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 400 Y strain (n=240) for 42 days. These broiler chicks were randomly distributed into 4 equal treatment groups with T0 (Control), T1, T2 and T3 with 60 birds in each group having 4 replicates of 15 birds each group. The treatments were T0 (Control, Standard broiler chicken diet as per BIS, 2007), T1 (Standard broiler chicken diet as per BIS, 2007 with oregano essential oil @ 0.15 gm/kg diet), T2 (Standard broiler chicken diet as per BIS, 2007 with probiotic (encapsulated Saccharomyces cerevisiae) @ 200 gm/ tonnes) and T3 (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+ (Ouwewand et al., 2010) 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) and can be defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). The probiotics are used in poultry for “competitive/exclusion” of bacterial pathogens (Barrow P et al., 1992). 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). 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 and increasing resistance to infection (Rekiel et al., 2007).
2. Materials and Methods

2.1 Experimental birds, feeding and management

The experiment was conducted 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 T0 (Control), T1, T2 and T3 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 [3]. 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 |
|--------|-------------------|----------------|-------------|
| T0     | Standard broiler chicken diet as per BIS, 2007. | 4             | 60          |
| T1     | Standard broiler chicken diet as per BIS, 2007+ oregano essential oil @ 0.15 gm/kg diet | 4             | 60          |
| T2     | Standard broiler chicken diet as per BIS, 2007 + probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/tones | 4             | 60          |
| T3     | Standard broiler chicken diet as per BIS, 2007 + oregano essential oil @ 0.15 gm/kg diet + probiotic (encapsulated *Saccharomyces cerevisiae*) @ 200 gm/tones T1) | 4             | 60          |
| Total birds |                  | 16            | 240         |

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 °C. Incubation procedure was conducted under aerobic (*E. coli* and *Salmonella*) and anaerobic (*Clostridium*) condition in the incubator. After incubation, typical colonies were counted.

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

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

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 (10⁷ CFU/gm) | Salmonella (10⁷ CFU/gm) | Clostridia (10⁷ CFU/gm) |
|-----------|------------------------|------------------------|----|--------------------------|-----------------------|------------------------|
| T0        | 69.88±1.06             | 174.63±2.9             | 5.83±0.09 | 6.34±0.3 | 4.06±0.67 | 2.27±0.02 |
| T1        | 73.88±3.92             | 176.63±3.49            | 5.86±0.14 | 5.66±0.84 | 5.09±0.17 | 2.05±0.06 |
| T2        | 74.75±2.3              | 177.63±5.79            | 5.56±0.05 | 5.2±0.79  | 4.13±0.62 | 1.81±0.27 |
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 T3 followed by T2, T1 and T0 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 T3 (Diet containing mixtures of essential oil with probiotic) and the lowest value was observed in the T0 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 T3 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 T3 had a numerically lower value than other groups whereas differences among the treatments T3, T2, T1 and T0 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 T3 differ significantly (P<0.05; Table 4) followed by T2 than T0 and T1 while treatment groups T1 and T2 differed non-significantly (P>0.05; Table 4). The lowest value was recorded in T3 followed by T2, T1 and T0 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 T3 differ significantly (P<0.05; Table 4) followed by T2 than T0, T1 while treatment groups T1 and T2 differed non-significantly (P>0.05; Table 4). The lowest value was recorded in T3 followed by T2, T1 and T0 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        | 60.4±0.77        | 67.3±0.52          |
| T1        | 60.97±1.33       | 69.05±0.82         |
| T2        | 63.58±0.4        | 73.04±1.37         |
| T3        | 63.6±0.67        | 74.01±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 T3 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 T3 and T1 showed statistical significance (P<0.05; Table 5) with higher numerical values than T1 and T2 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 T3 (~74.01±0.68 treatment group fed oregano oil with probiotic encapsulated Saccharomyces cerevisiae (200gm/tones) followed by treatment group T2 diet containing probiotic encapsulated Saccharomyces cerevisiae (200gm/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.

5. References

1. Al-Notour MQ, Alshawabkeh KM. Using varying levels of formic acid to limit growth of Salmonella gallinarum in contaminated broiler feed. Asian-Aust. J Anim. Sci. 2005; 18:390-395.
2. AOAC. Association of Official Analytical Chemist, official Method of Analysis of AOAC international, 19th Edn. Washington D.C., U.S.A, 2012.
3. Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M, Hernandez-Santana A. Effect of Dietary Inclusion of a Plant Extract Blend on Broiler Growth Performance, Nutrient Digestibility, Caecal Microflora and Intestinal Histomorphology. J Poult. Sci. 2017; 16(9):344-353.
4. Barrow PA. Probiotics for chicken (In Probiotics: The Scientific basis, ed. by R. Fuller). Chapman and Hall, London, 1992, 225-257.
5. BIS. Nutrient Requirements of Poultry. Bureau of Indian Standards, 5th revision, New Delhi, India, 2007.
6. Çabuk M, Bozkurt, Alicantek A, Akbaş Y, Küçükylmaz K, Effect of a herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks S. Afr. J Anim. Sci. 2006; 36(2).
7. Du E. Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with Clostridium perfringens. J. Anim. Sci. Biotechnol. 2016; 7:19-29.
8. Du E, Liping G, Zhiu L, Wang W, Liu D, Gu Y. In vitro antibacterial activity of thymol and carvacrol and their effects on broiler chickens challenged with Clostridium perfringens Du et al. J Anim. Sci. and Biotech. 2015; 6:58.
9. Fuller R. Probiotics in man and animals. J Appl. Bacteriol. 1989; 66:365-378.
10. Galal AAAE, El-Araby JE, Hassanin O, El-Said Omar A. Positive impact of oregano essential oil on growth performance, humoral immune responses and chicken interferon alpha signalling pathway in broilers. Adv. Anim. Vet. Sci. 2016; 4:57-65.
11. Gibson GR, Fuller R. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J Nutr. 2000; 130:391S-395S.
12. Hernandez, Madrid J, Garcì a’ Va, Orengo J, Megi ’As MD. Influence of two plant extracts on broilers performance, digestibility and digestible organ size Poult. Sci. 2004; 83:169-174.
13. Ilias G, Tzora A, Sarakatsianos I, Karamoutsios A, Skoufos S, Papaioannou N et al. The effectiveness of the use of oregano and laurel essential oils in chicken feeding. Ann. Anim. Sci. 2016; 16(3):779-796.
14. Jamroz D, Orda J, Kameł C, Wiliczkiewicz A, Wertelecki T, Skorupinska J. The influence of phytogetic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. Int. Jamroz d. et al. J Anim. and Feed Sci. 2003; 12:583-596.
15. Kabir SML, Rahman MM, Rahman MB, Rahman MM, Ahmed SU. The dynamics of probiotics on growth performance and immune response in broilers. Int. J Poult. Sci. 2004; 3:361-364.
16. Mehmet B, Kucukylmaz K, Caltia U, Ozylidiz Z, Cinar M, Cabuk M et al. Influences of an essential oil mixture supplementation to corn versus wheat-based practical diets on growth, organ size, intestinal morphology and immune response of male and female broilers., Ital. J Anim. Sci. 2012; 11(3):e54.
17. Mathlouthi N, Bouzaieehre T, Oueslati I, Reccoquifl Y, Hamdi M, Urdaci M et al. Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: In vitro antimicrobial activities and effects on growth performance. J Anim. Sci. 2012; 90:813-823.
18. Mead GC. Prospects for competitive exclusion treatment to control salmonellas and other foodborne pathogens in poult. Vet. J. 2000; 159:111-123.
19. Manafi M, Hedayati M, Mirzaie S. Probiotic Bacillus species and Saccharomyces boulardi improve performance, gut histology and immunity in broiler in chickens. South African Journal of Animal Science. 2018; 48(2).
20. Ouwehand AC, Tiitonen K, Kettunen H, Peuranen S, Schulze H, Rautonen N. In vitro effects of essential oils on potential pathogens and beneficial members of the normal microbiota. Veterinarni Medicina. 2010; 55:71-
21. Rekiel A, Wieczek J, Bielecki W, Gajewska J, Cichowicz M, Kulisiewicz J et al. Effect of addition of feed antibiotic flavomycin, or prebiotic BIO-MOS on production results of fatteners, blood biochemical parameters, morphometric indices of intestine and composition of microflora. Arch. Tierz. 2007; 50:172-80.

22. Sarica S, Corduk M, Yarim GF, Yenisehirli G, Karatas U. Effects of novel feed additives in wheat based diets on performance, carcass and intestinal tract characteristics of quail South Afr. J. of Anim. Sci. 2009; 39(2).

23. Yalçın S, Eser H, Yalçın S, Cengiz S, Eltan. Effects of dietary yeast autolysate (Saccharomyces cerevisiae) on performance, carcass and gut characteristics, blood profile, and antibody production to sheep red blood cells in broilers. J Appl. Poult. Res. 2013; 22:55-61.