Original Research

The Effect of Supplementation of Oregano Oil and Probiotic on Intestinal Microbes (Namely *E. coli spp.*, *Salmonella spp.*, *Clostridia spp.*) of the Broiler Chicken

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Abstract

To evaluate the effect of oregano oil and probiotic on total microbial count of intestine namely E. coli, Salmonella and Clostridia. On 42nd day, 8 birds from each treatment were slaughtered by decapitation. The digestive tracts were dissected and described Caecal content samples were taken aseptically and were transferred into sterile plastic bags and immediately transported in cold chain to the laboratory, samples diluted, cultured and checked for the presence of organisms in samples (E. coli, Salmonella, Clostridia) and compared with the control after incubation. Results were expressed as log10 colony-forming units per gram of ileal digesta (log10 CFU/gram). The intestinal microbial count namely E. coli, Salmonella, Clostridia were significantly decreased in T_3 than in groups T_2, T_1 and control. It was concluded that feeding of Oregano essential oil and probiotic- Saccharomyces cerevisiae exhibited beneficial effect on GIT and decreased the harmful microbial count of the intestine in the broiler chicken.

Key words: Broiler, Intestinal Microbes, Oregano Essential Oil, Probiotic

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Introduction

The poultry industry demands maximum performance from broilers as well as layer**.** Now a days, essential oils (EOs) are used in poultry feed, 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

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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). Awaad *et al*. (2014) 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) 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) the commercial probiotic multispecies probiotic having four *Bacillus species* and *Saccharomyces boulardii* (Microguard®) with a (Protexin®) and a commonly used antibiotic in broilers probiotic having 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). Probiotics are living microorganisms which upon ingestion in adequate amounts confer health benefits to host (FAO/WHO 2002). 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). It creates gut conditions that suppress harmful microorganisms and favor beneficial ones (Line *et al.*, 1998; Mead *et al.*, 2000). The antibacterial properties of EOs are well documented (Tihonen *et al*., 2010). The supplementation of curd as a probiotics showed a positive effect on blood biochemical and caecal *Lactobacilli* and *E. coli* count in broiler chicken. (Chaudhary *et al.*, 2017), So, the present study is aimed at investigating whether oregano oil and probiotic could decrease in microbial count namely *E. coli spp., Salmonella spp., Clostridia spp.* of intestine of birds or not.

Materials and Method

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 forty straight straight run "Ven-Cobb 400Y" strain commercial day-old broiler chicks were equally and randomly distributed into four groups. Each treatment was subjected to four replicates with fifteen chicks.

Management of Bird

Prior to experimental trial, the experimental broiler shed, premises and the equipments were thoroughly cleaned and disinfected. Saw dust was provided as a bedding material for the chicks. Immediately after arrival, all the chicks were provided glucose through drinking water. The desired brooding temperature was maintained using incandescent bulbs. All the groups were provided similar environmental and management

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conditions throughout the experimental period. The experimental chicks were offered ad-labium feed and fresh water. The standard floor, feeding and watering space was provided throughout the experimental period depending on their age group. 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) vaccine on 14th day of age by intraocular route and booster vaccination of Infectious Bursal disease (IBD) was done on 21st day and vaccination of Ranikhet disease with lasota strain on 28th day through drinking water.

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 chemical analysis of Maize and Soybean was carried out as per AOAC, 2012 (Table 2). The diets were formulated for prestarter, starter and finisher chickens by following BIS 2007 standards (Table 1).

Table 1: BIS (2007) Standard for broilers

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

Table 2: Chemical composition of feed ingredients (% DM basis)

S. 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

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.

Dietary Treatment

The experimental birds were subjected to the following dietary treatments.

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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

Total Microbial Count (*E. coli spp., Salmonella spp., Clostridia spp.*)

Caecal content of the specimens were taken aseptically and were transferred into sterile plastic bags and immediately transported in cold chain to the laboratory. One gram of each sample was diluted 1:9 (wt./vol) in sterile saline. All samples were subjected to 10 sequential dilutions 1:9 (vol/vol), and 0.1 ml of each sample was plated as duplicates by using spread plate method for *E coli*-EMB agar, *Salmonella-shegella* agar *and Clostridium*-nutrient agar. The samples were incubated for 22 ± 2 h at 37ºC. Incubation procedure was conducted under aerobic (*E. coli* and *Salmonella*) and anaerobic (*Clostridium*) condition by using incubator. After incubation, typical colonies were counted. The results were expressed as log10 colony-forming units per g of ileal digesta (log10 CFU/g). Results for each bacterium (*E. coli, Salmonella and Clostridia spp*.) were also checked about its presence.

Statistical Analysis

The results obtained from various parameters *i.e.* 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 ($P<0.05$).

Results and Discussion

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Total Microbial Count (*E. coli spp.*)

It was observed from the Table 4 that, data pertaining to values of *E coli* count between the treatment groups were found to be non-significant. The value of *E. coli* count (10^7 CFU/g) for each treatment was found to be 6.34^a±0.3, 5.66±0.84, 5.2±0.79 and 4.35±1 in T_0, T_1, T_2 and T_3 respectively with mean value of 5.38±0.39. It was observed that Treatment group T_3 had numerically lower value than other groups. Whereas, differences among the treatments T_3, T_2, T_1 and T_0 found to be non-significant. In contrast to the present results Mathlouthi *et al*. (2015) in vitro antimicrobial activities of 3 essential oils- oregano, rosemary and a commercial blend of essential oils (BEO) against pathogenic bacteria *Escherichia coli*, reported significant decrease in the bacterial concentration in the treatment. Total bacterial counts (coliforms particularly) in caecal contents were decreased for birds fed with blend of plant extracts containing oregano, fenugreek, chamomile and fennel decreased Attia *et al*. (2017). Du *et al*. (2015), Sarica *et al*. (2009), Giannenas *et al*. (2016), Manafi *et al*. (2018) also reported similar results.

Total Microbial Count (*Salmonella spp)*

It was observed from the Table 4 that, there was significant differences for *Salmonella* count between the treatment groups. The value of *Salmonella* count for each treatment was found to be 4.6±0.67, 5.09±0.17, 4.13±0.62 and 2.76±0.61 in T_0, T_1, T_2 and T_3 respectively with mean value of 4.14±0.31 10^7 CFU/gm. There were significant differences for *Salmonella* count between the treatment groups (Table 4). Treatment group T_3 differ significantly followed by T_2 than T_0 and T_1 while treatment groups $T_1$and T_2 differed non-significantly. The lowest value was recorded in T_3 followed by T_2, T_1 and T_0 treatment group respectively. The result of the present study was in accordance with Mathlouthi *et al*. (2015) who fed oregano and rosemary essential oil in broiler and observed and reported decreased salmonella Indiana population in intestine of birds in the treatment groups. Manafi *et al*. (2018) also found the similar results.

Total Microbial Count (*Clostridia spp*)

It was observed from the Table 4 that, the values of *Clostridia* count for each treatment were 2.27±0.02, 2.05±0.06, 1.81±0.27 and 1.37±0.34 in T_0, T_1, T_2 and T_3 respectively 10^7 CFU/gm of sample. There were significant differences for *Clostridia* count between the treatment groups (Table 4). Treatment group T_3 differs significantly followed by T_2 than T_0, T_1 while treatment groups T_1 and T_2 differed non-significantly. The lowest value was recorded in T_3 followed by T_2, T_1 and T_0 treatment group respectively.

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Table 4: Gut microbes of different dietary treatment

Treatment	*E. coli* count (10^7 CFU/gm)	*Salmonella* (10^7 CFU/gm)	*Clostridia* (10^7 CFU/gm)
T0	6.34^{a}±0.3	4.6^{b}±0.67	2.27^{b}±0.02
T1	5.66^{a}±0.84	5.09^{b}±0.17	2.05^{b}±0.06
T2	5.2^{a}±0.79	4.13^{ab}±0.62	1.81^{ab}±0.27
T3	4.35^{a}±1	2.76^{a}±0.61	1.37^{a}±0.34
Mean	**5.38±0.39**	**4.14±0.31**	**1.87±0.12**

Treatments in column bearing common superscripts doesn't differ significantly (P<0.05)

The results of the present study are in agreement with Du *et al*. (2015) who showed significantly decrease in the *clostridial* concentration when fed with the active ingredient of oregano oil in the broiler chicken diet.

Conclusion

The results obtained from this study was concluded that the performance of commercial broiler chickens fed with oregano (*Origanum vulgare*) oil as phytobiotic growth promoter with probiotic (encapsulated *Saccharomyces cerevisae*), has showed a positive impact on GIT and decreased the harmful microbial count of *E. coli, Salmonella* and *Clostridia spp* on GIT.

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