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Effect of feeding of oregano oil with probiotic on gut microbiota and nutrients digestibility of broiler chicken

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Abstract
To evaluate the effect of probiotics and essential oils on gut activities and metabolic studies of broiler, this experiment was carried out on day-old broiler chicks of Ven-Cobb 400Y strain (n=240) for 42 days. These broiler chicks were randomly distributed into 4 equal treatment groups with T_0 (Control), T_1, T_2 and T_3 with 60 birds in each group having 4 replicates of 15 birds each group. The treatments were T_0 (Control, Standard broiler chicken diet as per BIS, 2007), T_1 (Standard broiler chicken diet as per BIS, 2007 with oregano essential oil @ 0.15 gm/kg diet), T_2 (Standard broiler chicken diet as per BIS, 2007 with probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/ tonnes) and T_3 (Standard broiler chicken diet as per BIS, 2007 with oregano essential oil @ 0.15 gm/kg diet and probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/ tonnes). In the gut *salmonella* and *clostridia* count, ileal pH, dry matter digestibility and nitrogen retention showed significant (P<0.05) difference while *E Coli*. count, intestinal length and weight do not show the significant (P>0.05) difference between the groups. So supplementation of probiotics and essential oils modulate the activity of gut, increase the dry matter digestibility and nitrogen retention of broiler chicken.

Keywords: Broiler, probiotics, essential oil, gut, metabolic study

1. Introduction
India is the third-largest egg producer and the fourth-largest chicken producer in the world. In India, ICMR recommends a minimum of 180 eggs and 10 kg chicken per annum for a healthy adult human, which suggests that the Indian poultry market is laden with opportunities. Now a day's Essential oils (EOs) are used in poultry feed in the carrier oil as these have antimicrobial, antioxidant, antifungal, antiparasitic and antiviral properties. Besides this, other beneficial effects of EOs include improvement of enzyme secretion, appetite stimulation related to food digestion and immune response activation. The EOs are classified as "Generally Recognized as Safe (GRAS)" as endorsed by the Flavor and Extract Manufacturers Association (FEMA) and the Food and Drug Administration (FDA) from the USA and they are widely used in the food industry. Their antimicrobial mode of action consists of interactions with cell membranes that change the permeability for cations such as H+ and K+ (Ouwehand *et al.*, 2010) [20] but the antimicrobial activity of EOs cannot be attributed to one specific mechanism but rather to the interaction between the EOs chemical structure and a variety of targets in the bacterial cell. Therefore, the oregano essential oil can be used as an alternative to growth promoters in the animal diet with the replacement of antibiotics. Probiotic is the Greek word for "for life" (Gibson and Fuller, 2000) [11] and can be defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989) [9]. The probiotics are used in poultry for "competitive/exclusion" of bacterial pathogens (Barrow P *et al.*, 1992) [4]. The positive effects of probiotics on animals can result either from a direct trophic effect of the probiotic or a health effect, with probiotics acting as biological-regulators of the intestinal microflora and enhancing the host's natural attitude of defences. Supplementing broilers with microbial cultures provides beneficial bacteria to aid in nutrient absorption and enhance the microbial balance in the avian digestive tract. They create gut conditions that suppress harmful microorganisms and favour beneficial ones (Mead *et al.*, 2000) [18]. They have been shown to maintain health by reducing risk diseases, possibly through a reduction in proliferation of pathogenic species, maintaining microbiota balance in the gut boosting immune function by competitive exclusion (Kabir *et al.*, 2004) [15] and increasing resistance to infection (Rekiel *et al.*, 2007) [21].

2. Materials and Methods

2.1 Experimental birds, feeding and management

The experiment was carried out on day-old broiler chicks of Ven-Cobb 400Y strain (n=240) for 42 days. The experiment was conducted on a day old broiler chicks procured from Shree Krupa Poultry Hatcheries, Amravati Pvt. Ltd. Amravati. The experimental broilers chicks were allotted to 4 treatments with T_0 (Control), T_1, T_2 and T_3 with 60 birds in each group having 4 replicates of 15 birds each. Based on the chemical analysis (AOAC, 2012) [2] the diets were formulated for pre starter, starter and finisher chickens with standard BIS, 2007 [5]. Before the arrival of broiler chicks, the experimental pens, equipment was cleaned, disinfected and fumigated by using formaldehyde and potassium permanganate. The experimental birds were reared on deep litter system with sawdust as litter material. The *ad-lib* feeding and ample clean drinking water were made available during the experiment. The experimental broiler chicks were vaccinated for 'Ranikhet' disease and for 'Infectious Bursal Disease' as per standard vaccination protocol. The details of the feeding and supplementation ingredients of different treatment groups are given in table 1.

Table 1: Details of the Different dietary treatment in broiler chicken

Groups	Dietary Treatments	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

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

Table 3: BIS (2007) Standard for broilers

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

2.2 Estimation of Gut Parameters

On day 42, 2 broilers from each main group (8 birds per treatment) were randomly selected and slaughtered to determine each of intestinal weight, ileum pH, intestinal length, and microbial count. The carcasses of broilers were subsequently opened and the entire gastrointestinal tract was removed aseptically. Gut weight is determined directly after aseptic removal of intestine on digital weighing balance. To determine the pH, 10 gm of intestinal content from ileum were collected aseptically in 90 ml sterilized physiological saline (1:10 dilution) (Al-Natour and Alshawabkeh, 2005) [1] and pH was measured by using digital pH meter. The gut length was measured directly with the help of measuring tape. Caecal content of the specimens was taken aseptically and was transferred into sterile plastic bags and immediately transported in the 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-shigella* agar *and Clostridium*-nutrient agar. The samples were incubated for 22 ± 2 h at 37 ºC. Incubation procedure was conducted under aerobic (*E Coli* and *Salmonella*) and anaerobic (*Clostridium*) condition in the incubator. After incubation, typical colonies were counted.

2.3 Metabolic study

To observe nitrogen retention metabolic trial was carried out for five consecutive days at the end of 6th week of age. In this trial, eight birds from each group (two birds from each replicate) representing the average body weight of the group were randomly chosen. During the metabolic trial, the droppings from individual birds from different groups were collected daily on 24 hours interval. The excreta was made free of feathers and extraneous and then weighed. The 1/10threpresentative samples from each group were drawn and stored in a labelled wide mouth glass bottle containing 10% used for nitrogen analysis. However, 10 g dried faecal sample was stored in polythene bags use further for the nitrogen analysis as per (AOAC, 2012) [2].

2.4 Statistical Analysis

The data were 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 the General Linear Model.

3. Results and Discussions

The average value of Gut pH, Gut weight, Gut length and total bacterial count namely *E. coli*, *Salmonella*, *Clostridia* were determined at the end of the experiment after sacrificing eight birds from each treatment (two birds from each replicate) were statistically analyzed and the results are tabulated in Table 4.

Table 4: Gut parameters of different dietary treatment

Treatment	Intestinal weight (gm)	Intestinal length (cm)	pH	E.Coli count (10^7 CFU/gm)	Salmonella (10^7 CFU/gm)	Clostridia (10^7 CFU/gm)
T0	69.88^{a}±1.06	174.63^{a}±2.9	5.83^{b}±0.09	6.34^{a}±0.3	4.6^{b}±0.67	2.27^{b}±0.02
T1	73.88^{a}±3.92	176.63^{a}±3.49	5.86^{b}±0.14	5.66^{a}±0.84	5.09^{b}±0.17	2.05^{b}±0.06
T2	74.75^{a}±2.3	177.63^{a}±5.79	5.56^{a}±0.05	5.2^{a}±0.79	4.13^{ab}±0.62	1.81^{ab}±0.27

T3	76[a]±3.38	179.88[a]±3.56	5.55[a]±0.04	4.35[a]±1	2.76[a]±0.61	1.37[a]±0.34
Mean	73.63±1.43	177.19±1.97	5.7±0.05	5.38±0.39	4.14±0.31	1.87±0.12

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

3.1 Ileal pH
The ileal pH shows significant ($P<0.05$; Table 4) difference between the groups. The lowest pH was recorded in the treatment T_3 followed by T_2, T_1 and T_0 treatment groups. It was observed that the ileal pH of all treatment group was found to lower as compared to control. The significant ($P<0.05$; Table 4) pH values were observed among the treatments while T3 and T2 were significantly different ($P<0.05$; Table 4) as compared to T1 and T0. This results obtained in the present study are in agreement with Sarica *et al*. (2009) [22] who reported that the oregano essential oil (1 g Origanum onites L. /kg) reduced the pH of the cecal contents significantly ($P<0.05$) and Yalçın *et al*.(2013) [23] observed pH of jejunal and ileal digesta was decreased at the 2, 3, and 4 g/kg with supplementation of yeast autolysate (*saccharomyces cerevisiae*) compared with that of birds fed the control diet group.

3.2 Intestinal Length
The intestinal length between the treatment groups was found to be non-significant. ($P>0.05$; Table 4).The highest value was recorded in the treatment group T_3 (Diet containing mixtures of essential oil with probiotic) and the lowest value was observed in the T_0 control group. A similar result was found by Çabuk *et al*. (2006) [6] who examined the effects of a herbal essential oil mixture on the gut traits of broilers produced by a young (30 wks) or an old breeder (80 wks) flock. Length of intestine was not affected by the addition of the essential oil mixture to the diet. No significant results were seen for intestinal length. In Contrast to the present results by Manafi *et al.* (2018) [19] who observed increased villus height, and villus highest crypt depth ratio, and intestinal length in groups fed with 100 or 150 g/ton of Saccharomyces boulardii. Mehmet *et al*. (2012) [16] investigated the effect of chicken diet, supplementation with an essential oil mixture laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seeds oil, and citrus peel oil and reported a significant increase in intestinal length.

3.3 Intestinal Weight
The Intestinal Weight values were non-significant ($P>0.05$; Table 4) between the treatment groups. It was observed from the table that the highest intestinal weight was in the T_3 group whereas lowest intestinal weight was in control. By the present results, Mehmet *et al*. (2012) [16] reported non-significant values for the intestinal weight when the fed blend of essential oil mixture six different essential oils, i.e., oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seeds oil and citrus peel oil to broilers, Çabuk *et al*. (2006) [6] examined the effects of a herbal essential oil mixture on the gut traits of broilers produced by a young (30 wk) or an old breeder (80 wk) flock weight of intestine was not affected by the addition of the essential oil mixture to the diet. Hernandez *et al*. (2004) [12] reported non-significant results in terms of intestinal weight when fed essential oil extract from oregano, cinnamon, and pepper and *Labiatae* extracts from sage, thyme, and rosemary. While in contrast to the present results Jamroz *et al.* (2003) [14] and Çabuk *et al. (*2006) [6] found a significant increase in intestinal weight in broiler chickens when fed diets with different essential oils.

3.4 Total Microbial Count (*E.coli*)
It was observed that Treatment group T_3 had a 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 ($P>0.05$; Table 4). In contrast to the present results Mathlouthi *et al*. (2015) [17] *in vitro* antimicrobial activities of 3 essential oils [oregano, rosemary and a commercial blend of essential oils (BEO) against pathogenic bacteria *Escherichia coli*, reported a significant decrease in the bacterial concentration in the treatment. Total bacterial counts (coliforms particularly) in caecal contents were decreased for birds fed with a blend of plant extracts containing oregano, fenugreek, chamomile and fennel decreased Attia *et al*. (2017) [3]. Du E *et al.* (2015) [8], Sarica *et al.* (2009) [22], Ilias *et al*. (2016) [13], Manafi *et al*. (2018) [19] also reported similar results.

3.5 Total Microbial Count (*Salmonella*)
Total Microbial Count (*Salmonella*) values were found significantly ($P<0.05$; Table 4) different between the treatment groups. Treatment group T_3 differ significantly ($P<0.05$; Table 4) followed by T_2 than T_0 and T_1 while treatment groups $T_1$and T_2 differed non-significantly ($P>0.05$; Table 4). The lowest value was recorded in T_3 followed by T_2, T_1 and T_0 treatment group respectively. The results of the present study were by Mathlouthi *et al*. (2015) [17] who fed oregano and rosemary essential oil in broiler and observed and reported decreased salmonella Indiana population in the intestine of birds in the treatment groups. Manafi *et al*. (2018) [19] also found similar results.

3.6 Total Microbial Count (*Clostridia*)
Total Microbial Count (*Clostridia*) values were found significantly ($P<0.05$; Table 4) different between the treatment groups. Treatment group T_3 differ significantly ($P<0.05$; Table 4) followed by T_2 than T_0, T_1 while treatment groups T_1 and T_2 differed non-significantly ($P>0.05$; Table 4). The lowest value was recorded in T_3 followed by T_2, T_1 and T_0 treatment group respectively. The results of the present study are in agreement with Du E *et al*. (2015) [8] who showed a significant decrease in the *clostridial* concentration when fed with the active ingredient of oregano oil in the broiler chicken diet.

3.7 Metabolic Trial
The metabolic trial of 5 days duration was conducted on 8 birds from each treatment at the end of the experimental trial to study the dry matter digestibility, N- retention. The data collected was analyzed and presented in Table 5.

Table 5: Apparent nutrient metabolizability retention (% DM) as influenced by dietary treatments

Treatment	DM digestibility	Nitrogen retention
T0	60[a]±0.77	67.3[a]±0.52
T1	60.97[a]±1.33	69.05[a]±0.82
T2	63.58[b]±0.4	73.04[b]±1.37
T3	63.6[b]±0.67	74.01[b]±0.68
Total	62.04±0.5	70.85±0.66

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

3.7.1 Dry matter digestibility and nitrogen Retention

From Table.5, it was revealed that the T_3 group fed on a diet containing oregano essential oil and probiotic encapsulated *Saccharomyces cerevisiae* (200gm/tones) showed the numerically high value for dry matter metabolizability. Dry Matter Digestibility of T_2 and T_3 showed statistical significance ($P<0.05$; Table 5) with higher numerical values than T_1 and T_0 in all treatment group. The Nitrogen Retention (%) was statistically significant ($P<0.05$; Table 5) among different treatment groups. The highest nitrogen retention was found in T_3- 74.01±0.68 treatment group fed oregano oil with probiotic encapsulated *Saccharomyces cerevisiae* (200gm/ tones) followed by treatment group T_2 diet containing probiotic encapsulated *Saccharomyces cerevisiae* (200 gm/tones). These results are corroborating with Attia *et al.* (2017) [3] who observed optimum improvement in the N-retention in broiler chickens fed a blend of plant extracts containing oregano, fenugreek, chamomile and fennel at different dietary 100 ppm and 200 ppm levels. The apparent total tract digestibility of dry matter, crude protein, and ether extract were increased due to the inclusion of the plant extract blend or antibiotic oxytetracycline (OTC) leading to improvement in N- retention. The improvement in feed efficiency achieved with essential oil mixtures could be attributed to their positive effects on nutrient digestibility, as reported by Jamroz *et al.* (2003) [14] also noticed improved feed efficiency because of positive effects on the nutrient digestibility due to the favourable intestinal environment. The present results were also in agreement with Hernandez *et al.* (2004) [12] who studied the effect of 200 ppm essential oil extract from oregano, cinnamon, and pepper; and 5,000 ppm Labiatae extract from sage, thyme, and rosemary, fed with the standard diet and found that plant extract supplementation improved apparent whole-tract and, digestibility of the nutrients and dry matter digestibility.

4. Conclusions

Based on the result obtained in the present study it could be concluded that the performance in commercial broiler chickens fed with Oregano (*Origanum Vulgare*) Oil as Phytobiotic Growth Promoter with Probiotic (encapsulated *Saccharomyces cerevisiae*) showed a positive impact on gut microbiota, dry matter digestibility and nitrogen retention of broiler chicken.

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