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Supplementation of Clove Essential Oils and Probiotic to the Broiler's Diet on Performance, Carcass Traits and Blood Components

Research Article

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ABSTRACT

This study was carried out to determine the effect of different levels of clove essential oil, as a natural growth promoter and probiotic on performance, carcass traits and serum components of broiler chickens. A total of 240 straight run day-old commercial broiler chicks (Cobb 500) were distributed randomly into five groups. Each group was subdivided into four replicates with 12 chicks each. The control diet, diet with probiotic protexin®, and diets supplemented with 150, 300 and 450 ppm clove essential oil were fed to the chickens. The results showed that from 11-22 days, feed intake (FI), and body weight gain (BWG) were increased significantly in control group compared to the other groups. However, from 23-42 d and 0-42 days of the experiment, feed intake (FI) and body weight gain (BWG) were increased significantly in broilers fed 450 ppm clove essential oil compare to those of control group ($P<0.05$). Also, clove essential oil at the level of 450 ppm increased feed intake (FI), body weight gain (BWG) and improved feed conversion ratio **(**FCR) in finisher period ($P<0.05$). Results show that different levels of clove essential oil and probiotic had not any significant effect on dressing percentage, abdominal fat and internal organs percentage (liver, heart and gizzard) ($P>0.05$). The results revealed significant decrease in total cholesterol concentration in diets supplemented with probiotic and 450 ppm clove essential oil at 21 day of age ($P<0.05$). Total cholesterol concentration, low density lipoprotein (LDL), and cholesterol / high density lipoprotein (HDL) ratio at 42 day of age were significantly improved in probiotic protexin® group compare to those of other groups ($P<0.05$). In conclusion, dietary supplementation with 450 ppm clove essential oil improved feed intake (FI), body weight gain (BWG), feed conversion ratio **(**FCR) and decreased total cholesterol concentration ($P<0.05$). Probiotic could decrease serum cholesterol, low density lipoprotein (LDL), cholesterol / high density lipoprotein (HDL) and improve HDL / LDL at day 42 broiler chickens ($P<0.05$).

KEY WORDS blood factors, carcass yield, clove essential oil, feed intake, protexin®, weight gain.

INTRODUCTION

Antibiotics have been used in poultry nutrition for improving growth performance, preventing some specific pathogenic microorganism and increasing some useful microorganism in intestinal microflora over the years. However, antibiotics used as growth promoters in animal feeds have been banned recently due to potential development of antibiotic resistant human pathogenic bacteria. Nowadays, the possibility of using new natural alternative additives instead of antibiotics in animal diets is being researched (Weber *et al.* 2012).

Probiotics as alternatives of antibiotics are live microbial feed supplements which beneficially affect the host by im-

proving its intestinal microbial balance (Fuller, 2001; Midilli and Tuncer, 2001). There are viable single or mixed cultures of microorganisms that when given to animals or humans, beneficially affect the host by improving the properties of the indigenous microflora (Kabir, 2004). Protexin® is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts (*Lactobacillus plantarum* 1.89×1010 cfu/kg, *Lactobacillus delbrueckii subsp*. Bulgaricus 3.09×1010 cfu/kg, *Lactobacillus acidophilus* 3.09×1010 cfu/kg, *Lactobacillus rhamnosus* 3.09×1010 cfu/kg, *Bifidobacterium bifidum* 3.00×1010 cfu/kg, *Streptococcus salivarius subsp*. *Thermophilus* 6.15×1010 cfu/kg, *Enterococcus faecium* 8.85×1010 cfu/kg, *Aspergillus oryza* 7.98×109 cfu/kg, *Candida pintolopesii* 7.98×109 cfu/kg) (Vahdatpour *et al.* 2011). Other alternatives are plant extracts. Plant extracts contain many active components, including essential oils, which have a wide range of pharmocological activities. The essential oil extracted from aromatic plants have been shown antibacterial, anticoccidial (Gianneanas *et al.* 2003), antifungal (Janatan *et al.* 2003), antioxidant (Bostoglou *et al.* 2004) activities. Some results showed that aromatic plants and their extracts have a positive effect on growth performance in chickens (Guler *et al.* 2005) or have not any significant effect (Botsoglou *et al.* 2002). When the effect was positive, WG and FI were increased whereas the feed gain ratio was lowered when compared to control. Dietary essential oils can also improve digestion. A number of studies have reported the positive effect of spices or their active components on digestion process. They have been shown to stimulate bile salt secretion and digestive enzyme activities of intestinal mucosa and of pancreas (Hernandez *et al.* 2004). Clove extract is commonly used in the food industry because of its special aroma and natural safety. In addition, the essential oil from clove also exhibited strong antibacterial properties. Antiseptic, appetite and digestion stimulant (Kamel, 2001), anti inflammatory and antioxidant (Dragland *et al.* 2003) activities of clove and its ingredients have been reported. Clove essential oil has been studied very few as a performance enhancer. In this study, we aimed the use of clove essential oil and probiotic in broiler nutrition as a natural growth promoting substance instead of antibiotics. For this purpose, the different level of clove essential oil were added in standard diet, and studied to determine their effects on performance, carcass traits and blood components compared to control.

MATERIALS AND METHODS

A total of 240 mixed one day-old commercial Cobb 500 broiler chicks (initial weight 38±3 g) were divided randomly into five groups. Every group was replicated four times with 12 chicks each. The control diet was formulated to meet the nutrient requirements of broiler chicks, another diet was supplemented with 120 ppm probiotic protexin®, and the other diets were supplemented with 150, 300 and 450 ppm clove essential oil. Clove oil was dissolved in vegetable oil and then gently mixed with the above mentioned diets. The basal diet was formulated to meet or exceed the nutrient requirements of broilers as recommended by Cobb 500. The compositions of basal diets are shown in Table 1. The chicks were raised on floor pens (1 m^2) for 6 weeks and had free access to feed and water throughout the entire experiment. Lighting was provided 24 h per day for all the trials. The ambient temperature was gradually decreased from 33 to 25 on day 21 and was then kept constant.

The BW, FI and FCR were recorded at different periods. One bird per replicate was randomly chosen at 21 and 42 d of age. Then, blood samples were obtained from wing vein and centrifuged in order to obtain serum. Serums have been analyzed for cholesterol, HDL, LDL, cholesterol / HDL, HDL / LDL and triglycerides. After 6 h of fasting, they have been weighted to obtain live weight. Then they were slaughtered and their abdominal fat, liver, heart and gizzard were eviscerated, weighted and expressed as percentage of live weight of the bird. The data were analyzed using the GLM procedure of SAS 9.1 (SAS, 2004). Tukey HSD test was used to compare the means. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

The effects of different levels of clove essential oil and probiotic protexin® on growth performance of broiler chicks are presented in Tables 2, 3 and 4. From 11-22 days, FI and BWG were increased significantly in control group compared to other groups. But from 23-42 d and 0-42 days of experiment, feed intake and body weight gain were significantly increased in broilers fed 450 ppm clove essential oil compare to control group. Results showed that clove essential oil at the level of 450 ppm increased FI, BWG and improved FCR in finisher period ($P<0.05$). It can be interpreted that at the starter period, chicks refuse to consume the feed but they have been acclimatized to the especial aroma of the diets after some days. Thus, the FI increased during finisher and as overall periods.

The results showed that active materials in clove (ugeonol) are considered as digestion stimulatory factors, in addition to their antimicrobial activity against bacteria found intestine (Cabuk *et al.* 2003); Also, the improvement of body weight gain and feed conversion are due to active materials found in clove essential oil causing greater efficiency in utilization of feed, resulting in enhanced growth.

Table 1 Nutrient content of the basal diet over different periods of production

Ingredients (%)	Starter (1-10 d)	Grower (11-22 d)	Finisher (23-42 d)
Corn	55.59	60.77	62.69
Soybean meal (44% CP)	36.74	31.04	28.31
Soybean oil	3.56	4.07	5.05
Common salt	0.30	0.30	0.35
Dicalcium phosphate	1.85	1.79	1.66
Limestone	1.25	1.22	1.15
DL-methionine	0.21	0.22	0.23
L-lysine HCl	0.00	0.09	0.06
Mineral and vitamin premix[1]	0.50	0.50	0.50
Calculated composition			
Metabolizable energy (ME) (kcal/kg)	2990	3085	3176
Crude protein (CP) (%)	21.00	19.00	18.00
Calcium (%)	1.00	0.96	0.90
Available P (%)	0.50	0.48	0.45
Na (%)	0.20	0.17	0.16
Lysine (%)	1.20	1.10	1.05
TSAA	0.89	0.84	0.82

[1] Vitamin premix per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant 100 mg.
Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 50 mg; Mn (MnSO4.H2O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO4.5H2O): 10 mg; I (KI, 58% I): 1 mg and Se (NaSeO3, 45.56% Se): 0.2 mg.

Table 2 The effects of clove essential oils and probiotic protexin® on daily feed intake of broilers

Treatments	Starter (0-10 d)	Grower (11-22 d)	Finisher (23-42 d)	Overall (0-42 d)
Control (unsupplemented diet)	26.08	116.38^{a}	181.51^{b}	122.79
Probiotic protexin®	26.49	109.88^{ab}	186.52^{b}	122.87
150 ppm clove essential oil	25.50	113.81^{ab}	184.99^{b}	123.29
300 ppm clove essential oil	27.76	106.86^{ab}	185.29^{b}	121.64
450 ppm clove essential oil	27.19	105.69^{b}	201.57^{a}	128.09
SEM	0.545	2.240	3.312	1.811
P-value	0.073	0.019	0.006	0.159

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effects of clove essential oils and probiotic protexin® on daily weight gain of broilers

Treatments	Starter (0-10 d)	Grower (11-22 d)	Finisher (23-42 d)	Overall (0-42 d)
Control (unsupplemented diet)	16.96	59.75^{a}	74.87^{b}	56.04^{b}
Probiotic protexin®	17.57	52.37^{bc}	82.48^{b}	56.99^{b}
150 ppm clove essential oil	16.46	57.83^{ab}	80.54^{b}	57.71^{b}
300 ppm clove essential oil	17.12	52.32^{bc}	83.56^{b}	57.33^{b}
450 ppm clove essential oil	17.53	51.08^{c}	100.74^{a}	64.38^{a}
SEM	0.435	1.265	2.699	1.326
P-value	0.393	0.0006	0.0001	0.0033

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 The effects of clove essential oils and probiotic protexin® on feed conversion ratio of broilers

Treatments	Starter (0-10 d)	Grower (11-22 d)	Finisher (23-42 d)	Overall (0-42 d)
Control (unsupplemented diet)	1.54	1.95	2.43^{a}	2.19
Probiotic protexin®	1.51	2.09	2.26^{ab}	2.15
150 ppm clove essential oil	1.55	1.97	2.31^{ab}	2.14
300 ppm clove essential oil	1.62	2.04	2.21^{ab}	2.12
450 ppm clove essential oil	1.55	2.06	2.00^{b}	1.99
SEM	0.026	0.041	0.074	0.052
P-value	0.078	0.102	0.014	0.122

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These results agree with the work of Lee *et al.* (2004) who found that adding the essential oil to the diet of broilers improved their growth performance.

There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulatory properties and antimicrobial effects (Kamel, 2001).

It has been reported that clove oil is super-rich in manganese, trace minerals necessary for protein and carbohydrate metabolism, the synthesis of fatty acid and cholesterol and contain in lesser amounts, and omega3 fatty acid so these could be improve broiler performance (Mukhtar Ahmed, 2011). A number of studies have reported the positive effect of spices or their active components on digestion process. They have been shown to stimulate bile salt secretion and digestive enzyme activities of intestinal mucosa and of pancreas (Hernandez *et al.* 2004). There are many studies that suggested dietary essential oil can improve digestion and increase the performance of broiler chickens. Lee *et al.* (2004) reported that thymol and cinnamaldehyde at the level of 100 ppm stimulate secretion of pancreatic enzyme such as amylase, lipase, trypsin and chymotrypsin in broiler chickens and induce to increase their performance. But Kalavathy *et al.* (2003) used eugenol, the main component of clove essential oil, at the concentration of 1000 and 850 ppm, and concluded that these levels impaired the absorption of alanine by rat jejunum. The author reported that these two principles inhibit the activity of Na-K- ATPase which is located in enterocyte and consequently impair transport process in the intestine. However, the exact concentration in diet has not established yet. The results showed that probiotic did not have any significant effect on performance (FI, BWG, and FCR) in different periods (P>0.05). Probiotic efficacy may depend on factors such as microbial species composition (e.g., single or multistrain) and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors (Mountzouris *et al.* 2010).

Rahimi and Khaksefidi (2006) reported that during stressful conditions such as transportation to growing site, overcrowding, vaccination, chilling or overheating, probiotic will be effective and can be alleviated by early establishment and maintenance of a favourable microbial population in the digestive tract, so the effect of dietary probiotics on growth performance become apparent when chickens are subjected to suboptimal condition such as less digestible diet or a less clean environment.

Results show that different levels of clove essential oil and probiotic had not any significant effect on dressing percentage, abdominal fat and internal organs percentage (liver, heart and gizzard) (P>0.05).

However in starter period (Table 6), probiotic had decreased numerically the abdominal fat in 42 days of age (P>0.05).

These observations are correlated with the data published by Midilli and Tuncer (2001) and Homma and Shinohara (2004). The effect of different levels of clove essential oil and probiotic on blood factors is presented in Table 7 at 21 and 42 day of age.

The results showed that probiotic and 450 ppm clove essential oil had significantly decreased the level of cholesterol on 21 day of age (P<0.05).

The main components of essential oils inhibit hepatic 3-hydroxy-3 methylglutaryl coenzyme A (HMG-COA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis. As a result, a hypocholesterolemic effect of essential oils can be expected (Lee *et al.* 2004). Also, according to Clegg and Mbada (1980) a 5% inhibition of HMG-COA reductase lowered serum cholesterol by 2% in poultry.

Table 5 The effects of clove essential oils and probiotic on carcass of broilers at 21d of age

Treatments	Carcass*	Breast	Thigh	Heart	Liver	Gizzard	Pancreas
Control (unsupplemented diet)	63.94	16.86	15.58	0.58	3.42	2.58	0.48
Probiotic protexin®	59.87	16.71	15.36	0.66	3.51	2.61	0.50
150 ppm clove essential oil	62.26	16.79	15.42	0.61	3.61	2.60	0.45
300 ppm clove essential oil	62.96	16.80	15.28	0.55	3.44	2.63	0.53
450 ppm clove essential oil	60.98	16.79	15.40	0.64	3.56	2.60	0.45
SEM	1.022	0.129	0.110	0.022	0.022	0.036	0.026
P-value	0.174	0.950	0.166	0.088	0.088	0.862	0.308

* Carcass and all organs are calculated as percentage of organ weight (g) to live weight without skin (g).
SEM: standard error of the means.

Table 6 The effects of clove essential oils and probiotic on carcass of broilers at 42 d of age

Treatments	Carcass*	Breast	Thigh	Heart	Liver	Gizzard	Fat	Pancreas
Control (unsupplemented diet)	60.75	19.33	18.20	0.55	2.26	1.62	1.32	0.31
Probiotic protexin®	60.49	20.12	18.90	0.57	2.31	1.71	1.00	0.32
150 ppm clove essential oil	60.68	19.51	18.56	0.57	2.42	1.44	1.17	0.33
300 ppm clove essential oil	60.08	19.72	17.91	0.47	2.34	1.79	1.39	0.23
450 ppm clove essential oil	61.82	21.08	19.47	0.54	2.09	1.64	1.13	0.29
SEM	2.080	1.012	0.623	0.034	0.176	0.132	0.102	0.052
P-value	0.979	0.759	0.509	0.352	0.747	0.500	0.192	0.710

* Carcass and all organs are calculated as percentage of organ weight (g) to live weight without skin (g).
SEM: standard error of the means.

Table 7 The effects of clove essential oils and probiotic on blood components at 21 and 42 d

Treatments	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Cholesterol / HDL	HDL / LDL	Triglycerides (mg/dL)
21 d						
Control (unsupplemented diet)	146.85^{a}	49.85	71.66	3.13	0.70	78.87
Probiotic protexin®	117.35^{b}	56.25	65.30	2.17	0.86	81.22
150 ppm clove essential oil	132.97^{ab}	56.25	66.80	2.38	0.84	85.37
300 ppm clove essential oil	146.32^{a}	55.65	65	2.73	0.85	84.87
450 ppm clove essential oil	127^{b}	52.65	62.15	2.46	0.85	86.02
SEM	3.85	5.41	2.03	0.309	0.086	3.311
P-value	0.0002	0.884	0.133	0.345	0.667	0.505
42 d						
Control (unsupplemented diet)	137.02^{a}	59.84	93.88^{a}	2.34^{a}	0.64^{b}	106.07
Probiotic protexin®	96.02^{b}	69.65	51.93^{b}	1.42^{b}	1.34^{a}	102.62
150 ppm clove essential oil	138.21^{a}	61.85	76.86^{ab}	2.29^{a}	0.81^{b}	100.27
300 ppm clove essential oil	139.87^{a}	62.40	70.85^{ab}	2.35^{a}	0.87^{b}	100.40
450 ppm clove essential oil	133.35^{a}	62.81	69.80^{ab}	1.94^{ab}	0.90^{b}	93.100
SEM	4.11	4.201	4.903	0.146	0.074	4.609
P-value	0.0001	0.580	0.015	0.024	0.008	0.403

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

At 42 days of age, supplementation with probiotic protexin® decreased significantly the cholesterol, LDL and cholesterol / HDL ratio, also improved the HDL / LDL ratio. Similar cholesterol depressing effect due to probiotic supplementation in broiler chicken was observed by some researchers (Joy and Samuel, 1997; Azadegan Mehr *et al.* 2007).

Some researchers reported that probiotic supplementation reduced the serum LDL cholesterol (Kalavathy *et al.* 2003) and triglycerides (Santos *et al.* 1995) in broiler chicken. Also, it is reported that some of the microorganisms present in the probiotic preparation could utilize the cholesterol present in the gastro intestinal tract for their own metabolism, and reduce the amount of cholesterol absorption (Mohan *et al.* 1995).

Lactobacillus, which has a high bile salt hydrolytic activity, is responsible for deconjugation of bile salts (Sarono, 1995). Deconjugated bile acid are less soluble at low pH and less absorb in the intestine and is more likely to be excreted in feces (Klaver and Van der Meer, 1993). In addition, probiotic microorganism inhibits hydroxymthyl glutaryl coenzyme A (HMG-COA) reductase, an enzyme involved in the cholesterol synthesis (Fukashima and Nakano, 1995).

The most important mechanism by which probiotic eliminates cholesterol probably be through reducing lipid absorption in intestine by binding bile acids, which results in increased cholesterol elimination and hepatic synthesis of new bile acid (Zhang *et al.* 2003). This is in agreement with the results of Mohan and Anjames (Mohan and Anjames, 1988) and Azadegan Mehr *et al.* (2007). Different levels of clove essential oil did not have any significant effects on HDL, LDL and triglycerids (P<0.05) (Table 7).

CONCLUSION

Reports on the value of this essential oil on poultry nutrition are scarce. This study showed that supplementation of 450 ppm clove essential oil in broiler diets significantly improved body weight gain and feed conversion ratio in whole period of the experiment (0-42 d) and decreased total cholesretol composition in 21 day of age. Also, the supplementation with probiotic protexin®, significantly decreased the total cholesterol, LDL and cholesterol / HDL ratio and improved HDL / LDL ratio (P<0.05). Thus, clove essential oil could be considered as a potential growth promoter for poultry.

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