Original Research

Effect of Supplementation of Essential Oils on Performance of Broiler, Meat Quality and Immune Status

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Abstract

An experiment was conducted to evaluate the performance of broiler, meat quality and immune status by supplementation of essential oils. 360 day old Vencobb^{400} straight run chicks were weighed and distributed randomly into six treatment groups viz, A, B, C, D, E and F with three replicates of 20 chicks each. The treatment group A served as control without supplementation of any essential oil. The treatment groups B, C, D, E and F were supplemented with essential oils through feed @ Garlic oil 100mg /kg. Garlic oil 200mg/kg, Neem oil 100mg/kg. Neem oil 200mg/kg and Garlic oil 100mg + Neem oil 100mg/kg of feed, respectively. The pre-starter, starter and finisher rations were formulated as per BIS (2007). The weekly cumulative weight gain feed consumption and feed conversion ratio were significantly affected (P <0.01) by supplementation of essential oils. Significantly lower mortality was observed for essential oils supplemented groups compared to control group. The TBA value (mg/kg) of broiler for breast and thigh meat at 15th day of storage revealed significant (P < 0.01) influence. The significant differences (P < 0.01) were observed for serum titer values at 42nd day of age. Neem essential oil @ 100mg/kg supplemented group (D) had numerically higher antibody titer against IBD. The total cost of broiler production was Rs. 140.62, 138.23, 141.76, 140.10, 142.75 and 141.39 for treatment groups A, B, C, D, E and F, respectively. The net profit per bird was highest for Treatment group D (Rs.20.00), followed by B (Rs.19.95), C (Rs.19.92), A (Rs.19.22), E (Rs.17.64) and F (Rs.17.33). It is concluded that supplementation of garlic @ 200 mg/kg of feed is beneficial from the point of production parameters. However, supplementation of garlic and Neem essential oil @ 100mg/kg of feed alone in broiler ration is beneficial from the point of oxidative stability, economical gain and immune status of commercial broiler chickens.

Key words: Broiler Performance, Essential Oil, Meat Quality, Supplementation

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Introduction

Many natural compounds including herbs, herb spices, essential oils (EOs) and others have been shown to express promising effects on growth performance, gut health, meat quality, immune status and health of poultry. The chemical constituents of most plant EOs are also recognized as safe in general are commonly used in the food industry. Medicinal plants are used in pharmaceuticals, neutraceuticals, cosmetics and food supplements and even as traditional source of medicines because of their anti-tumor, anti-arthritic and anti-thrombotic functions (Thomson and Ali, 2003). During the last two decades considerable research has been done in exploring the beneficial effects of the growth promoters and identifying suitable alternative to antibiotics.

EOs could be obtained through various methods like fermentation, extraction; however, steam distillation is used as the most common methods for commercial use. EOs extracted from herb and spices are complex mixture of various compounds, which consists of aromatic and volatile substances, generally recognized as safety admitted by the food and drug administration (Cross *et al.,* 2007, Kirsti *et al.*, 2010). EO is a mixture of fragment, volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated (Oyen and Dung, 1999). EOs are found to have antibacterial ability and also exhibit antioxidant, anti-inflammatory, anticarcinogenic, digestion stimulating and hypolipidemic activities (CCC). The improvement in feed efficiency achieved with EO mixtures could be attributed to their positive effects on nutrient digestibility (Langhout, 2000, Madrid *et al*., 2003, Hernandez *et al*., 2004 and Jamroz *et al*., 2005). Many EOs stimulate the growth of beneficial microbes and limit number of pathogenic bacteria in poultry (Wenk, 2000). The performance promoting effect of EO, extract, powder or principal component of thyme has been demonstrated in poultry (Ciftci *et al.*, 2009, Feizi and Nazeri, 2011). EOs have been proven to control pathogens due to their antimicrobial activity (Dorman and Deans 2000), to have antioxidative potential (Hui, 1996) by delaying lipid oxidation in broiler meat and to enhance digestion (Brugalli, 2003) by stimulating the endogenous enzymes.

There are some promising results on the use of EOs and other natural products as performance enhancers. EO from plant extracts has distinct biological functions, such as antimicrobial (Solo´rzano-Santos and Miranda-Novales, 2012), antifungal (Rasooli and Abyaneh, 2004; Rasooli *et al*., 2008; Verma *et al.,* 2011) or antioxidant activities (Botsoglou *et al*., 2002, Fasseas *et al*., 2007). Supplementation of poultry diets with EO has been reported to have beneficial effects on the intestinal microflora (Cross *et al*., 2007) as well as digestive enzymes (Jang *et al.,* 2007). Therefore, EOs could be well considered as potential, viable and natural alternatives for antibiotic growth promoters and the study to assess the effect would of great help to the commercial poultry farming community.

The EO like garlic oil which is extracted from garlic and garlic is widely used in all parts of a spice and herbal medicine. The major active ingredient of garlic is allicin, S-ally cycteine. Garlic has been found to

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demonstrate antimicrobial activity (Adibmoradi *et al.,* 2006) in broiler chicks. Some studies, however suggested that commercial garlic oil, garlic powder and commercially available garlic extract may be hypocholesterolemic (Aporn *et al.,* 2008). Garlic preparation and extracts have been shown to exhibit antiatherosclerotic, antimicrobial, hypolipidemic, antithrombotic, antihypertensive, antidiabetic, antithrombotic effects (Mansoub and Nezhad, 2011).The effect of garlic as an EO on the growth performance of a broiler and positive impact on gut microflora, contributing to some extent to health maintenance (Dieumou *et al.,* 2009).It also has been recognized for its strong stimulating effect on the immune system, in addition to its positive effects on digestion in birds due to very rich aromatic essential content of garlic (Demir *et al.,* 2005).

Neem (*Azardiranchta indica*) is an indigenous plant of Asian subcontinent known for its useful medicinal properties like antibacterial and hepatoprotective (Kale *et al.,* 2003). In general, Neem leaf extracts may be used therapeutically to control respiratory problems, constipation and also as health promoter (Agarwal, 2002). The different components of Neem e.g. leaves, kernel cake, Neem oil and extracts etc. having beneficial effects to improve broiler performances and performances of birds as well (Akilandeswari *et al.,* 2003). Neem leaves and its constituents have been demonstrated to exhibit anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antioxidant, antimutagenic and anticarcinogenic properties (Subapriya and Nagini, 2005). Neem also plays an important role in strengthening the immune system of the body. Increase in antibodies against new castle and infectious bursal disease viruses have been observed when Neem product is incorporated in poultry feed (Dhurrani *et al.,* 2008). Scanty literature is available on effects of EOs of garlic, Neem and their synergetic properties. Now consequent to the increasing concern about the potential public health problems due to antibiotic resistance, poultry nutritionists are being challenged to develop alternatives for antibiotic growth promoters. It is suitable appropriate if herbal alternatives to antibiotic growth promoters can be confirmed, poultry nutritionists could formulate a ration that would meet the needs of the poultry industry.

Materials and Methods

Experimental Site

The experiment was carried out at Broiler unit of Department of poultry Science, College of Veterinary and Animal Science, Parbhani, affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur, India.

Formulation of Experimental Ration

The required quantity of feed ingredients used in the present experiment were purchased from local market and rations were prepared as per BIS (2007), at feed mixing plant. Department of Poultry science, College

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of Veterinary and Animal sciences, MAFSU, Parbhani (Table1). The pre-starter ration was offered for first seven days of age, starter ration was offered from 8th day to 20thday and finisher ration was offered thereafter up to 42nd day of age. The *iso-caloric and iso-nitrogenous* levels of feeding practices were followed for all the treatment groups throughout the experimental period. Garlic and Neem essential oils were purchased from local market and added in the feed at two different dose levels viz. garlic essential oil 100mg and 200mg per kg of feed; Neem essential oil 100 and 200mg per kg of feed, respectively. Similarly, is also used in combination each @ 100mg/kg of feed.

Table 1: Percent ingredient and nutrient composition of (basal diet) pre-starter, starter and finisher rations with supplementation of EOs

Feed Ingredients	Pre-Starter	Starter	Finisher
Maize	55.45	55.2	60.2
*Vegetable oil	1.6	3	4
Soya-bean meal	40.15	39	33
Di-calcium phosphate	1.5	1.5	1.5
Limestone powder	1	1	1
Salt	0.3	0.3	0.3
Total	100	100	100
Supplements/Additives (g/100kgs)			
Mineral mixture	300	300	300
Vitamin mixture	150	150	150
Methionine	180	190	160
Lysine	170	130	100
Choline chloride	60	60	60
Coccidiostat	60	60	60
Crude protein (%)(calculated)	23.05	22.12	20.26
Metabolizable energy (Kcal/kg)(calculated)	3015.32	3101.82	3213.32

**An essential oils are added in vegetable oil in the diet as per the dose rate mentioned in experimental design*

Experimental Birds and Data Collection

The experiment was carried out on commercial straight run broilers for a period of 42 days. Total 360 day old Vencobb400 broiler chicks were obtained from private hatchery. On arrival, the chicks were weighed and distributed randomly into six treatment groups viz, A, B, C, D, E and F with three replicates of 20 chicks each. Experimental design used for housing of broilers is presented in Table 2. An ideal feeding, watering and floor space were provided to all groups throughout the experimental period as per standards. Standard vaccination schedule was followed.

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Table 2: Experimental design for housing of broiler with supplementation of EOs

Treatment Group	Treatment Group Details	No. of Birds/Pen/ Replication	No. of Replicates	Total No. of Birds
A	Control –Basal Diet without Essential oil	20	3	60
B	Control diet + 100mg garlic oil / kg diet	20	3	60
C	Control diet+ 200mg garlic oil / kg diet	20	3	60
D	Control diet + 100mg Neem oil / kg diet	20	3	60
E	Control diet + 200mg Neem oil / kg diet	20	3	60
F	Control diet + 100mg garlic oil / kg diet + 100mg of Neem oil / kg diet in combination	20	3	60
		Total		360

Performance Parameters

The cumulative weight gain, feed consumption, FCR and mortality were recorded replicate wise at weekly interval. The economics of broiler production was calculated by taking into consideration the cost of chick, cost of total feed consumed by bird, cost of different litters, vaccination, medication expenses. However, Gross profit per bird was calculated by subtracting the cost of production per bird from the price fetched per bird after selling it in the local market on live weight basis.

Quality Characteristics of Meat

Thiobarbutaric Acid (TBA) Value of Meat

At the end of the trial each bird from the replicate, meat samples collected studies were analyzed for their chemical properties viz. thiobarbituric acid. The chemical properties were assessed in breast meat on 0 day (fresh sample) and 15thday of meat sample collection. The breast and thigh meat samples from these birds will be stored at -18 degrees C and their oxidative stability i.e. lipid oxidation will be evaluated by thiobarbituric acid values (TBA) at 0th and 15th day of storage. The meat sample were stored at 4^{0}C in LDPE (low density polyethylene) bags and their oxidative stability i.e. thiobarbituric acid value was determined. 5 ml of aliquot of TCA extract was mixed with 5ml of TBA reagent in a test tube. TBA reagent was prepared according to the method of Pearson (1968), by dissolving 0.2883 g of thiobarbituric acid in sufficient quantity of 90% acetic acid with slight warming; the volume was made up to 100ml with 90% acetic acid. The test tube containing samples were kept in a water bath at 100^{0}C for 30 min along with control test tube containing a blank of 5ml of 10% TCA and 5ml of TBA reagent. After cooling the tubes in running water for about 10 min the optical density was measured at 530nm in a spectro-photometer

Immune Status

Antibody Titre against RD (Ranikhet Disease)

Antibody titre against RD (Ranikhet disease) was estimated by PDRC laboratory by Haemagglutination (HA) Test. It was performed in 'U' bottom Microtitre plate as per O.I.E. procedure (1992) with slight

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modification 50 ul of 0.1 M PBS. PH 7.2 was added upto 11th well of 'U' shaped microtiter plate. 50 ul of antigen (virus RD) was added in 1st well and serial two fold dilution of antigen was made upto 11th well and well was kept as control. 50 ul of C-RBC 1% v/v was added to each well. After gentle mixing, plate was incubated at room temperature for 45 minutes and the results was recorded as HA titre. The original virus was diluted to contain 8 HA units and was used as HA antigen in the HI test.

Haemagglutination Inhibition (HI) Test

The test was carried out as per the procedure of O.I.E. (1992) with few modifications. 50 µl of 0.1 M PBS. PH 7.2 was added upto 11th well of 'U' shaped microtiter plate. 50 µl of test serum was added in 1st well and serial two fold dilution of serum was done. 50 µl of 8 HA units of antigen was added in each well. After this 50 µl of C-RBC 1% (v/v) added in each well and mixed gently. The plate was incubated at room temperature for 45 min. The HI titre was expressed as highest dilution of serum causing complete inhibition of antigen.

IBD Titre

An indirect ELISA for IBD antibody test kit was developed by PDRC (Poultry Diagnostic and Research Centre, Pune) and ELISA antibody titre against IBD (Infecitious Bursal Disease) was estimated at PDRC laboratory, Pune.

Enzyme-Linked Immunosorbent Assay (ELISA)

The antigen coated plates (coated with the inactivated viral antigen on microtitre plates) and the ELISA kit reagents were adjusted at room temperature prior to the test. The test serum was diluted; five hundred folds (1:500) prior to the assay with sample diluents provided. 100 ul of diluted serum was then put into each well of the plate. This was followed by addition of 100 ul of undiluted negative control (specific pathogen free serum in phosphate buffer with protein stabilizers and sodium azide preservative (0.1 % w/v). 100 ul of positive control was also added (antibodies specific to IBD in phosphate buffer with protein stabilizers and sodium azide preservative (0.1 % w/v). The plate was then incubated for 30 minutes at room temperature. Each well was then washed 4 times with washing buffer containing 0.05% Tween 20 in powdered phosphate buffered saline (300 ul per well). A 100 ul of conjugate reagent (sheep anti-chicken alkaline phosphatase in Tris buffer with protein stabilizers, inert red dye and sodium azide preservative (0.15 w/v) was added into each well and the plate was incubated at room temperature for 30 minutes.

Each well was washed again 4 times with the washing buffer. 100ul of substrate reagent (p-Nitro phenyl phosphate dissolved in Oiethanolamine buffer with enzyme co-factors) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 ul of stop solution (Sodium Hydroxide in Oiethanolamine buffer) was dispensed into each well to stop the reaction. The absorbance

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values were measured and recorded at 405 nm wavelength using ELISA microtitre Plate reader (Ahmed and Ali, 2002).

Statistical Analysis

Data, thus collected were subjected to statistical analysis by using Randomized Block Design by Snedecor, and Cochran, (2007). The treatment means were compared by critical differences (CD) and Analysis of Variance.

Results and Discussion

Cumulative Weight Gain

The analysis of variance for overall cumulative weight gain (Table 4) revealed highly significant ($P < 0.01$) difference among various treatment groups. From the data (Table 3) it was revealed that the birds of treatment group C receiving diet supplemented with 200 mg garlic E.O. /kg recorded significantly higher cumulative gain in weight at 6th week. The findings in the present study regarding significant improvement of the cumulative body weight gain with supplementation of garlic oil were in the line with Raeesi *et al.* (2010); Aji *et al.* (2011); Hassan *et al.* (2013); Eid *et al.* (2014); Kharde *et al.* (2014); Sadd *et al.* (2015); Karangiya *et al*. (2016). It is inferred that garlic essential oil has a growth promoting properties due to bioactive components such as Sulphur containing organic compound dialkyl polysulphides which possesses antimicrobial activity. An allicin in garlic promotes performance of intestinal microflora thereby improving the digestion and enhancing utility of energy leading to improve growth rate (Pourali *et al.*, 2010). However, this growth promoting property was not expressed in the birds receiving diet supplemented with garlic @ lower dose rate (100mg/kg of feed) on the contrary decreasing trend was found for growth which was not explained. Similarly, the birds receiving diet supplemented with Neem EO at different dose levels did not show any improvement in the growth. The non-significant influence on body weight gain observed in the present study with supplementation of Neem oil were similar to the findings of Nidaullah *et al.* (2010), Bonsu *et al.* (2012) and Nnenna and Okey (2013). In contrast to present findings there are some reports in which birds exhibited improved body weight gain (Wankar *et al.*, 2009; Zanu *et al.*, 2011; Ansari *et al.*, 2012; Adeyemo and Akanmu, 2013 and Alam *et al.*, 2015).

Cumulative Feed Consumption

From the data (Table 3 & Table 4) observed that, the birds of treatment group B fed with garlic essential oil @ 100mg/kg of feed recorded significantly lower feed consumption. However non-significant differences were observed among the rest of the treatment groups. Cumulative feed consumption at 6th week revealed decreasing trend for garlic and Neem essential oil supplemented treatment groups compared to control (A).

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Table 3: Weekly cumulative weight gain (g), feed consumption (g), FCR and mortality at different age groups supplemented with EOs

Age (weeks)	Groups/Treatments					
	Cumulative weight gain					
	A (Control diet)	B (100 mg Garlic E.O./kg)	C (200 mg Garlic E.O./kg)	D (100 mg Neem E.O./kg)	E (200 mg Neem E.O./kg)	F (100 mg Garlic and Neem E.O./kg)
I	127.18	121.88	128.05	123.13	119.42	121.04
II	338.38	311.78	349.52	341.05	326.85	340.29
III	622.52	606.6	641.53	617.33	627.71	624.37
IV	1091.42	1039.12	1162.43	1129.28	1108.13	1140.02
V	1520.47	1504.02	1579.75	1552.63	1550.28	1552.17
VI	2062.93	2042.31	2086.73	2066.6	2070.38	2047.76
Overall Mean	960.48^{b}	937.62^{c}	991.33^{a}	971.67^{b}	967.13^{b}	970.94^{b}
Cumulative Feed Consumption						
I	141.6	143.92	145.27	142.55	137	138.63
II	469.48	457.37	485.67	483.67	458.67	482.63
III	951.23	947.92	979.23	960.27	982.49	979.25
IV	1788.98	1750.07	1809.03	1773.45	1812	1802.28
V	2634.66	2585.62	2656.39	2611.33	2669.275	2641.8
-VI	3730.64	3593.64	3660.41	3662.4	3704.7	3652.45
Overall mean	1619.43^{a}	1579.68^{b}	1622.67^{a}	1605.61^{a}	1627.39^{a}	1616.17^{a}
Cumulative FCR						
I	1.11	1.18	1.13	1.16	1.15	1.15
II	1.39	1.47	1.39	1.42	1.4	1.42
III	1.53	1.56	1.53	1.55	1.57	1.57
IV	1.61	1.68	1.55	1.57	1.63	1.58
V	1.73	1.72	1.68	1.68	1.72	1.7
VI	1.81	1.76	1.75	1.77	1.79	1.78
Overall mean	1.53b	1.56^{a}	1.50^{c}	1.52^{b}	1.54^{b}	1.53^{b}
Mortality (%)						
Overall	3.33	1.66	1.66	0	1.66	0

Means of different superscript differ significantly each other

These reports are similar to the finding of Fadlalla *et al.* (2010) and Eid and Iraqi (2014) who reported that feed intake decreased, probably due to the associated flavor factor, the need of chicken to get adopted to supplementation. Since garlic has pungent smell it may be lead to lower diet palatability. In contrast to this, Raeesi *et al.* (2010) found that feed intake was significantly higher for control group ($P< 0.01$) compared to other group fed on diets containing garlic.

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Feed Conversion Ratio

The statistical analysis of variance for cumulative feed conversion ratio of broilers with different EOs revealed significant ($P < 0.01$) influence on feed conversion ratio (Table 3& 4).

Table 4: ANOVA for cumulative weight gain, feed consumption and FCR of broilers with different age groups supplemented with Eos

Sources		Cumulative Weight Gain		Cumulative Feed Consumption		Cumulative Feed Conversion Ratio	
	df	MS	F	MS	F	MS	F
Treatments	5	1840.78	6.88**	1810.318	3.856687**	0.002111	3.851818**
Weeks	5	3339916.5	12479.15**	11009615	23454.8**	0.311142	568.1008**
Error	25	267.64		469.3971		0.000548	
Total	35						

*** P<0.01*

Mortality

The superior cumulative feed conversion ratio was observed for treatment group C. The poor feed conversion ratio was recorded for treatment group A (control diet without supplementation of EOs.). A critical observation of data at 6th week revealed improvement in FCR for garlic and Neem essential oil supplementation compared to control. The improvement could be attributed to allicin an organosulphur compound contained in garlic that promotes performance of intestinal flora thereby enhanced digestion (Pourali *et al.*, 2010). Additionally, Willam and Lossa (2001) suggested that the improved digestibility of nutrients leads to more balanced flora with potential to reduce the proportion of pathogenic bacteria, there is also evidence in the literature that the garlic supplementation significantly enhanced villus and goblet cell number in the duodenum, jejunum and ileum of the birds and thus entire absorption process of nutrients (Tatara *et al.*, 2005 and Mosoud, 2006). Similar finding were observed by Dhurrani *et al*. (2008); Mahmood *et al.* (2009); Fadlalla *et al.* (2010); Onu *et al*. (2010); Pourali *et al*. (2010); Aji *et al.* (2011); Ansari *et al.* (2012); Elagib *et al.* (2013); Eid *et al.* (2014); Kharde *et al.* (2014); Saad *et al.* (2015). They revealed improvement in FCR in EOs supplemented groups. In contrast to present results, Chowdhury *et al.* (2002) and Ademola *et al.* (2011) concluded that garlic oil supplemented @ 100mg/kg diet in laying hens resulted in poor feed conversion ratio, reported non-significant differences in feed efficiency in laying hens fed with sundried garlic essential oil @ 10, 20 and 40mg/kg by stomach tube. This contraindication between studies could possible due to the difference in form, source, concentration of plants, diet, management and environmental condition.

Non-significant influence was observed on mortality pattern in different groups indicating no influence of supplementation of EO on livability. Similar findings in accordance with present study were reported by Ansari *et al.* (2012); El-tazi *et al.* (2014a) observed no any significant influence of Neem and garlic

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component on mortality rate of birds. In contrast to present findings, El-tazi *et al.* (2008); Fadlalla *et al.* (2010); Bonsu *et al.* (2012); Eid *et al.* (2014) showed significant decreased mortality rate by supplementation of garlic and Neem component in birds' diet.

Quality Characteristic of Meat

TBA Values (mg malondialdehyde/kg tissue of meat)

TBA value (mg/kg) of broiler breast and thigh meat at 15th day of storage with different essential oils revealed significant ($P < 0.01$) influence (Table 5) on keeping quality of meat, indicating improvement in shelf life of meat. These findings concluded that EOs were rich in different antioxidant properties. Increased antioxidant activity resulted in increased protection against the stresses caused by lipid oxidation, the decreased TBA values concluded that the shelf life of meat can be improved by dietary supplementation of garlic, Neem essential oil and their combination (Botsoglou *et al.*, 2002). Similar findings were observed by Ancsin *et al.* (2009) who revealed that garlic oil supplementation significantly reduced malondialdehyde concentration of meat. Onibi *et al.* (2009), Jang *et al.* (2008) revealed decreased malanaldehyde concentration with increasing level of garlic powder. Choi *et al.* (2010) concluded that TBA reactive substances values were significantly reduced by inclusion garlic powder. Killi *et al*. (2015) found dietary supplementation of Neem leaf powder, decrease in the TBRS protein carbonyls. Kiranmai *et al.* (2015) reported the antioxidant potential of various parts of Neem in both *in vivo* and *in vitro* experiments and stated that ethanolic extract of Neem contains a number of antioxidants. In contrast, to present study Mariutti *et al.* (2011) showed that the garlic exhibited non-significant effect as antioxidant.

Table 5: The effect of storage time on lipid oxidation of meat quality (TBRS) of broiler supplemented with EOs

Treatments	TBA value (mg/kg) (Breast meat)				TBA value (mg/kg) (Thigh meat)			
	0 day		15th day		0 day		15th day	
	Mean	S E	Mean	S E	Mean	S E	Mean	S E
A	0.064	0.004	0.275^{a}	0.032	0.088	0.009	0.347^{c}	0.012
B	0.059	0.005	0.074^{c}	0.005	0.071	0.005	0.093^{c}	0.029
C	0.055	0.004	0.071^{c}	0.008	0.062	0.007	0.074^{c}	0.025
D	0.053	0.009	0.069^{c}	0.01	0.065	0.006	0.069^{c}	0.005
E	0.049	0.004	0.063^{c}	0.004	0.056	0.006	0.064^{c}	0.004
F	0.062	0.01	0.118^{b}	0.027	0.066	0.004	0.101^{b}	0.015
CD	0.023		0.062		0.022		0.061	
CV %	19.21		73.28		20.24		85.19	

Immune Status

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RD Titre

An antibody titre against Ranikhet disease (RD) at 42nd day of age was influenced by dietary supplementation of combination of 100 mg garlic and Neem EOs are presented in (Table 6). Significant influence of garlic and Neem essential oil in combinations on antibody titer against Ranikhet disease (RD) inferred improved immune status of broiler. These effects may be due to interaction between dietary inclusion and synergetic effect of two essential oils i.e. garlic and Neem. In accordance with present study Nidaullah *et al*. (2010); Saima *et al.* (2014); Kharde *et al.* (2014) reported significant effect on antibody titre against Ranikhet disease (RD) by supplementation of garlic and Neem. In contrast, Pourali *et al.* (2010); EL-Latif *et al.* (2013) and Fallah *et al.* (2014) reported that non-significant effect on antibody titre against Ranikhet disease (RD). Non-significant influence of EOs on antibody titre against Infectious bursal disease (IBD) in the present study is in accordance with El-latif *et al.* (2013). They reported non-significant effect on antibody titer against Infectious bursal disease by supplementation of garlic essential oil.

Table 6: Antibody titre against Ranikhet disease and Infectious bursal disease (IBD) at 21st and 42nd day of age of broiler supplemented with EOs

Treatments	Ranikhet disease (RD) Titer				Infectious bursal disease (IBD) Titer			
	21st day		42nd day		21st day		42nd day	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	9.33	1.76	53.33[b]	13.33	371	51.45	990.83	155.28
B	20	7.21	50.00[b]	10.41	340.5	15.73	1094.67	173.11
C	10.67	1.33	44.00[b]	12.86	430.5	27.22	846.5	85.81
D	8.33	3.93	70.33[b]	17.23	567.67	26.86	1267.67	166.77
E	12	3.06	87.00[b]	4.04	486.33	122.88	982.17	187.18
F	9.33	1.33	146.66[a]	20.28	394	59.66	1207.67	107.95
CD	12.99		48.78		12.99		48.78	
CV%	58.27		55.02		58.27		55.02	

Economics of Broiler Production

The results of economical evaluation indicated that the diet supplemented with garlic and Neem @ 100 mg/kg of feed gained more net profit than that of treatment group A (control). The profitability was highest for treatment group B (9.59) followed by D (9.50). The increase in the profitability was due to lower cost of production. The present results are in agreement with findings of Oleforuh-Okoleh *et al.* (2014). They revealed that garlic extract supplementation had highest revenue and net return. The supplementation of Neem essential oil @ 200 mg/kg and combination of garlic and Neem oil @ 100mg/kg showed decrease in profit/kg of body weight when compared with control. In accordance with present study Nidaullah *et al*. (2010) observed decreasing trend in the mean gross return per chick of the Neem and garlic treated groups. Karangiya *et al*. (2016) also concluded non-significant effects of garlic supplementation on economics.

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Table 7: Economics of broiler production supplemented with essential oils

S. No.	Particulars	A	B	C	D	E	F
1	Chick cost (Rs)	26	26	26	26	26	26
2	**Feed consumption**						
i)	Pre-starter	141.6	141.92	145.27	142.55	137	138.63
Ii)	Starter	809.63	789.32	833.97	817.72	816.75	840.62
iii)	Finisher	2779.4	2659.95	2681.18	2702.13	2750.93	2673.2
	Total	3730.64	3593.19	3660.41	3662.4	3740.7	3652.45
3	**Feed cost /kg**						
i)	Pre-starter	28.3	28.73	29.16	28.69	29.08	29.12
Ii)	Starter	29.32	29.75	30.18	29.71	30.1	30.14
iii)	Finisher	28.39	28.82	29.25	28.78	29.17	29.21
4	**Feed cost /bird**						
i)	Pre-starter	4.02	4.13	4.22	4.07	3.96	4.01
Ii)	Starter	23.72	23.47	25.13	24.27	24.56	25.31
iii)	Finisher	78.89	76.63	78.41	77.76	80.21	78.07
	Total	106.62	104.23	107.76	106.1	108.75	107.39
5	**Misc. cost (Rs)**	8	8	8	8	8	8
6	**Production cost**	140.62	138.23	141.76	140.1	142.75	141.39
7	**Av. Live wt. (g)**	2103.1	2081.34	2127.39	2106.6	2110.41	2088.48
8	**Sale receipt @ 76/kg wt**	159.82	158.18	161.68	160.1	160.39	158.72
9	**Net profit/ bird**	19.22	19.95	19.92	20	17.64	17.33
10	**Net profit /kg (Rs)**	9.1	9.59	9.36	9.5	8.36	8.29

Conclusion

The overall results from the present study lead to conclusion that the supplementation of garlic @ 200mg/kg of feed is beneficial for the production parameters. However, supplementation of garlic and Neem EOsl @ 100mg/kg of feed alone in broiler ration is beneficial from the point of oxidative stability, economical gain and immune status of commercial broiler chickens.

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