Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 1432-1438

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.162

Evaluation of Antimicrobial Effect of *Emblica officinalis* Fruit Powder on Intestinal Micro-biota in Broilers Chicken

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Keywords

E. coli, *Lactobacilli*, Micro-biota, Phytobiotics

Article Info

Accepted:
12 March 2018
Available Online:
10 April 2018

A B S T R A C T

To study the effect of dietary supplementation of amla fruit powder on intestinal microbiota of broiler chicken, 300 commercial broiler chicks were randomly distributed into six treatments having five replicates consisting of ten birds each. The chicks fed with standard basal diet in two different growth phases i.e. starter (0-28d) and finisher (29-42 d). The first group was kept as control (T_1) and given the basal diet without antibiotic, while second group (T_2) was given basal diet with antibiotic. In third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups, basal diet was supplemented with amla fruit powder @0.25%, 0.50%, 0.75% and 1%, respectively. The birds were weighed fortnightly to calculate performance parameters *viz.* feed intake, body weight change and FCR. Then one bird from each replicate was slaughtered and ileal contents were collected aseptically and serially diluted upto six dilutions and 0.1 ml of each dilution was poured and spread uniformly on MacConkey lactose agar for *E. coli* and MRS for *lactobacilli* and incubated at 37ºC for 24 hours. The average number of colonies was multiplied by reciprocal of the dilution factor and expressed as log Cfu/g of contents. Due to supplementation of phytogenic feed additive microbial load of gram negative *E. coli* decreased and beneficial gram positive *Lactobacilli* increased significantly at higher level of amla supplementation as compare to control group. Feeding diets containing phytobiotics may result in inhibition of growth and colonization of entero-pathogenic microbes in the digestive tract and favours growth of beneficial bacteria i.e. *Lactobacilli*, thus contributing to the balance of gut microflora.

Introduction

The practice of feeding livestock with sub therapeutic levels of antibiotics has been in use for over fifty years. Antibiotics improve the production performance and utilization of nutrients in meat producing chicks. However, the use of antibiotics is being placed under more and more pressure, as consumers increasingly fear that their use in the rations of poultry leads to the formation of resistance against bacteria which are pathogenic to humans (Langhout, 2000). Recently, the emphasis is being directed towards the search of herbal formulations which could be effective for amelioration of stress and leads

to increase in production of birds. Several Indian herbs are reported to possess adaptogenic, antistress and immunomodulator properties (Sapcota *et al.,* 2005; Wadhwa *et al.,* 2007). Herbs and their bio-active constituents possess a broad antimicrobial activity (Dorman and Deans *et al.,* 2000). Herbs and their extracts stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of poultry (Langhout, 2000; Wenk, 2000). Herbs or phytochemicals can influence selectively the microorganisms by an antimicrobial activity or by a favourable stimulation of the eubiosis of the microflora. The mechanism by which the majority of herbal feed additives exert their antibacterial effect is by acting on the bacterial cell wall structure, denaturing and coagulating proteins. The amla fruit due to presence of saponins, phenols and tannins have potent antimicrobial activity against both Gram positive and Gram negative bacteria.

Materials and Methods

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee, 12/CPCSEA Dated 6.2.2017 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Experimental design

Completely Randomized Design (CRD) was used as experimental design at uniform and standard management practices.

Birds and management

A total of 300 commercial broiler chicks (Ven Cobb strain) were randomly distributed into six treatments having five replicates consisting of ten birds each. The chicks fed with standard basal diet in two different growth phase i.e. starter (0-28d) and finisher (29-42 d). The first group was kept as control (T_1) and given the basal diet without antibiotic, while in second group (T_2) basal diet was given with antibiotic.

The diet in third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with amla fruit powder @0.25%, 0.50%, 0.75% and 1%, respectively. The birds were weighed fortnightly to calculate growth performance parameters *viz.* feed intake, body weight gain and FCR. Then, one bird from each replicate was slaughtered and ileal contents were collected aseptically, samples were weighed (1 gm), transferred to sterile tubes and homogenized with sterile 0.9% normal saline solution.

Then the solutions were mixed on vortex and serial dilutions of samples were made up to six dilutions, 0.1 ml of each dilution was poured and spread uniformly on MacConkey lactose agar for *E. coli* and MRS for *Lactobacilli* and incubated at 37^0C for 24 hours. The average number of colonies was multiplied by reciprocal of the dilution factor and expressed as log Cfu/g of contents.

Figuring and composition of diets

Basal ration was formulated as per BIS (2007) to fulfill the metabolizable energy (ME) and crude protein requirements of birds. Level of crude protein in starter (0-4weeks) and finisher (4-6weeks) ration was 22 percent and 20 percent, respectively. The respective ME content was 3000 and 3200 KCal/kg are presented in Table 1. All feed ingredients, additives and supplements used in the experiment were procured in one lot before the start of the experiment. The ingredients, additives and supplements used in the diet

formulation were maize, soybean meal, vegetable oil, fish meal, mineral mixture, vitamins, coccidiostat, lysine, DL- methionine and amla fruit powder. The sources, composition and mixing rate of additives/supplements used in ration formulations are presented in Table 2. The experimental chicks were reared under deep litter system. The floor of the pens was thoroughly cleaned, disinfected before scattering of the bedding material. Well chopped dry wheat straw was used as bedding material to form the litter. The straw was evenly spread upto 5 cm thickness. The litter was regularly racked to avoid any lump formation. Wooden brooders fitted with bulb in the centre were used in each pen for brooding.

The feeding programme consisted of a starter diet until 28 days and a finisher diet from 29 to 42 days of age. Weighed amount of feed was offered on paper sheets for first 3 days and thereafter, in the automatic feeders up to 28 days of age. Afterwards, the feeds were offered through hanging feeders maintained at appropriate heights. The chicks were provided *ad libitum* clean drinking water through the plastic waterers during first two weeks of the experiment. Thereafter, bigger plastic waterers were used till the end of the experiment. Individual body weight of chicks was recorded at 0 day age and thereafter fortnightly. At the end of the experiment, one bird from each replicate was slaughtered ethically by mechanical stunning followed by exsanguinations.

Statistical analysis

Data was analysed statistically as described by Snedecor and Cochran (1994). Analysis of variance was used to study the differences among treatment means and they were compared by using Duncans Multiple Range Test (DMRT) as modified by Kramer (1956).

Results and Discussion

Gut flora

Data pertaining to *E. coli* and *Lactobacilli* count of ileal content of the experimental birds under different dietary treatments are presented in Table 3. Log value for *E. coli* (log cfu/g) ranged from 4.60 to 6.89 among different dietary treatments. Significant reduction in the *E. coli* count was observed inT_2 and T_6 group. Other treatment groups also showed significant reduction in the *E. coli* count as compared to control group (T_1). Total *Lactobacilli* (log cfu/g) ranged from 4.84 (T_2) to 7.08 (T_5) and significantly higher value was recorded in the T_5 and T_6 group supplemented with 0.75% and 1% amla powder. Our study was in consonance with finding of Bostami *et al.,* (2017) who revealed that, there was negative impact of medicinal herb supplementation on the gram negative microbes and *E. coli* and positive impact on the *Lactobacillus* counts. Therefore, it was observed that medicinal herb *Emblica officinalis* supplementation significantly suppress ($P<0.05$) the pathogenic *E. coli* and non-pathogenic *Lactobacillus* count was significant higher in amla supplemented groups as compared to control group. Jamroz *et al.,* (2005) reported that plant extract supplement also significantly increased the *Lactobacillus* numbers following an application of natural plant extract. Similarly, Siddiqui *et al.,* (2015) studied the effect of different dietary levels of *Nigella sativa* seed powder on *Escherichia coli* and total viable bacterial count in excreta of broilers and found *E. coli* and total bacterial counts were significantly decreased by *Nigella sativa* seed powder supplemented diets irrespective of inclusion levels. Castillo *et al.,* (2006) reported that different herbs are able to enhance the growth of *Lactobacilli* this indicates that herbs have the ability to increase the beneficial bacteria.

Table.1 Chemical composition of feed ingredients used in ration formulation

Ingredient	CP (%)	CF (%)	EE (%)	TA (%)	Lysine* (%)	Methionine* (%)	ME* (kcal/kg)
Maize	9.11	2.44	3.44	2.25	0.18	0.15	3300
Soybean meal	45.15	3.93	3.16	8.47	2.57	0.76	2230
Fish meal	47.40	1.79	5.16	26.62	1.42	1.42	2210

*Calculated values (Singh and panda 1992)

Table.2 Ingredient composition of experimental diets during different phases of growth

Ingredient (kg /100 kg of feed)	0-4wks	4-6 wks
Maize	58	60
Soybean meal	30	25
Fish meal	7	7
Vegetable oil	3	6
Mineral mixture	2	2
Feed additives (g/100 kg feed)		
Spectromix	10	10
Spectromix BE	20	20
Veldot	50	50
Choline chloride	50	50
Lysine	50	50
DL-methionine	150	150

Composition, sources and rate of mixing of feed additives/supplements

Spectromix: Powder (Ranbaxy Animal Health, New Delhi). Each gm. contained VitaminA-82,500 IU, Vit D3-12000 IU, Vit B2-50 mg and Vit.K-10mg.Mixing rate: 10 g/100Kg of feed.

SpectromixBE: Powder (Ranbaxy Animal Health, New Delhi). Each gm. Contained Vit.B1- 8mg, Vit.B6- 16mg, Vit.B12- 80mg, niacin-120mg, calcium pentothenate-80mg, Vit. E-160 mg, Lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium 260 mg. Mixing rate: 20g/100kg of feed.

Veldot: Venkeys- Dinitro-O-Toluamide (Coccidiostat). Mixing rate: 50g/100kg of feed.

Choline chloride: Contain 60 percent choline. Mixing rate: 50g/100kg of feed.

Lysine: Contained 98% lysine. Mixing rate: 50g/100kg of feed.

DL-methionine: Contained 98% methionine. Mixing rate: 150g/100kg of feed.

Table.3 *E. coli* (log cfu/g) and total *Lactobacilli* (log cfu/g) count of the ileal content of the experimental birds under different dietary treatments

Treatments	*E. coli* (log cfu/g)	*Lactobacilli* (log cfu/g)
T_1	6.89^{a}±.09	5.96^{c}±.35
T_2	4.60^{d}±.19	4.84^{d}±.23
T_3	6.56^{a}±.08	6.19^{bc}±.23
T_4	5.84^{b}±.26	6.77^{ab}±.18
T_5	5.19^{c}±.15	7.08^{a}±.02
T_6	4.88^{cd}±.17	7.03^{a}±.03

Means bearing different superscripts in a column differ significantly (P<0.05)

The antimicrobial activity of plant secondary metabolites has been attributed to a number of terpenoid and phenolic compounds (Helander *et al.,* 1998; Chao *et al.,* 2000). The antimicrobial mode of action is supposed to be due to the hydrophobicity of these phenolic compounds and due to the fact that these compounds can enter the bacterial cell membrane leading to disintegration of cell membrane, leakage of ions and eventually cell death (Burt, 2004). Feeding diets containing phytobiotics may result in inhibition of the growth and colonization of entero-pathogenic microbes in the digestive tract, thus contributing to the balance of gut microflora (Harris *et al.,* 2001) and promoting the growth performance and health of birds (Adibmoradi *et al.,* 2006). Savage *et al.,* (1996) reported supplementation of oligosaccharides may have a prebiotic effect through an increase in production of lactic acid, thus increasing the proliferation of beneficial bacteria and reducing the presence of Gram-negative bacteria, *in vitro* and *in vivo* studies has confirmed that phytobiotic in animal nutrition can stimulate feed intake, antimicrobial, coccidiostatic and immune-stimulatory action (Panda *et al.,* 2006). Enhancement of intestinal activities of trypsin, lipase and amylase (Lee *et al.,* 2004) and improved gut morphological characteristics (Jamroz *et al.,* 2003) are the major mechanisms through which phytoadditives exert their beneficial effect on the nutrient digestibility. The beneficial influence of the phytogenic feed additives on improved performance and feed conversion ratio could be also explained due to the antioxidant activity of bioactive compounds such as carvacrol, thymol, cineol and pinene (Faleiro *et al.,* 2005; Hazzit*et al.,* 2006) as well as from improved enzyme activity in the alimentary tract, stimulation of useful and inhibition of pathogenic microflora. This eventually resulted in improved absorption and utilization of nutrients (Windisch *et al.,* 2008; Frankic *et al.,* 2009). Based upon the above study, it can be concluded that amla fruit powder can be effectively supplemented as an alternative to antibiotics as growth promoter in poultry ration and best results were obtained at 0.75% amla supplementation level (T_5) regards to gut ecology. Antibacterial effects of amla on gram negative bacteria and beneficial effect on gram positive bacteria results in production of more lactic acid by *Lactobacilli* in gut which further leads to decreased pH in intestine thereby at low pH *Coliforms* are not able to colonise themselves within intestinal mucosa, which interns cause less sloughing of tissue due to decreased toxin produced by them. It also results in favourable environment due to production of short chain fatty acids by *Lactobacilli* which cause effective migration of enterocytes along the tip of villi, resulted in enhanced growth of intestinal villi and thus leading to improved nutrient absorption and better utilization.

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How to cite this article:

Rajesh Dalal, Parveen Kumar Ahlawat, Sonu, Vinus, V.S. Panwar, B.S. Tewatia and Nancy Sheoran. 2018. Evaluation of Antimicrobial Effect of *Emblica officinalis* Fruit Powder on Intestinal Micro-biota in Broilers Chicken. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 1432-1438.
doi: https://doi.org/10.20546/ijcmas.2018.704.162