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Original Article

Effect of Blend of Essential Oils on Growth Performance, Carcass Characteristics, Meat Quality, Intestinal Morphology, Serum Biochemistry, and Immune Response of Broiler Chickens

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Essential oils, growth, carcass characteristics, meat quality, serum biochemistry.

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ABSTRACT

The purpose of this study was to assess the impact of a blend of essential oils from eucalyptus, citrus, bromohexene HCl, thymole and camphor on the growth performance, carcass characteristics, meat quality, intestinal morphology, serum biochemistry, and immune response of broiler chickens. A total of 240 day-old chicks were divided into four groups, each with six replicates containing ten birds. The experiment was conducted under a completely randomized design (CRD). Different concentrations of the oil blend (0mL/kg, 0.15mL/kg, 0.30mL/kg and 0.45mL/kg) were added to the diet. The findings showed that, in comparison to the other groups, the birds that were given a blend of essential oils at concentrations of 0.30 and 0.45 mL/kg showed improved weight gain, feed efficiency, carcass yield, villus height, crypt depth, and greater immune response against Newcastle disease vaccination ($p<0.05$). Nonetheless, there was no statistically significant difference in the yields of the breast and thighs, feed consumption, mortality, weights of the liver, wing, heart, and gizzard, or abdominal fat between the treatments. The addition of a blend of essential oils at the doses of 0.30 and 0.45 mL/kg significantly lowered the pH of the meat in comparison to the other groups ($p<0.05$). All blood biochemical markers, including total serum protein, albumin, globulin, glucose, cholesterol, triglyceride, and uric acid, revealed no variations between the treatments. In conclusion, adding 0.30mL/kg of a blend of essential oils to broiler diets may be the optimum level to improve overall performance without adversely affecting the blood biochemical profile.

INTRODUCTION

The global market for poultry products is expanding and growing. The poultry industry is at an excellent position due to the quality purification of genetic potential through the selection and improvement of poultry feed additives, especially through feed formulation. Poultry feed often contains feed additives. In order to increase efficiency, productivity and output, feed additives are essential components of the feed formulation process. Feed additives are non-nutritious substances that are necessary for growth and development. The proper use of feed additives in poultry diets may boost feed conversion ratios and bird output (Khan & Iqbal, 2016). A balanced formulation with effective feed additives is responsible for better growth and performance (Pirgozliev *et al.,* 2019). Antibiotic feed additives have been used in the poultry industry for the last ten years to boost bird immunity, development, and efficiency. Antibiotics used in non-therapeutic doses as growth stimulants cause antimicrobial resistance in birds, which has negative consequences (Robinson *et al.,* 2019). Essential oils have great potential to act as an alternative to antibiotics, improving growth performance with lesser side effects (Rehman, 2023).

Plant leftovers that may or may not be toxic are used to extract essential oils (Zhai *et al.,* 2018). According to Krishan & Narang (2014), essential oils can improve chicken metabolism and feed consumption. Essential oils are known to have antioxidants, anti-inflammatory, and digestive efficiency properties (Craig, 2001; Jang *et al.,* 2007; Fernandez-Panchon *et al.,* 2008), all of which are linked to increased production and bird health. Volatile oils that are derived from essential oils come from a variety of plants that have characteristics such as antibacterial, antiviral, antifungal, immune-boosting, digestive, and anti-heat stress (Gopi *et al.,* 2014).These essential oils are increasingly being utilized in poultry diets because they help with digestion and increase output. This might be owing to birds' remarkable gastro-intestinal microbial growth (Wade *et al.,* 2018). The addition of mixed essential oils to chicken diets has a positive effect on gut morphology, intestinal lining and causes growth improvement (Tiihonen *et al.,* 2010). According to Cabuk *et al.* (2014), the supplementation of a blend of essential oils improved the growth rate of birds, increased goblet cells of the small intestine, increased the height of villi, and also increased the feed conversion ratio. In light of the above, this study was designed to assess the impact of a blend of essential oils on the growth performance, carcass characteristics, meat quality, gut morphology, serum biochemistry, and immune response of broiler chicken.

MATERIALS AND METHODS

Experimental site, design, and husbandry

The research was carried out at the Faisal Chicks (Pvt.) Ltd. Poultry Farm in Multan, Punjab, Pakistan. A total of 240 day-old chicks were divided into four groups (T_1, T_2, T_3, and T_4), each of which had six replicates and contained ten birds. The experiment was conducted under a completely randomized design (CRD). Different concentrations of the oil blend (Arsilvan Super), 0mL/kg (T_1), 0.15mL/kg ($T_{2)}$, 0.30mL/kg ($T_{3)}$ and 0.45mL/kg ($T_{4)}$, were added to the diets. The blend of essential oils was prepared from eucalyptus, citrus, bromohexene HCl, thymole and camphor. The calculated and analyzed values of essential oils in the different experimental diets is presented in Table 1. The chicks in the experiment were reared in 24 floor pens with adequate litter (rice husk). In each pen (1×1 m^2), there were two round feeders and a nipple drinker for *ad libitum* feeding and the supply of clean, fresh drinking water. In the hatchery, the chicks were vaccinated against infectious bronchitis (IB) and Newcastle disease (ND). The brooding temperature and relative humidity (RH) were maintained at 34 ± 1.1°C and 62 ± 3%, respectively, throughout the first week after hatching. Following that, the temperature gradually dropped till reaching 24°C on day 21 with a 65% RH. Throughout the investigation, a lighting regimen of 23L: 1D was used. Throughout the duration of the trial, *iso-nitrogenous* and *iso-caloric* diets were used. Treatment diets were created (NRC, 1994) to address the particular nutritional needs of broilers during the starter (1 to 11 day), grower (12 to 21 day), and finisher (22 to 35 day) stages (Table 2). The diets were fed as crumbs in the starter phase and as pellets during the grower and finisher phases. The experiment was conducted under rules and regulations approved by the Ethical Review Committee of the Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan.

Table 1 – Calculated and analyzed values of essential oils in diets.

Treatment	Calculated value of essential oils in feed	Analyzed value of essential oils in feed
T_1	0	0
T_2	0.15	0.11-0.14
T_3	0.30	0.24-0.27
T_4	0.45	0.37-0.42

Data collection

Growth performance and carcass characteristics

Daily feed offered, weekly body weight, and daily mortality data were collected and used to calculate the weekly feed intake, cumulative feed intake, weekly weight gain, total weight gain, weekly feed efficiency, overall feed efficiency, and mortality percentage following the method adopted by Khan *et al.* (2019). Three broilers from each replicate that were closest to the average weight of that replicate at the end of the experiment were chosen, held off-feed for four hours, and then slaughtered in line with Halal laws, with bleeding permitted for about three to four minutes. After that, the carcass yield (excluding skin, shanks, and head) and the relative weights of the liver, gizzard, heart, and abdominal fat were calculated as a percentage of the live weight following the methodology used by Khan *et al.* (2019).

Meat quality

Meat quality parameters were studied at the end of the experimental trial. Three birds from each replicate were selected and slaughtered. The pH of

Table 2 – Composition of the basal diet for different development phases.

Ingredients (%)	Development Phases		
	Starter (1-11 days)	Grower (12- 21 days)	Finisher (22-35 days)
Corn	52.00	54.00	57.00
Rice tips	4.90	5.20	5.34
Canola meal	4.05	4.90	5.00
Corn gluten 60 %	2.00	2.00	2.00
Soybean meal	30.00	26.00	22.00
Canola oil	2.00	3.21	4.20
$CaCO_3$	1.20	1.00	0.90
DCP.$2H_2O$	2.20	2.15	2.10
Lysine-SO_4	0.60	0.50	0.40
DL-Methionine	0.15	0.13	0.12
Threonine	0.10	0.06	0.04
Sodium chloride	0.20	0.25	0.30
Sodium bicarbonate	0.10	0.10	0.10
Vitamin premix[1]	0.20	0.20	0.20
Minerals premix[2]	0.30	0.30	0.30
Total	100.00	100.00	100.00
Nutrient			
Crude protein	23.00	22.00	21.00
ME (kcal/kg)	3000	3100	3200
Digestible Lysine	1.21	1.21	1.01
Digestible Methionine	0.54	0.50	0.45
Digestible Methionine + Cysteine	0.88	0.81	0.76
Arginine	1.25	1.16	1.07
Threonine	0.80	0.74	0.68
Tryptophan	0.19	0.18	0.16
Calcium	0.90	0.85	0.80
Available phosphorus	0.50	0.48	0.46
Sodium	0.16	0.16	0.16
Potassium	0.55	0.55	0.55
Chloride	0.30	0.30	0.30

[1]Provided per kg of diet: vitamin A, 11,000 IU; vitamin D_3, 2,560 IU; vitamin E, 44 IU; vitamin K, 4.2 mg; riboflavin, 8.5 mg; niacin, 48.5 mg; thiamine, 3.5 mg; d-pantothenic, 27 mg; choline, 150 mg; vitamin B_{12}, 33 µg.

[2]Provided per kg of diet: copper, 8 mg; zinc, 75 mg; manganese, 55 mg; iodine, 0.35 mg; selenium, 0.15 mg.

the meat was examined using a pH meter. Firstly, meat was grounded finely, followed by water addition at a 1:9 ratio. The meat was homogenized and then its pH was evaluated using a pH meter. According to Pi *et al.* (2005), the lightness (L*), redness (a*), and yellowness (b*) of a sample of breast meat are used to determine its color. Meat color was examined with a colorimeter. A meat sample was suspended in an inflated bag and kept at 2-4°C for one to two days in order to measure drip loss from the breast fillet using the gravimetric method (also known as the Honikel bag method (Warner, 2014)). This procedure provides information about the meat sample's capacity to hold water (Warner, 2017).

Intestinal morphology

For jejunum tissue sampling, 3 birds were slaughtered from each replicate. These samples were preserved in formalin 10% for 48 hours. Thereafter, the tissue samples were washed in tape running water, followed by an alcohol treatment. Samples were embedded in paraffin after slicing into 4 micrometers slices with the help of microtome. The mounted samples were stained with hematoxylin and eosin. The height of the villi was measured using a microscope, according to the methodology described by Panda *et al.* (2009). The villus height was calculated from the crypt-villus junction to the brush border at the tip. Crypt depth was measured at the level of the opposing crypt epithelial cells' basement membranes.

Blood biochemistry and immune response

Using a 5ml disposable syringe without anti-coagulant, blood samples (3ml/sample) were taken at the moment of slaughter from three birds per experimental unit (18 birds/treatment). Blood was processed and the serum was separated, and examined for total protein (TP), albumin (AB), globulin (GB), glucose (GL), cholesterol (CH), triglycerides (TR), and uric acid (UA) following the methodology used by Khan *et al.* (2019). One week before sampling, all birds were vaccinated against ND and IB through the drinking water, and the immunological responses were determined following Khan *et al.* (2019).

Statistical analysis

One-way ANOVA was used to analyze the data in compliance with the CRD (SAS). The means of the various treatments were compared at a 5% probability level using Duncan's Multiple Range test (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Growth performance

Data on growth performance indicators showed significant differences in weight gain (WG) and feed efficiency (FE) between the birds. When compared to the other groups, birds that received the oil blend at the levels of 0.30 and 0.45mL/kg had higher WG and FE ($p<0.05$). However, feed intake (FI) and mortality (M) did not vary amongst regimens (Table 3). The profitability of chicken production is influenced by the growth performance characteristics of FI, WG, FE, and M. It is possible that essential oil supplements improved performance by enhancing antioxidant and digestive enzymes, improving nutrient digestibility, and improving intestinal health in the current investigation, which could account for the improvement in growth rate and feed efficiency (Mountzouris *et al.,* 2011; Gopi *et al.,* 2014). Similar to this, Abdel-Wareth *et al.* (2019) came to the conclusion that dietary supplements containing essential fatty acids enhance broiler chicken performance by encouraging more effective nutrient utilization. This improvement is seen in terms of body weight and feed conversion ratio. Elbaz *et al.* (2022) conducted a similar investigation to find out how broiler chicken performance would be affected by dietary supplementation with either garlic, lemon essential oil, or a mixture of the two. According to the obtained data, the average body weight of broilers given essential oils either in combination or separately improved. Similarly, Su *et al.* (2021) looked into how essential oils affected growth performance and came to the conclusion that essential oils had a favorable effect on growth. Amad *et al.* (2011) had similar findings, stating that phytogenic feed additives increased apparent ileal digestibility of nutrients at the ages of 21, 35, and 42 days. Combinations of feeding oils have also been shown to have synergistic effects on growth rates (Bassett, 2000; Langhout, 2000; Alcicek *et al.,* 2003; Denli *et al.,* 2004). Contrarily, some studies (Case *et al.,* 1995; Botsoglou *et al.,* 2002) found that impacts on animal performance were non-significant. The differences in the inclusion levels of essential oils, the sources of herbs utilized to create the blend of essential oils, the composition of the basal diet, or the microbiological environment in which the birds were raised may be the elements contributing to this variation between investigation results.

Table 3 – Effect of essential oils blend on the growth performance of broiler chicken[1].

Treatments[3]	Parameters[2]			
	CFI (g/bird)	WG (g/bird)	FE (g/g)	M (%)
T_1	3293.60	1982.00[b]	0.60[b]	2.80
T_2	3285.80	1995.60[b]	0.61[b]	3.20
T_3	3279.80	2140.00[a]	0.65[a]	2.00
T_4	3276.80	2156.40[a]	0.66[a]	1.60
SEM	4.903	18.657	0.006	0.275
p-value	0.669	0.0001	0.0001	0.147

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten birds per replicate.

[2]CFI: cumulative feed intake, WG: weight gain, FE: feed efficiency, M: mortality.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

Carcass characteristics

There were notable differences amongst the birds for carcass yield (CY). The birds that received a blend of essential oils at 0.30 and 0.45mL/kg showed enhanced ($p<0.05$) CY compared to the other groups. All other variables, including breast yield (BY), thigh yield (TY), wing weight (W), liver weight (L), gizzard weight (G), heart weight (H), and abdominal fat weight (ABF), did not vary across treatments (Table 4). Carvacrol, the active ingredient in essential oils, stimulates pancreatic secretions, and this enhanced digestive output can lead to greater digestion and nutrient absorption, which in turn can improve carcass yield (Ragaa *et al.,* 2016). Additionally, greater weight gain (Table 3) may have an impact on CY. In line with the current findings, Alçiçek *et al.* (2003) fed birds essential oils from several wild herbs at the doses of 24, 48, and 72 mg/kg meal and discovered that carcass output was increased, with the level of 48 mg/kg causing the most noticeable effects. According to Khattak *et al.* (2014), broiler fortification with a combination of essential oils exhibited the best increase in carcass weight. In contrast to the current study, Cázares-Gallegos *et al.* (2019) conducted a study to evaluate the effects of Mexican oregano essential oil (MOO; Lippia berlandieri Schauer) feed supplementation on broiler growth performance, carcass parameters, meat quality, and sensory evaluation. Supplementing the diet with 1000 mg/kg of Mexican oregano essential oil raised slaughter weight, but not carcass yield.

As stated above, the present investigation found no significant variations between treatments for BY, TY, W, L, G, H, and ABF. Similarly, no significant improvements in carcass attributes were observed when cinnamon oil was added to broiler feed (Ciftci *et al.,* 2009). In contrast, Fascina *et al.* (2012) observed that adding organic acids and phytogenic additives—which contain essential oils—to broiler meals significantly enhanced carcass properties. When 100 ppm of the essential oils obtained from cinnamon and cloves were added to the diet, the breast muscle weight increased noticeably (Isabel & Santos, 2009).

Meat quality

The outcomes for meat quality metrics, including meat lightness (L), meat redness (a), meat yellowness (b), meat drip loss (DL) and meat pH, are shown in Table 5. In comparison to other groups, supplementation with the blend of essential oils at 0.30 and 0.45mL/kg decreased meat pH significantly ($p<0.05$). However, the differences between treatments for all other

Table 4 – Effect of essential oils blend on the carcass characteristics of broiler chicken[1].

Treatments[3]	Parameters[2]							
	CY (%)	BR (%)	TH (%)	W (%)	L (%)	G (%)	H (%)	ABF (%)
T_1	67.18[b]	25.73	20.26	8.00	2.32	1.78	0.48	0.89
T_2	67.20[b]	25.50	20.35	8.01	2.31	1.79	0.48	0.88
T_3	69.57[a]	24.42	21.45	8.06	2.31	1.77	0.49	0.86
T_4	69.84[a]	24.21	21.52	8.08	2.29	1.78	0.46	0.87
SEM	0.400	0.300	0.233	0.015	0.008	0.007	0.006	0.007
p-value	0.007	0.186	0.074	0.179	0.711	0.812	0.510	0.406

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.

[2]CY: carcass yield, BR: breast yield, TH: thigh yield, W; wing weight, L: liver weight, G: gizzard weight, H: heart weight, ABF: abdominal fat.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

measures, including meat lightness, meat redness, meat yellowness, and meat drip loss, were not statistically significant ($p>0.05$). Similar conclusions were reached by Kahraman *et al.* (2015), who looked into the effects of rosemary essential oil on chicken fillet meat quality. They found that the lightness of the meat decreased, and its redness significantly increased, indicating that the use of essential oils can enhance meat quality. Similarly, a study was carried out to determine the impact of an essential oil blend on the growth and quality of meat. The alteration in meat consistency and color was evident in the results (Tekce *et al.,* 2020). Mexican oregano essential oil's impact on carcass characteristics and meat quality was studied by Cázares-Gallegos *et al.* in 2019. They found that essential oils can improve the redness of the meat. Moreover, another study conducted to determine how essential oils affected broiler chick growth and meat quality also showed considerable increase in growth with better meat quality (Isabel & Santos, 2009).

Table 5 – Effect of essential oils blend on the meat quality of broiler chicken[1].

Treatments[3]	Parameters[2]				
	L	a	b	DL	pH
T_1	52.52	5.11	14.33	2.00	5.94[a]
T_2	53.39	5.14	14.09	1.99	5.86[ab]
T_3	52.30	5.16	14.52	1.91	5.65[b]
T_4	54.56	5.14	14.41	1.90	5.63[b]
SEM	1.133	0.085	0.260	0.049	0.046
p-value	0.910	0.998	0.955	0.829	0.023

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten birds per replicate.

[2]L: meat lightness, a: meat redness, b: meat yellowness.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

Intestinal morphology

Values for the parameters of intestinal morphology show the state of gut health. When villus length and crypt depth are shorter, the bird absorbs nutrients at a slower pace because there is less surface area available for absorption, and enterocytes are less developed. As a result, the bird performs poorly due to reduced nutrient absorption (Paiva *et al.,* 2014). Additionally, the gut microbiota is regulated and balanced by essential oils and organic acids. This effect may be helpful in improving gut morphology (Zeng *et al.,* 2015).

Significant variations in gut morphology measures between treatments were seen for villus height (VH) and crypt depth (CD). In comparison to the other groups, birds receiving a blend of essential oils at 0.30 to 0.45mL/kg showed increased VH and CD. The VH: CD ratio, however, had no significant differences between regimens (Table 6). The fact that essential oils balanced and controlled the intestinal microbiota may be the cause of the increased VH and CD in groups receiving a blend of essential oils at 0.30 to 0.45mL/kg. The positive effects of this addition might improve gut morphology (Zeng *et al.,* 2015). Furthermore, improved intestinal morphology may be due to the anti-inflammatory and anti-oxidation mechanisms of essential oils (Du *et al.,* 2016; Gao *et al.,* 2019). Similar to the current work, Emami *et al.* (2012) tested the impact of fructooligosaccharides and peppermint essential oil on the growth efficiency and gut morphology of broilers. Results showed a considerable increase in villus length and crypt depth, particularly in the jejunum. Similarly, another study has been conducted on broiler chickens to determine how essential oils affected the morphology and microbiology of the intestines, with different doses – 100, 200, 300, and 600 mg/kg of feed – being given to different groups. When the birds were slaughtered on day 42, it was discovered that the villus length had greatly increased, and both gut morphology and gut microbiota dramatically improved (Ghazanfari *et al.,* 2014). Bozkurt *et al.* (2012) also investigated how an essential oil blend affected growth performance, gastrointestinal morphology, and gut microbiota. At day 42, it was noted during the slaughter that villus length and crypt depth had increased. Finally, Hajati *et al.* (2015) studied the impact of essential oils on the gut microbiota and intestinal morphology of broiler chicks during heat stress, finding that the depth and length of the crypts both increased significantly.

Table 6 – Effect of essential oils blend on the intestinal morphology of broiler chicken[1].

Treatments[3]	Parameters[2]		
	VH (μm)	CD (μm)	VH:CD
T_1	1059.52[b]	305.72[b]	3.48
T_2	1072.70[b]	308.80[b]	3.48
T_3	1218.62[a]	343.36[a]	3.56
T_4	1244.01[a]	343.66[a]	3.62
SEM	20.014	6.263	0.047
p-value	0.0001	0.022	0.679

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.

[2]VH: villus height, CD: crypt depth.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

Blood biochemistry

All of the blood biochemical markers examined in the current study – TP, AB, GB, GL, CH, TR, and UA – showed no statistically significant differences between treatments (Table 7). No toxicity was visible, and blood biochemistry profiles were within the expected range. These findings showed that blood chemistry did not change at the age of marketing, proving that the blend of essential oils is safe for birds to consume. These results are consistent with those reported by Hagan *et al.* (1967), who found no obvious evidence of toxicity when rats were fed diets containing thymol at the level of 1,000 and 10,000 mg/kg for 19 weeks. This could be predicted based on the quick metabolic conversion and excretion of essential oils from the body. Similarly, Khattak *et al.* (2014) investigated the effects of Tecnaroma Herbal Mix PL on the blood biochemistry of broilers. This unique commercial preparation is made from a natural mixture of essential oils from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme. In terms of blood biochemistry, there were no treatment changes ($p>0.05$). Oladokun *et al.* (2021) evaluated the effects of a combination of essential oils and the methods used for their administration on the growth performance, blood biochemistry, intestinal morphology, immunological condition, and antioxidant status of broiler chickens. Treatments had no effect on the blood plasma parameters tested in this study ($p>0.05$). Similar conclusions were reached by Santos *et al.* (2019), who investigated the effects of microencapsulated essential oil supplementation on the haematological profile, hepatic function, and histopathology of mixed-sex broilers. They found no differences ($p>0.05$) between the treatments and the positive control in the mean levels of total proteins, albumin, globulin, serum albumin:globulin ratio, total cholesterol, triglycerides, or glucose. In some other studies, no remarkable changes in the total cholesterol level were observed due to feeding essential oils and organic acids (Popović *et al.,* 2016; Vinus *et al.,* 2017). Belenli *et al.* (2015) also showed no effect of essential oils on broilers' glucose levels. However, some studies contradict our findings, showing that blood glucose levels were significantly raised by the

Table 7 – Effect of essential oils blend on the serum biochemistry of broiler chicken[1].

Treatments[3]	Parameters[2]						
	TP (g/dL)	AB (g/dL)	GB (g/dL)	GL (mg/dL)	CH (mg/dL)	TR (mg/dL)	UA (g/dL)
T_1	4.09	2.27	1.54	135.65	165.10	88.31	3.99
T_2	4.10	2.27	1.53	136.50	164.68	87.98	4.00
T_3	4.10	2.28	1.53	137.05	161.71	85.72	3.99
T_4	4.11	2.27	1.54	135.65	159.76	85.63	3.97
SEM	0.006	0.008	0.004	0.514	1.365	1.404	0.011
p-value	0.706	0.890	0.846	0.749	0.491	0.875	0.864

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.

[2]TP: total protein, AB: albumin, GB: globulin, GL: glucose, CH: cholesterol, TR: triglyceride, UA: uric acid.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

addition of essential oil and organic acid compared to the control group (Rahman *et al.,* 2015; Elnaggar & EL-Maaty, 2017; Elnaggar & El-Tahawy, 2018; Iqbal *et al.,* 2021).

Immune response

The body's antibody response to ND vaccination varied significantly between treatments. When compared to other groups, birds given a blend of essential oils at 0.30 and 0.45mL/kg had higher antibody titers against ND after vaccination. Antibody titer against IB, however, did not differ significantly between treatments (Table 8). The fact that essential oils have a scavenging action on free radicals, which rapidly improves the immune system, may explain why birds given a combination of essential oils at doses of 0.30 and 0.45 mL/kg had higher antibody titers against ND vaccination than other groups (ELnaggar & El-Tahawy, 2018). Similarly, an evaluation of the impact of essential oils with and without ND vaccination was conducted. The results indicated that essential oils have a significant potential to raise antibody titers against ND, since the group treated with essential oils in addition to the ND vaccine had less clinical signs and postmortem lesions related to Newcastle disease (Aksu & Bozkurt, 2009). Al-Hadid (2016) examined how five different plants' essential oils affected antibody titers against the vasogenic strain of ND. It was found that a combination of essential oils was particularly effective against the highly contagious viral disease known as Newcastle disease in chicken. Similar to this, a study was carried out to determine the impact of a blend of three different essential oils on the defense mechanisms and carcass traits of birds. Essential oils were found to significantly improve yield and enhance immunity (Chowdhury *et al.,* 2018). A study was conducted by Galal *et al.* (2015) to determine how essential oils affected the immunological system of broiler chicks. It was discovered that essential oils boosted the bird's innate and humoral immunity. An *et al.* (2019) studied the impact of ginger extract on laying hen immunity, antioxidant capacity, and production performance. According to this study, ginger extract has the power to lessen inflammatory response in addition to enhancing the antioxidant capacity and immunological function of the birds.

Table 8 – Effect of essential oils blend on the immune response of broiler chicken[1].

Treatments[3]	Antibody titers[2]	
	ND (HI titer, log_2)	IB (ELISA titer)
T_1	4.30[b]	3446.38
T_2	4.31[b]	3459.38
T_3	4.35[a]	3543.78
T_4	4.36[a]	3581.29
SEM	0.009	21.801
p-value	0.007	0.065

[a-b]Treatment means within a column bearing the different letters are significantly different ($p<0.05$).

[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.

[2]Hens were vaccinated via drinking water using commercially available ND (La Sota) and IB (H 120) vaccines, one week before blood samples were taken.

[3]T_1: diet without blend of essential oils (control), T_2: diet with 0.15mL/kg blend of essential oils, T_3: diet with 0.30mL/kg blend of essential oils, T_4: diet with 0.45mL/kg blend of essential oils.

CONCLUSIONS

The results of this study indicate that it is possible to change production characteristics of broiler chicken by manipulating dietary essential oils. The addition of a blend of essential oils to broiler diets at dose of 0.30mL/kg can improve overall performance without adversely affecting blood biochemical profiles.

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M. T. Khan, M. Arslan, A. Javaid, T. Asad and M. Azhar contributed to the conception and design of the study; M. F. Rehman contributed to the acquisition of data; U. Farooq, Q. Nisa, E. Bughio and S. Liaqat contributed to the analysis of data; S. Aslam, F. Ali and H. Ali contributed to the interpretation of data; F. Wadood, M. A. Gondal, M. Rauf and S. Nazir drafted the work; D. Fouad, F. S. Ataya and M. Safdar have substantially revised it; and A. S. Magsi and M. Qumar have approved the submitted version.

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

All authors of the present study declare that there is no conflict of interest, financial or otherwise.

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