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The effect of supplementation of oregano oil and probiotic on blood biochemicals and immune response of broiler chicken

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Abstract
Objective: To evaluate the effect of oregano oil and probiotic on blood biochemicals and immune response of Broiler Chicken.
Methods: The blood biochemical parameter was estimated by autoanalyzer using standard kit of AGD, biochemicals (P), Ltd., and Mumbai, India. Serum samples were used for Haemagglutination Inhibition (HI) test as per procedure of O.I.E (1992) to detect the antibody titer against New Castle Disease.
Result: The Biochemical analysis showed that group T_3 oregano essential oil + probiotic in basal feed of broiler higher protein concentration in blood serum, lower cholesterol, BUN value was significantly dropped than control in the treatment group. The values for haemagglutination inhibition test were highest for treatment (T3 group).
Conclusion: From the result obtained, it was concluded that feeding of oregano essential oil and probiotic *Saccharomyces cerevisiae* exhibited beneficial effect on the growth performance of broiler chicken.

Keywords: Probiotics, oregano essential oil, blood biochemistry, haemagglutination inhibition, broiler

1. Introduction
The poultry industry demands maximum performance from broilers as well as layer**.** Now a days, essential oils (EOs) are used in poultry feed, as these have antimicrobial, antioxidant, antifungal, antiparasitic and antiviral properties. Beside this, other beneficial effects of EOs include appetite stimulation, improvement of enzyme secretion related to food digestion and immune response activation. Recently, use of EOs in broiler chickens has drawn attention due to their advantageous properties. Many kinds of natural substances, prebiotics and probiotics have been supplemented to broilers to increase poultry production by activating intestinal function (B. Shanmuga Priya and S. Saravana Babu, 2013) [20]. Sarica *et al*. (2009) [21], The use of essential oils in combination with the enzyme complex, a probiotic and a mannanoligosaccharide with or without the enzyme complex in the wheat based diet significantly reduced the intestinal viscosity compared to the control diet, these treatments negatively decreased plasma total cholesterol and triglyceride. Awaad *et al*. (2014) [2] The effect of a specific combination (SC) of carvacrol (active constitute of oregano essential oil), cinnamaldehyde on productive performance and immune response in broiler chickens. Edward *et al*. (2002) [7] the effect of dietary supplementation of oregano oil (Origanum sp.) medicinal plant on, not significant effects on blood biochemical parameters and immune system of broiler chickens. Manafi *et al*. (2016) [13] new multispecies probiotic containing four Bacillus species and Saccharomyces boulardii (Microguard®) with a commercial probiotic (Protexin®) and a commonly used antibiotic in broilers probiotic containing four Bacillus species and Saccharomyces boulardii (Microguard®) at 150 g/ton showed increase in blood serum concentration of high density lipoprotein, triglyceride in probiotic-supplemented broilers. The probiotics are used in poultry for "competitive/exclusion" of bacterial pathogens (Barrow P *et al*., 1992) [3]. Probiotics are living microorganisms which upon ingestion in adequate amounts confer health benefits to host (FAO/WHO 2002) [8].
Exactly how supplemental dietary microbial products function in the digestive system is not known, but some suggested mechanisms are that they 1) Provide nutrients, 2) Aid in digesting foods and 3) Inhibit harmful bacteria (Owings *et al.,* 1990). They create gut conditions that suppress harmful microorganisms and favor beneficial ones (Line *et al*., 1998; Mead *et al*., 2000) [12, 14]. The antibacterial properties of EOs are well documented (Tihonen *et al*., 2010) [22].
So the present study is aimed at investigating the weather essential oil and probiotic could improve growth performance, blood biochemicals and immune response alterations would be observed in these birds.

2. Material and method

2.1 Birds and housing

The present study was conducted to evaluate the effect of feeding oregano (*Origanum vulgare*) oil as phytobiotic growth promoter with probiotic on Intestinal Histomorphology of Broiler Chicken. Two hundred fourty straight run "Ven-Cobb 400Y" strain commercial day-old broiler chicks were equally and randomly distributed in to four groups. Each treatment was subjected to four replicates with fifteen chicks.

2.2 Management of Bird

Prior to experimental trial, the experimental broiler shed, its premises and the equipments were thoroughly cleaned and disinfected. Saw dust was provided as a bedding material for the birds. Immediately after arrival, all the chicks were provided glucose through drinking water. The desired brooding temperature was maintained using electric bulbs. All the groups were provided similar environmental and managemental conditions throughout the experimental period. The experimental chicks were offered feed and fresh water ad-libitum. The identical floor, feeding and watering space was allotted to the experimental birds during the entire experimental period. The experimental chicks were housed in 16 different pens. Each pen was accommodating 15 birds. The experimental birds were vaccinated against Ranikhet disease through intraoccular route on 7th day with B1 strain, Infectious bursal disease (IBD) on 14th day of age by intraocular route and booster vaccination of Infectious bursal disease (IBD) Invasive intermediate strain (B2K) was carried out on 21th day and vaccination of Ranikhet disease with lasota strain on 28th day through drinking water.

2.3 Procurement of Feed Ingredients

The good quality feed ingredients were procured from local market for preparation of experimental diets. Oregano essential oil was procured from karma essential oil pharmaceuticals. the probiotic (encapsulated *Saccharomyces cerevisiae*) was sponsored by Venkateshwara Pvt. Ltd were subjected to chemical analysis in the laboratory at Department of Animal Nutrition, PGIVAS, and Akola. The chemical analysis of Maize and Soybean was carried out as per AOAC, 2012 [1] (Table2). The diets were formulated for prestarter, starter and finisher chickens with standard BIS 2007(Table 1).

Table 1: BIS (2007) Standard for broilers

BIS (2007)			
	Prestarter	**Starter**	**Finisher**
CP (%)	23	22	20
ME (kcal/kg)	3000	3100	3200

Table 2: Chemical Composition of Feed ingredients (% DM basis)

Sr. No.	Particulars	Maize	Soya-DOC
1	Dry matter	91.07	92.1
2	Crude protein	9	44
3	Crude fibre	2.35	6.3
4	Ether Extract	3.58	1.5
5	Total ash	1.65	2.38
6	Nitrogen free extract	83.42	58.42

2.4 Dietary treatment

The experimental birds were subjected to the following dietary treatments.

Table 3: Details of the dietary treatment

Groups	Dietary Treatments No. of bird in replicate	No. of Replicate	No. of Birds
T_0	Standard broiler chicken diet as per BIS, 2007.	4	60
T_1	Standard broiler chicken diet as per BIS, 2007+ oregano essential oil @ 0.15 gm/kg diet	4	60
T_2	Standard broiler chicken diet as per BIS, 2007+probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/ tones)	4	60
T_3	Standard broiler chicken diet as per BIS, 2007+oregano essential oil @ 0.15 gm/kg diet+ probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/ tonnes T_1)	4	60
	Total birds	16	240

2.5 Biochemical Observations

Serum Albumin, Serum globulin, BUN (Blood Urea Nitrogen), Serum Cholesterol and Total Protein were estimated by auto analyzer using standard kit of AGD, biochemicals (P), Ltd., and Mumbai, India.

2.6 Immune parameter

The birds from the experimental trial were assessed for the antibody titer against the New Castle Disease (ND) 42nd days of age. Two birds from each replication and a total of eight birds from each treatment were randomly selected for the blood collection at the end of 42nd day of age. The blood samples were collected via wing vein from each bird. The serum was separated by centrifugation at 3000 RPM for 20 minutes and decanted into clean, sterile plastic vials and stored under deep freeze at –18 ºC to –20 ºC. These serum samples were used for Haemagglutination Inhibition (HI) test as per procedure of O.I.E (1992) [15] to detect the antibody titer against New Castle Disease.

2.7 Statistical Analysis

The results obtained from various parameters blood biochemistry and immune responses were expressed as means ±SE (standard error). The data was analyzed by using

Statistical Package for the Social Sciences (SPSS) Version 17.0. The differences between means were subjected to ANOVA by univariate analysis using General Linear Model. A 'P' value less than 5% was considered as statistically significant (P<0.05).

3. Result and Discussions

3.1 Blood Biochemical Observation

The results pertaining to total protein, Albumin, Globulin, BUN, and Serum cholesterol are presented in Table 4.

Table 4: Blood biochemical observations

Group	Total protein	Globulin	Albumin	BUN	Cholesterol
T0	3.83^{a}±0.05	1.84^{a}±0.06	1.99^{b}±0.03	0.9^{c}±0.03	180.75^{c}±1.33
T1	3.87^{a}±0.02	1.92^{ab}±0.06	1.9^{ab}±0.05	0.81^{bc}±0.04	160.75^{b}±3.71
T2	3.99^{b}±0.04	2.1^{bc}±0.06	1.9^{ab}±0.03	0.78^{b}±0.02	153.63^{a}±1.65
T3	4.09^{b}±0.05	2.26^{c}±0.08	1.83^{a}±0.05	0.59^{a}±0.03	147.25^{a}±1.83
Pooled Mean	3.95±0.03	2.03±0.04	1.92±0.02	0.77±0.02	160.59±2.51

Treatment mean end in a column bearing common superscripts doesn't differ significantly (P<0.05)

3.1.1 Total protein

The treatment group T3 and T2 fed with mixture of oregano oil with probiotic and probiotic alone respectively, differed significantly from T0 and T1 with numerically highest value in T3 (oregano essential oil and probiotic encapsulated Saccharomyces cerevisiae) followed by T2, T1 (Oregano essential oil), (Probiotic encapsulated Saccharomyces cerevisiae) and T0 control diet. The effect of oregano oil and probiotic supplementation on protein metabolism may be related to improvement of intestinal amino-acids absorption in acidic conditions that consequently enhances protein synthesis. The results obtained in the present study are in accordance with Gopi *et al.* (2013) [10] who studied the effects of broiler chicks fed ration contains 1.5% *S. cerevisiae* had significantly increased total protein. Paryad and Mahmoudi (2008) [17] reported that chicks fed 1.5% yeast had the higher (P<0.05) total plasma protein values.

3.1.2 Globulin (mg/dl)

The globulin levels for all treatment groups including control were found to be significant. The highest value for Globulin was observed in T_3 -2.26±0.08 group, where oregano essential oil and probiotic encapsulated *Saccharomyces cerevisiae* was supplemented. The lowest value was observed in the treatment group T_0–1.84±0.06 *ie*. control group. While each Treatment group is differed numerically as well as statistically from each other and T_3 was having highest numerical value followed by T_2, T_1, T_0 for the globuline. The present investigation was in agreement of Prayad *et al*., (2008) [17] showed significant (P<0.05) increase in the globulin concentration with inclusion of 1.5% of yeast,which helps in raise colloidal osmotic pressure in the blood and increase the humoral mediated immunity.

3.1.3 Albumin (mg/dl)

Table 4. Revealed that a difference among all treatments was found to be significant. Albumin was observed in T_3 was significantly differed than other treatment groups followed by T2, T1 showed higher significance than T0. While highest value for albumin was seen in the control group. These results of the present study are in accordance with Prayad *et al*. (2008) [17] who showed an increase in the albumin concentration with inclusion of 1.5% of yeast was significant (P<0.05).

3.1.4 BUN (mg/dl)

The differences among all treatments were significant. The lowest value of BUN was observed in T3 - 0.59±0.03 treatment group. While highest value was seen for the control group. The data showed each treatment differed from each other significantly. Whereas analysis of variance showing no significant results. In this experiment, the BUN level in the serum of broilers significantly decreased in the treatment groups, thus showing that diet fed in T_3 (oregano essential oil and probiotic encapsulated *Saccharomyces cerevisiae*) group and followed by T_2 (probiotic encapsulated *Saccharomyces cerevisiae*) and T_1 (oregano essential oil) improves protein synthesis in broilers, decreases protein decomposition speed and increases the efficiency of nitrogen use. This is probably due to the ability of essential oil and probiotic to promote the growth of animals, improve feed conversion and therefore increase absorption and utilization of feed proteins and increase protein deposits.

3.1.5 Cholesterol (mg/dl)

The serum cholesterol value was found significant among all treatment groups. Highest numerical value of serum cholesterol was observed in control whereas lowest value of serum cholesterol was in group T3. However, the differences in serum cholesterol from groups T1, T2 and T3 were statistically significant with each other. The present investigation was in agreement of Yalcin *et al*., (2013) [23] who reported that yeast autolysate supplementation in laying reduced the levels of serum cholesterol and triglyceride significantly, Prayad *et al*., (2008) [17] showed that the feeding oregano essential oil and cinnamon essential oil significantly reduced the plasma total cholesterol and triglyceride levels compared to the control diet. While similar results were also seen in the study conducted the Sarica *et al.* (2009) [21], Pietras (2001) [18], Cenesz *et al.* (2008) [5] and Chitra *et al.* (2004) [6] who reported that supplementation of probiotics showed highly significant (P≤0.01) reduction of serum cholesterol level. Saad *et al*. (2014) [19] evaluated the blood biochemistry of chicken with probiotic mannan oligosaccharide in its diet; Study showed that there was significant decrease in serum cholesterol in blood.

3.2 Immune parameter

HI titres indicated non-significant differences in different groups; highest titer was seen for the T_3. (Table 5) The findings of the present study are in accordance with Manafi *et al*. (2016) [13], Gao *et al.* (2008) [9] and Haldar *et al.* (2011) [11] who observed the higher antibody titer in broilers supplemented with yeast autolysate.

Table 5: Haemagglutination inhibition titer for NDV at 42 days of age

Treatment	HI Titer
T0	4.33^{a}±0.45
T1	4.58^{a}±0.54
T2	4.75^{a}±0.48
T3	5.04^{a}±0.47
Pooled Mean	4.68±0.24

Treatment mean end in a column bearing common superscripts doesn't differ significantly (P<0.05)

Table 6: ANOVA for Haemagglutination inhibition titer for NDV at 42 days of age

Source	Df	HI Titer		
		MSS	'F' value	Sig.
Treatment	3	.707	.373	.773
Error	28	1.896	-	-
Total	31	-	-	-

NS – Non-significant

* – Significant at 5% level (P<0.05

4. Conclusion

From the results obtained, it is concluded that the performance in commercial broiler chickens fed with Oregano (*Origanum Vulgare*) Oil As Phytobiotic Growth Promoter With Probiotic (encapsulated *Saccharomyces cerevisae*) In Broiler Chicken showed a positive impact on growth, blood biochemical, immune parameter and improves protein synthesis in broilers, decreases protein decomposition speed and increases the efficiency of nitrogen use in broiler chickens without affecting the health of broiler chickens.

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