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Research Article

Effect of phytobiotic supplementation on growth performance, blood profile and immunity of broiler chicks

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Abstract

Phytobiotics are plant derived bioactive compounds, including herbs, spices, essential oils (EOs) and oleoresins. This study was conducted to evaluate the effect of phytobiotic supplementation (combination of Oregano and Thyme EO) at different dosages on growth performance, blood profile and immunity of broiler chicks. One-day old, 48 broiler chicks were randomly divided into four groups based on the dosage of essential oils; T_o (control group), T_1, T_2 and T_3 received Oregano EO + Thyme EO at 100+100, 200+200 and 300+300 mg/kg of diet respectively. All chicks were maintained under controlled conditions of temperature, humidity and light hours for 45 days. Feed conversion ratio and weight gain were calculated fortnightly for six weeks. Blood samples were collected on 15th, 30th and 45th day. Results indicated that EOs enhanced growth performance and reduced FCR. The highest weight of 2536.33±40.80g and lowest FCR 2.00±0.05 were recorded on 45th day. Measured blood parameters were also increased in all treatment groups as compared to the control group. High antibody titer was reported against Newcastle Disease Virus and Infectious Bursal Disease. Among all treated groups, the combined dose of EOs at T_2 level resulted in the highest weight gain followed by T_3, T_1 and the lowest in T_o. The present study revealed that Oregano and Thyme EOs as dietary supplements significantly ($p<0.05$) improved the overall performance of broiler chicks and can be used as growth promoters. However, further studies are required to determine the combined effects of Oregano Thyme EOs on meat and yolk quality.

Keywords: Blood Profile; Growth Performance; ELISA; Oregano Essential Oil; Phytobiotics; Thyme Essential Oil

Introduction

Phytobiotics are natural bioactive compounds derived from plants, added to the diets, to improve the productivity of livestock. These phytogenic feed additives include a wide range of herbs, spices and extracts of some valuable ingredients mainly in form of essential oils (EOs) and oleoresins [1]. Leaves, flowers, roots and whole plants are used to obtain these products. Phytobiotics are used in human nutrition as flavoring agents, food preservatives and are well known for their pharmacological effects as well [2]. These compounds have been used for a long time as an alternative medicine to improve human health [3]. The addition of phytobiotic supplementation in poultry production as a natural growth promoter has gained much interest in recent years [4].

Different biological attributes of phytobiotics include anti-oxidant, anti-stress, anti-microbial and nutrigenomic effects on immunity development. These characteristics have made them an appealing choice to be used as animal growth promoters [5, 6]. Antioxidants can positively affect the stability of animals' feed, can increase the quality and the storage time of the products obtained from animals [7] and likewise enhance the palatability of food, promote and improve gut development [8].

EOs derived from the different plants can serve as a defensive tool against different bacterial diseases in poultry [9]. Essential oil (EO) of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum verum*), anise (*Pimpinella anisum*), citrus peel [10], whole seeds or extracts of oregano, thyme, garlic (*Allium sativum*) rosemary, sage (*Salvia officinalis*), black cumin (*Nigella sativa*) and chili (*Capsicum annum*) are most commonly used feed additives in commercial poultry nutrition, used either singly or in a combination of these or other phytobiotics [2].

Thyme is a perennial herbaceous plant of Lamiaceae family, an important aromatic herb commonly used for both medical and culinary purposes. This medicinal plant is recommended because of number of therapeutical properties of its EOs, usually known as thyme oil. [11]. Thymol, a monoterpenoid phenol is a major component in thyme oil [12]. Thyme has proven to be useful in different intestinal infections caused by bacteria and fungi and can also improve the functioning of the liver and stimulate appetite. Thyme oil and thymol also have insecticidal activities [13]. Thyme oil has been used in traditional medication and has many important properties like antimicrobial, antioxidative, antibacterial, antiseptic, antifungal, antispasmodic and antiviral [14]. It is also used for veterinary medicines [15]. Active components of thyme oil have positive effects on poultry production and health [16].

Oregano is also a member of family Lamiaceae [17]. For decades, the plant has been used as a medicinal herb for the treatment of numerous diseases in ethnopharmacological preparations [18]. Various principal actions of Oregano EO include broad-spectrum antimicrobial activity, antiparasitic, stomachic, antispasmodic, diuretic activity and as an immunomodulatory agent. The bioactive component (carvacrol) of oregano is responsible for its pharmacodynamic effects [19]. Antimicrobial properties of Oregano EO can reduce the total viable bacterial count in the carcass of broilers and other pathogens especially salmonella [20]. Oregano EO has also shown antioxidant activity [9]. Positive effects on antibacterial, anti-inflammatory, coccidiostat, digestive secretion, immune stimulation and feed intake have been recorded when broiler chicks were fed with diet having these EOs [21].

As a number of biological properties are attributed to EOs of Oregano and Thyme and so, they also positively affect the performance of broiler when given individually. This study was designed to evaluate the combined effect of Oregano EO and Thyme EO when given at different dosages on different health parameters of broiler chicks.

Materials and Methods

Essential oils (EOs)

Thyme Essential oil (TEO) and Oregano Essential oil (OEO) were purchased from the local market of Gujranwala, Punjab, Pakistan.

Research animals and dosage design (Oral)

Gallus gallus domesticus were used as research animals. One-day old, 48 broiler chicks were weighed at the start of the experiment and the average weight of 47 grams was recorded. The chicks were randomly divided into four groups based on dietary supplementation. Each group had 3 replicates, each replicate contained 4 chicks

so, in total having 12 chicks in each group. Treatment groups and dosage design for the experiment were as:

Control Group provided with basal diet, untreated i.e., without any EO (T_o)

OEO+TEO at 100+100 mg/kg of diet (T_1)

OEO+TEO at 200+200 mg/kg of diet (T_2)

OEO+EO at 300+300 mg/kg of diet (T_3)

Housing and maintenance of animals

Chicks were maintained under controlled conditions at Cheema Protein Form Gakhar, Gujranwala, Punjab Pakistan. At the age of one day, temperature was maintained at 33°C and was reduced to 26.7°C by the end of 3 weeks by decreasing 0.3°C on daily and was finally maintained at 24±2°C. The photoperiod consisted of 23 hours of light and 1 hour of darkness and 64% relative humidity was maintained during the whole period of experiment.

Vaccination schedule

All chicks were vaccinated against Newcastle Disease Virus (NDV) in three shots: first at beginning of the experiment, the second shot (ND clone) on the 7th day of the experiment through eye drops and the third shot (ND La Sota) via drinking water on 17th day of the experiment and for Infectious Bursal Disease (IBD) in a single shot on the 11th day of the experiment via eye drops.

Treatment duration

The experiment was conducted from January to February and lasted for 45 days. EOs of Oregano and Thyme were given mixed in 14-NSS broiler pre-starter special crumbs (1-14th days), 14-NGS broiler starter-grower crumbs (15th -30th days) and 15-NFS broiler finisher special crumbs (31st – 45th day of age). EOs were added mg/kg of diet. Water and food were given *ad libitum*. Fortnightly, three samplings were made for 6 weeks.

Evaluated parameters

Following three parameters were evaluated in this experiment.

A) Growth Performance (Feed Conversion Ratio)

B) Blood Profile (Compete Blood Profile)

C) Immunity (Antibody Titer)

A) Growth performance (Feed Conversion Ratio)

Body weight for each group was measured separately on the 15th, 30th and 45th day of the experiment after 12 hours of fasting by using a digital balance. Feed intake and body weight gain were noted according to treatment groups and Feed Conversion Ratio (FCR) was calculated using formula: FCR = Feed intake/Weight gain [22].

Sample collection

Blood samples (for blood profiling and serum) were collected by venipuncture in the wing (brachial) vein of the chicken [23].

I) Blood profile

For blood collection, the wing of the chicken was pulled towards the outside and vein was located on the underside of the wing between the biceps and triceps. Some feathers were plucked and the site for puncture was disinfected with alcohol swabs. The needle was inserted in the vein almost parallel to the skin and the plunger was pulled slightly which created a vacuum and allowed the blood to flow in the syringe. After removing the needle, pressure was applied to that area to stop further bleeding [23].

3ml of blood was taken from birds of each group. Blood was immediately transferred into EDTA.K_3 tubes containing anticoagulant EDTA, tubes were gently inverted three times to avoid any coagulation and samples were rested at room temperature. Tubes were labeled according to treatment groups and replicates.

II) Serum (For Antibody Titer)

For serum separation, 2ml of blood was transferred into gel & clot activator tubes (containing no anti-coagulant). Samples were rested for 30 minutes to 1hr at 37°C. Tubes were placed in the horizontal position to aid in blood clotting and serum separation. Separated serum samples were then stored in properly labeled Eppendorf tubes at -20°C [24].

B) Blood profile (Complete Blood Count)

To analyze the blood profile, Complete Blood Count (CBC) was done. Blood samples collected in EDTA.K_3 tubes were used for CBC. Semi-Automatic Chemistry Analyzer (Model HKTE0112 Guangzhoun Hekang Biotechnology Co., Ltd China) was used for blood profiling and CBC was done within 24 hours of blood collection [25]. Measured parameters included (Hemoglobin) Hb, (Hematocrit) HCT, (Mean Corpuscular Hemoglobin Concentration) MCHC, (Mean Corpuscular Volume) MCV, (Mean Corpuscular Hemoglobin) MCH, Erythrocyte count (RBCs), Leukocyte count (WBCs) and Platelets.

C) Immunity (Antibody Titer)

Immunity was checked for two diseases: Newcastle Disease Virus (NDV) and Infectious Bursal Disease (IBD). The serum for NDV was analyzed by Hemagglutination Inhibition Test (HAI) and the standard method of testing by [26] was used. For IBD, Indirect ELISA (Enzyme-Linked Immunosorbent Assay) was performed and assay procedure [27] was followed. Antibody titer was checked by using serum samples stored in Eppendorf tubes. Serum samples were separated from the blood collected on the 15^{th}, 30^{th} and 45^{th} day of the experiment after the administration of EOs.

Data analysis

Data were analyzed by using Statistical Package for Social Sciences (IBM SPSS, 21). Mean (±S.E.M) was calculated for each of the parameters. Moreover, Variations between the groups were determined by ANOVA test and a post hoc test (Tukey HSD) was applied to check the extent of variations found in the groups. The value of $p<0.05$ was considered statistically significant.

Results

The findings of this study showed that the EOs of Oregano and Thyme positively affected the growth performance of broiler chicks and reduced the FCR. Similarly, the blood profile and immunity of broiler chicks were also improved as compared to the control group.

Effect of OEO + TEO on growth performance

Treatment with the combination of OEO + TEO at 100+100 mg/kg, 200+200 mg/kg and 300+300 mg/kg of diet, resulted in increased body weight of the broiler (Figure 1). Statistical data analysis also indicated that the mean values of weight measured on the 15^{th}, 30^{th} and 45^{th} day of the experiment for treatment group T_1 (962.34±18.62, 1513.00±8.96, 2374±25.65), T_2 (1111.00±7.71, 1724±6.96, 2536±40.80) and T_3 (1012.70±10.54, 1613.66±4.40, 2436.66±6.06) were significantly ($p<0.05$) higher than those of control group T_o (791.20±8.01, 1317.00±9.29, 1317.00±9.29). All treatment groups showed an increase in weight when compared to the control group. However, maximum weight gain was recorded by treatment group T_2 given a combination of both oils i.e., OEO + TEO at 200+200 mg/kg of diet.

However, treatment with OEO + TEO at the same dosage resulted in decreased FCR over the study duration (Figure 2). Statistical data analysis also showed that mean values for FCR calculated on the 15^{th}, 30^{th} and 45^{th} day of the experiment for treatment group T_1 (3.32±0.01, 2.75±0.03, 2.27±0.02), T_2 (2.97±0.07, 2.38±0.02, 2.00±0.05) and T_3 (3.23±0.01, 2.58±0.01, 2.16±0.01) were reduced significantly when compared to control group T_o (3.62±0.04, 3.33±0.01, 2.99±0.05). All treatment groups showed decreased FCR which indicated improved performance. But, the lowest FCR was recorded by T_2 group i.e., OEO + TEO at 200+200 mg/kg of diet.

Effect of OEO + TEO on immunity

OEO + TEO when given at 100+100 mg/kg, 200+200 mg/kg and 300+300s mg/kg of diet, resulted in increased antibody titer against NDV (Figure 3). Moreover, the statistical data analysis also illustrated that mean values for antibody

titer measured on the 15th, 30th and 45th day of the experiment for treatment group T_1 (213.00±7.85, 305.00±10.47, 381.33±81), T_2 (277.33±5.20, 395.33± 458.00±5.77) and T_3 (233.33±7.68, 3.44.66±8.81, 424.66±8081) were significantly ($p<0.05$) higher than those of control group T_o (108.6±8.81, 187.0±7.09, 261.3±8.19). All treatment groups showed increased antibody titer against NDV when compared to the control group. However, maximum antibody titer was recorded by treatment group T_2 i.e., OEO + TEO at 200+200 mg/kg of diet.

Likewise, antibody titer against IBD after treatment with the combination of OEO + TEO at 100+100 mg/kg, 200+200 mg/kg and 300+300 mg/kg of diet, also increased (Figure 4). Moreover, the statistical data analysis also illustrated that the mean values of antibody titer measured on the 15th, 30th and 45th day of the experiment for treatment group T1 (2579.00±75.36, 3541.33±61.76, 3956.00±45.76), T2 (3002.33±77.39, 4109.66±65.68, 4382.66±29.06) and T3 (2762.66±54.13, 3814.00±81.83, 4146.66±81.22) were significantly ($p<0.05$) higher than those of control group T_o (2141.00±26.38, 2835.00±56.21, 3384.00±60.00). All treatment groups showed increased antibody titer against IBD when compared to the control group. However, maximum antibody titer was recorded by treatment group T_2 i.e., OEO + TEO at 200+200 mg/kg of diet.

Effect of oregano EO + Thyme EO on blood profile

Treatment with the combination of OEO and TEO at 100+100 mg, 200+200 mg and 300+300 mg/ kg of diet resulted in improved blood profile. All measured hematological parameters were improved in all treated groups when compared to the control group. The highest parameters in comparison to that of the control group were recorded by OEO + TEO at 200+200 mg/kg of diet. Whereas, maximum values of MCV were recorded at OEO + TEO at 100+100 mg/kg of diet (Table-1).

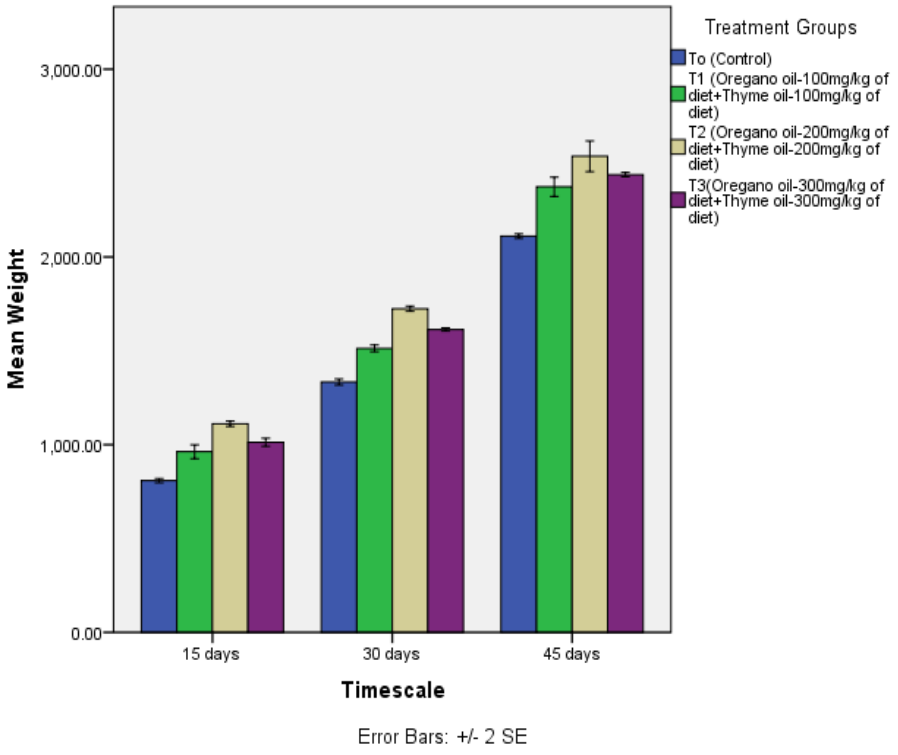

Figure 1: Concentration and Time-Dependent Effects of Oregano EO + Thyme EO on Body Weight of Broiler at Different Time Scales (15th, 30th and 45th Days)

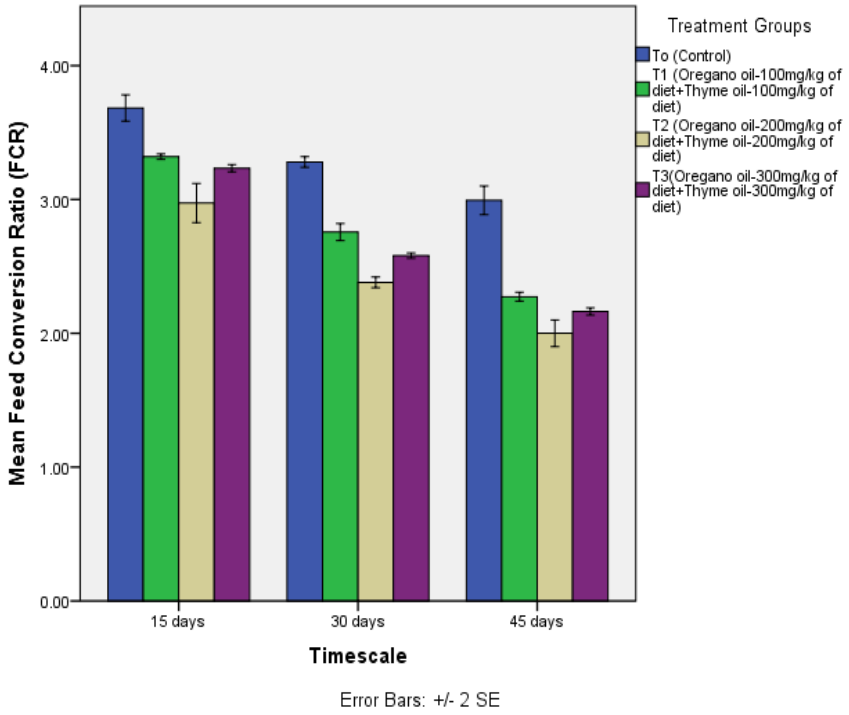

Figure 2: Concentration and Time-Dependent Effects of Oregano EO + Thyme EO on Feed Conversion Ratio of Broiler at Different Time Scales (15th, 30th and 45th Days)

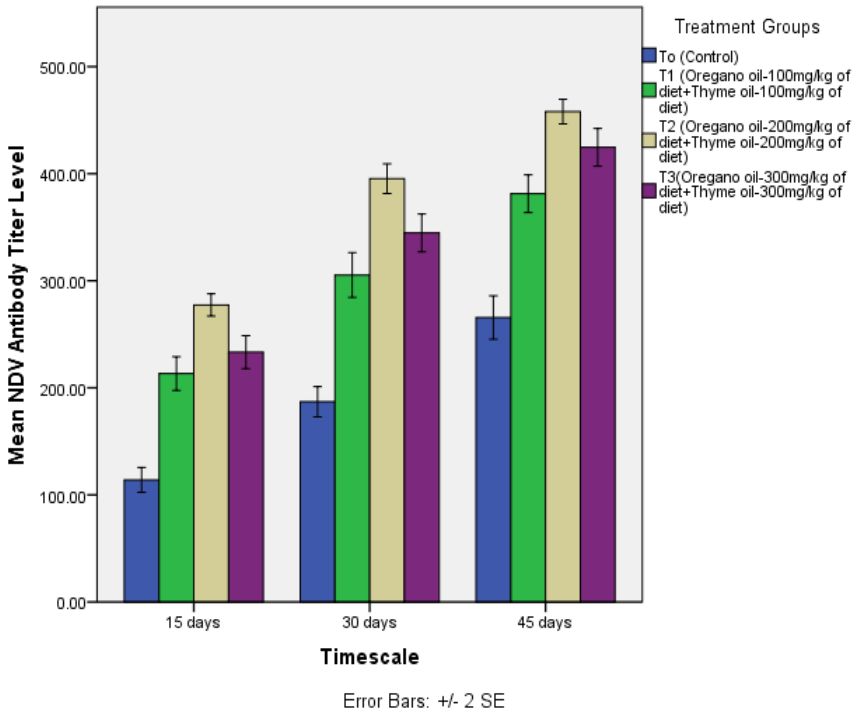

Figure 3: Concentration and Time-Dependent Effects of Oregano EO + Thyme EO on Antibody Titer level against Newcastle Disease Virus (NDV) of Broiler at Different Time Scales (15th, 30th and 45th Days)

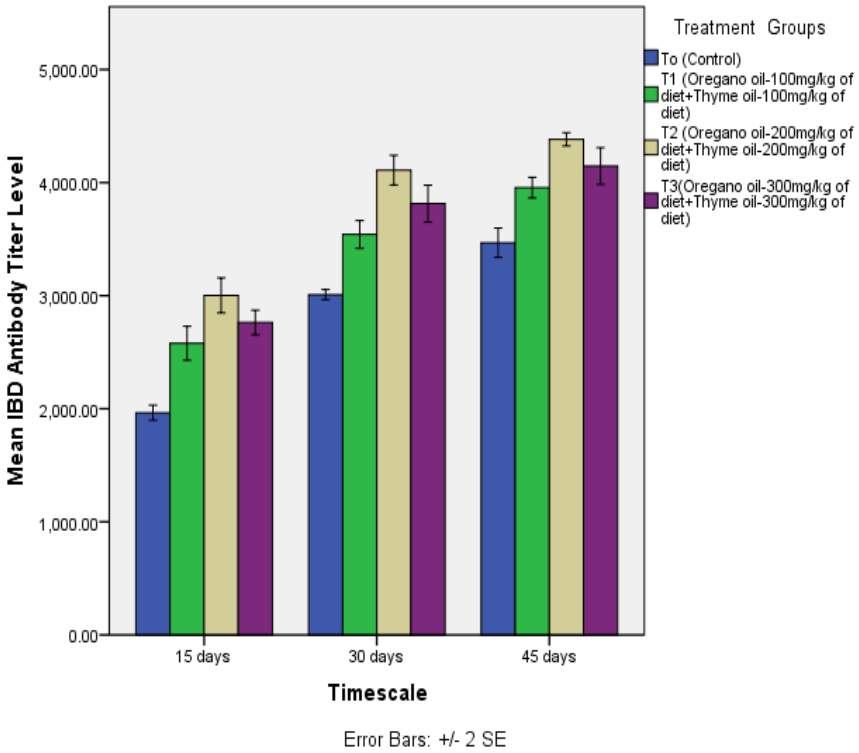

Figure 4: Concentration and Time Dependent Effects of Oregano EO + Thyme EO on Antibody Titer level against Infectious Bursal Disease (IBD) of Broiler at Different Time-Scales (15th, 30th and 45th Days)

Table-1. Effect of Different Dosage of Oregano EO +Thyme EO on Blood Profile of Broiler Chicks at 15th, 30th and 45th Days

Parameters	Duration	Concentration Used			
		Control	100+100 mg/kg of diet	200+200 mg/kg of diet	300+300 mg/kg of diet
Hb (g/dL)	15th Day	7.34±0.08^{a}	8.85±0.03^{b}	9.81±0.043^{d}	9.20±0.28^{c}
RBCs (x10^{12} /l)		2.53±0.01^{a}	2.77±0.03^{b}	2.93±0.01^{d}	2.84±0.02^{c}
HCT (%)		22.56±0.08^{a}	26.60±0.11^{b}	27.13±0.08^{d}	26.13±0.14^{c}
MCV (fL)		87.40±0.11^{a}	100.93±0.63^{b}	95.46±0.49^{d}	98.10±0.61^{c}
MCH (pg)		36.66±0.23^{a}	38.93±0.37^{b}	41.56±0.33^{d}	39.99±0.12^{c}
MCHC (g/dL)		26.26±0.88^{a}	28.26±0.08^{b}	30.03±0.18^{d}	29.16±0.14^{c}
WBCs (x10^{12} /l)		3.09±0.01^{a}	3.20±0.09^{b}	3.41±0.48^{c}	3.34±0.04^{c}
Platelets (x10^{12} /l)		118.00±1.15^{a}	129.66±0.88^{b}	136.33±1.45^{d}	134.00±1.15^{c}
Hb (g/dL)	30th Day	7.96±0.06^{a}	9.97±0.02^{b}	10.46±0.08^{d}	10.01±0.04^{c}
RBCs (x10^{12}/l)		2.59±0.01^{a}	2.81±0.01^{b}	2.99±0.01^{d}	2.88±0.01^{c}
HCT (%)		23.13±0.14^{a}	27.23±0.12^{b}	28.66±0.23^{d}	28.00±0.18^{c}
MCV (fL)		88.13±0.12^{a}	103.26±1.02^{b}	97.10±0.36^{d}	99.40±0.26^{c}
MCH (pg)		37.06±0.88^{a}	39.73±0.41^{b}	41.56±0.49^{d}	40.86±0.14^{c}
MCHC (g/dL)		26.90±0.11^{a}	29.46±0.12^{b}	31.10±0.57^{d}	29.96±0.17^{c}
WBCs		3.12±0.01^{a}	3.38±0.01^{b}	3.55±0.01^{c}	3.47±0.01^{c}

($x10^{12}$ /l)					
Platelets ($x10^{12}$ /l)		120.00±0.57^{a}	134.00±1.15^{b}	141.00±0.88^{d}	138.33±1.45^{c}
Hb (g/dL)	**45th Day**	8.79±0.07^{a}	10.38±0.05^{b}	11.20±0.15^{d}	10.74±0.12^{c}
RBCs ($x10^{12}$ /l)		2.61±0.08^{a}	2.90±0.08^{b}	3.14±0.02^{d}	3.00±0.08^{c}
HCT (%)		24.00±0.26^{a}	27.96±0.08^{b}	29.73±0.23^{d}	28.63±0.08^{c}
MCV (fL)		89.30±0.55^{a}	106.33±1.45^{b}	99.93±0.60^{d}	102.26±1.89^{c}
MCH (pg)		37.63±0.14^{a}	42.43±0.08^{b}	44.50±0.34^{d}	43.53±0.20^{c}
MCHC (g/dL)		27.56±0.14^{a}	30.23±0.20^{b}	31.56±0.08^{d}	30.76±0.08^{c}
WBCs ($x10^{12}$ /l)		3.17±0.01^{a}	3.48±0.01^{b}	3.63±0.01^{c}	3.56±0.01^{c}
Platelets ($x10^{12}$ /l)		122.33±0.88^{a}	137.33±1.76^{b}	145.00±1.52^{d}	140.66±0.88^{c}

Means with different subscripts in a row are significantly different from one another ($p \leq 0.05$) Tukey's Test

Discussion

The weight of the broiler was increased over the course of treatment and FCR was reduced significantly which indicated better performance. The highest weight gain and lowest FCR were recorded on the 45th day of the study. Possible mechanisms of phytobiotic essential oil in promoting growth performance of broilers may be due to better utilization of feed as compared to the control group [28] which resulted in better sedimentation of muscle proteins [29], stimulating effect on digestive enzyme, appetite [30] or *Lactobacillus* proliferation [31]. Hematological parameters including Hb, RBCs, HCT, MCV, MCH and MCHC and antibody titer against NDV and IBD was also significantly improved.

A study was conducted to evaluate the effect of the mixture of oregano, ziziphora and peppermint. Results showed that when used in combination of 1%, 0.5% and 0.5% improved the weight gain, FCR, carcass yield and decreased the abdominal fat disposition [32]. In another experiment, blend of EOs of oregano, thyme and garlic resulted in significant improvements in weight gain, relative growth rate and FCR and decreased oocyte count in broiler chicks. The study concluded that blend of EOs improved the overall performance of broiler chicks and can be used as an anticoccidial phytobiotic [33]. Likewise, a study reported that Oregano EO when added to diet at 300, 600 and 900 mg /kg positively influenced the growth performance, hemogram and leukogram at 300 mg/kg of oregano EO [34]. Another study [35] evaluated the effect of thyme oil at 1.0 g/kg, 1.5 g/kg and 2.0 g/kg on growth performance for 28 days when raised in a hot climate and reported that the group that received oil at 1.0 g/kg of feed showed better FCR as compared to other treatment groups. Similar results were for oregano EO in an experiment on broiler chicks and were divided into four groups. The four groups were given oregano EO at different doses i.e., 0.0, 100 ppm, 150 ppm and 200 ppm for 1-42 days of age. At the end of the experiment highest weight gain and lowest FCR was reported in the group that received 200 ppm of oregano oil [36]. Similar results were reported by [37-41] indicating that oregano EO and thyme EO when given individually improved the weight gain, FCR and blood profile as well.

Conclusions

Phytobiotic supplementation (Oregano EO + Thyme EO) showed a significant increase ($p<0.05$) in growth performance, blood profile and immunity of broiler chicks at T_2 dose. The addition of these oils stimulates digestive enzymes which resulted in better utilization of feed and promoted increased

body weight and decrease FCR accordingly, improving overall performance. Supplementation of EOs also positively affected the hematological parameters and enhanced the immunity of broiler chicks in all treated groups as compared to control groups. Since phytobiotic supplementation is effective in enhancing growth performance by increasing body weight, therefore, it should be supplemented in the diets of other commercial animals as well to improve their growth performance.

Author's contributions

Conceived and designed the experiments: MN Javed & R Iqbal, Performed the experiments: MN Javed, Analyzed the data: M Hussain, MF Malik & A Razaq, Contributed reagents/ materials/ analysis tools: MN Javed & R Iqbal, Wrote the paper: MN Javed & R Iqbal.

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