	3.35	2.79	2.09	2.02	2.9000^{a}	3.4800^{ab}
2	3.27	2.65	2.13	1.95	2.7300^{a}	3.4000^{ab}
4	3.35	2.79	2.21	2.07	2.7000^{a}	3.4300^{ab}
6	3.37	2.70	2.14	1.96	2.8500^{a}	3.6200^{a}
8	3.35	2.60	2.08	1.92	2.7700^{a}	3.5100^{ab}
0 (control +ive)	3.43	2.83	2.04	2.10	2.7700^{a}	3.2500^{b}
0 (control -ive)	3.41	2.78	2.15	1.81	1.3800^{b}	0.7800^{c}
SEM	0.04	0.08	0.06	0.08	0.1300	0.1000
p-values	0.20	0.33	0.66	0.19	<0.0001	<0.0001

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Table 7: Antibody titers against IBDV of the broiler chickens fed a diet supplemented with star anise (*Illicium verum* Hook. f)

Treatments	Age (weeks)					
(g kg^{-1} diet)	1	2	3	4	5	6
1	3.11	2.28	1.71	1.43	1.9300^{a}	2.2700^{a}
2	3.11	2.11	1.70	1.49	1.8300^{a}	2.0400^{b}
4	3.16	2.48	1.85	1.53	1.8500^{a}	1.9000^{b}
6	3.19	2.52	1.77	1.58	1.7900^{a}	1.9900^{b}
8	3.12	2.31	1.70	1.58	1.7400^{a}	1.9500^{b}
0 (control +)	3.10	2.34	1.79	1.52	1.7000^{a}	1.5800^{c}
0 (control -)	3.10	2.32	1.83	1.34	1.1600^{b}	0.8800^{d}
SEM	0.05	0.09	0.07	0.06	0.1000	0.0600
p-values	0.86	0.10	0.22	0.12	<0.0001	<0.0001

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Table 8: Antibody titers against IBV of the broiler chickens fed a diet supplemented with star anise (*Illicium verum* Hook. f)

Treatments	Age (weeks)					
(g kg^{-1} diet)	1	2	3	4	5	6
1	2.80	2.31	1.94	1.820^{a}	2.0100^{a}	2.3300^{ab}
2	2.60	2.08	1.92	1.900^{a}	2.0800^{a}	2.0900^{ab}
4	2.86	2.38	1.95	1.910^{a}	2.0700^{a}	2.2400^{ab}
6	2.75	2.21	1.82	2.020^{a}	2.1200^{a}	2.3800^{a}
8	2.59	2.19	1.87	1.860^{a}	2.0000^{a}	2.2200^{ab}
0 (control +)	2.71	2.34	1.87	1.900^{a}	1.9400^{a}	2.0200^{b}
0 (control -)	2.73	2.20	1.88	1.410^{b}	1.0600^{b}	0.7400^{c}
SEM	0.08	0.08	0.10	0.090	0.0900	0.1100
p-values	0.10	0.15	0.54	0.002	<.0001	<.0001

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

supplemented with 1 g kg^{-1} of star anise had a higher antibody titer against IBDV compared with the other treatments and the control (Table 7). The immune response of the broiler chickens against IBV was similar to that observed for the NDV vaccine (Table 8). It was found that the broiler chickens that were fed the diet containing 6 g of anise/kg of diet showed the highest antibody titers compared with the other treatments and the positive control group. The results were also in agreement with previous reports (Al-Beitawi *et al*., 2010) found that the use of anise seeds in broiler chickens resulted in improved antibody titers against NDV, IBDV and IBV. Additionally, Bayram *et al.* (2007) reported that chickens fed a diet containing anise seed had the highest antibody titers compared with the control. However, the results are in disagreement with Soltan *et al.* (2008) who found that anise supplementation had no significant effects on the antibody titer against Newcastle disease, according to a hemagglutination inhibition test. The improvement in the antibody titers against NDV, IBDV and IBV in the present study may be due to the effect of the active components of star anise such as anethole, eugenol, methylchavicol, anisaldehyde and estragol (Ciftci *et al*., 2005).

In addition, star anise has antioxidant activities (Singh *et al*., 2006), antibacterial effects (Iauk *et al*., 2003), antiviral activities (Song *et al*., 2007) and antifungal effects (Mugnaini *et al*., 2012). Furthermore, *Illicium verum* has also shown sedative and anti-inflammatory effects (Jin *et al*., 2013). Another explanation is that *Illicium verum* has immunomodulatory effects via the manipulation of the expression of chemokines and cytokines (Lee *et al*., 2011; Sung *et al*., 2012a, b; Sung and Kim, 2013). The differences between this study and others may be attributed to one of the following reasons: the majority of the previous studies used either anise seed (*Pimpinella anisum*) or mixture of compounds or essential oils, different inoculation levels, different types of chickens, different sources of anise or different experiment conditions and feed compositions.

Table 9: Relative weights of the immune organs of the broiler chickens fed a diet supplemented with different levels of star anise (*Illicium verum* Hook. f)

Age groups	21 days			42 days		
(g kg^{-1} diet)	Thymus	Bursa	Spleen	Thymus	Bursa	Spleen
1 g	0.42	0.26	0.090	0.38	0.17^{a}	0.11
2 g	0.40	0.22	0.090	0.37	0.16^{a}	0.09
4 g	0.37	0.25	0.090	0.43	0.16^{a}	0.11
6 g	0.35	0.28	0.100	0.43	0.19^{a}	0.10
8 g	0.32	0.28	0.100	0.43	0.17^{a}	0.09
Control +ve	0.34	0.23	0.090	0.37	0.18^{a}	0.08
Control -ve	0.43	0.24	0.080	0.35	0.10^{b}	0.09
SEM	0.03	0.03	0.009	0.05	0.02	0.01
p-values	0.17	0.63	0.470	0.87	0.01	0.43

The means within a column with different superscripts are significantly different ($p \leq 0.05$)

Lymphoid organs: The relative weights of the lymphoid organs (bursa, spleen and thymus) are presented in Table 9. There were no significant changes in the relative weights of the lymphoid organs at 21 and 42 days of age. Although, the group fed the diet containing 6 g of star anise/kg of diet showed a greater increase in the relative weight of the bursa at 42 days of age, the results were not significant. These results are in agreement with Soltan *et al.* (2008) who found that anise supplementation had no effect on the relative weight of the bursa or spleen whereas the highest level of anise that was used (1.50 g kg^{-1}) had a negative effect on the relative weights of the spleen, bursa and thymus.

CONCLUSION

Under the experimental conditions used in this study, it can be concluded that Chinese star anise could be used as natural feed additive to enhance the performance and immune response of broiler chickens. Moreover, chickens fed a diet inoculated with 1 or 2 g of star anise/kg of diet exhibited better performance compared with the other groups. The group supplemented with 1 g kg^{-1} of star

anise showed the highest antibody titers against IBD whereas the chickens supplemented with 6 g kg^{-1} of star anise had higher antibody titers against ND and IB. More studies are still needed to illustrate how star anise affects the gastrointestinal tract and immune system of broiler chickens. We also recommend conducting similar experiments under field conditions.

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REFERENCES

Al-Beitawi, N.A., S.S. El-Ghousein and A.H. Nofal, 2010. Antibiotic growth promoters and anise seeds in broiler diets. Jordan J. Agric. Sci., 5: 472-481.

Al-Kassie, G.A.M., 2008. The effect of anise and rosemary on broiler performance. Int. J. Poult. Sci., 7: 243-245.

Amad, A. A., K. Manner, K.R. Wendler, K. Neumann and J. Zentek, 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult. Sci., 90: 2811-2816.

Bayram, I., I. Cetingul, B. Akkaya and C. Uyarlar, 2007. Effects of aniseed (*Pimpinella anisum* L.) on egg production, quality, cholesterol levels, hatching results and the antibody values in blood of laying quails (Coturnix coturnix japonica). Archiva Zootechnica, 10: 73-77.

Castanon, J.I.R., 2007. Review history of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci., 86: 2466-2471.

Ciftci, M., T. Guler, B. Dalkilic and O.N. Ertas, 2005. The effect of anise oil (*Pimpinella anisum* L.) on broiler performance. Int. J. Poult. Sci., 4: 851-855.

Dzamic, A., M. Sokovic, M.S. Ristic, S. Grijic-Jovanovic, J. Vukojevic and P.D. Marin, 2009. Chemical composition and antifungal activity of *Illicium verum* and *Eugenia caryophyllata* essential oils. Chem. Nat. Compounds, 45: 259-261.

Eckert, N.H., J.T. Lee, D. Hyatt, S.M. Stevens and S. Anderson *et al*., 2010. Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets. J. Applied Poult. Res., 19: 59-67.

Edens, F.W., 2003. An alternative for antibiotic use in poultry: Probiotics. Revista Brasileira de Ciencia Avicola, 5: 75-97.

Iauk, L., A.M. Lo Bue, I. Milazzo, A. Rapisarda and G. Blandino, 2003. Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. Phytother. Res., 17: 599-604.

Jin, K.S., Y.N. Oh, J.Y. Lee, B.Y. Son and W. Choi *et al*., 2013. Anti-oxidative and anti-inflammatory activities of seven medicinal herbs including Tetrapanax papyriferus and Piper longum Linne. Korean J. Microbiol. Biotechnol., 41: 253-262.

Lee, J.B., C. Yamagishi, K. Hayashi and T. Hayashi, 2011. Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from *Pimpinella anisum*. Biosci. Biotechnol. Biochem., 75: 459-465.

Mothana, R.A.A. and U. Lindequist, 2005. Antimicrobial activity of some medicinal plants of the islend Soqotra. J. Ethnopharmacol., 96: 177-181.

Mountzouris, K.C., V. Paraskevas, P. Tsirtsikos, I. Palamidi, T. Steiner, G. Schatzmayr and K. Fegeros, 2011. Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol., 168: 223-231.

Mugnaini, L., S. Nardoni, L. Pinto, L. Pistelli, M. Leonardi, F. Pisseri and F. Mancianti, 2012. *In vitro* and *in vivo* antifungal activity of some essential oils against feline isolates of *Microsporum canis*. J. Med. Mycol., 22: 179-184.

Park, I.K. and S.C. Shin, 2005. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). J. Agric. Food Chem., 53: 4388-4392.

Pavithra, P.S., V.S. Janani, K.H. Charumathi, R. Indumathy, S. Potala and R.S. Verma, 2010. Antibacterial activity of plants used in Indian herbal medicine. Int. J. Green Pharm., 4: 22-28.

Pelicano, E.R.L., P.A. De Souza, H.B.A. De Souza, F.R. Leonel, N.M.B.L. Zeola and M.M. Boiago, 2004. Productive traits of broiler chickens fed diets containing different growth promoters. Braz. J. Poult. Sci., 6: 177-182.

Radulovic, N., V. Stankov-Jovanovic, G. Stojanovic, A. Smelcerovic, M. Spiteller and Y. Asakawa, 2007. Screening of *in vitro* antimicrobial and antioxidant activity of nine Hypericum species from the Balkans. Food Chem., 103: 15-21.

Rao, R.R., K. Platel and K. Srinivasan, 2003. *In vitro* influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. Nahrung, 47: 408-412.

SAS., 1999. SAS User's Guide. Version 8, SAS Institute, Raleigh, NC., USA.

Simsek, U.G., T. Guler, M. Ciftci, O.N. Ertas and B. Dalkilic, 2005. The effect of an essence oil mix (derived from oregano, clove and anise) on body weight and carcass characteristic in broiler. World Poult. El-Sev., 16: 31-34.

Singh, G., S. Maurya, M.P. Delampasona and C. Catalan, 2006. Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. J. Sci. Food Agric., 86: 111-121.

Soltan, M.A., R.S. Shewita and M.I. El-Katcha, 2008. Effect of dietary anise seeds supplementation on growth performance, immune response, carcass traits and some blood parameters of broiler chickens. Int. J. Poult. Sci., 7: 1078-1088.

Song, W.Y., Y.B. Ma, X. Bai, X.M. Zhang and Q. Gu *et al*., 2007. Two new compounds and anti-HIV active constituents from *Illicium verum*. Planta Med., 73: 372-375.

Sung, Y.Y. and H.K. Kim, 2013. *Illicium verum* extract suppresses IFN-γ-induced ICAM-1 expression via blockade of JAK/STAT pathway in HaCaT human keratinocytes. J. Ethnopharmacol., 149: 626-632.

Sung, Y.Y., W.K. Yang, A.Y. Lee, D.S. Kim, K.J. Nho, Y.S. Kim and H.K. Kim, 2012a. Topical application of an ethanol extract prepared from *Illicium verum* suppresses atopic dermatitis in NC/Nga mice. J. Ethnopharmacol., 144: 151-159.

Sung, Y.Y., Y.S. Kim and H.K. Kim, 2012b. *Illicium verum* extract inhibits TNF-α-and IFN-γ-induced expression of chemokines and cytokines in human keratinocytes. J. Ethnopharmacol., 144: 182-189.

Wang, G.W., W.T. Hu, B.K. Huang and L.P. Qin, 2011. *Illicium verum*: A review on its botany, traditional use, chemistry and pharmacology. J. Ethnopharmacol., 136: 10-20.

Yadav, A.S. and D. Bhatnagar, 2007. Chemo-preventive effect of Star anise in N-nitrosodiethylamine initiated and phenobarbital promoted hepato-carcinogenesis. Chemico-Biol. Interactions, 169: 207-214.