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Effect of Two Different Phytobiotic Mixtures on Production Performance and Gut Health in Broilers

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ABSTRACT

Background: In broiler production, antibiotics are used to prevent/control infectious diseases and as a growth promoter. The incorrect application of antibiotics increases the risk of residues in chicken tissues. In human's continuous exposure of antibiotic residue containing meat results antimicrobial resistance. So the demand towards antibiotic free meat is increasing. In this condition studies related with alternatives to antibiotic feeding in broilers gaining importance. Among the alternatives, phytogenic feed additives have antimicrobial and growth promoting effect without causing side effects to the birds. The current study aimed to evaluate two different phytobiotic mixtures on production performance and gut health in broilers.

Methods: The study of six weeks duration was carried out between the months of July and August, 2019, at Poultry Farm Complex, Department of Poultry Science, Veterinary College and Research Institute, Namakkal, Tamil Nadu, which falls in the North-Western agro climatic zone of the state. 150 day old chicks (vencobb) used to evaluate two different phytobiotic mixtures on production performance and gut health of birds.

Result: Birds fed with phytobiotic mixture (II) - *E. officinalis* fruits + *M. kenigii* leaves + *S. aromaticum* buds (1:1:1) at 2 kg/ton of feed resulted in significant improvement in bodyweight, weight gain and lactobacillus count was observed. So this phytobiotic mixture recommended as alternative to antibiotic feed additive in broilers.

Key words: Broiler, Gut health, Phytobiotic mixture, Production performance.

INTRODUCTION

In commercial broiler production, antibiotics have been used extensively, to prevent or control infectious diseases and also to serve as a growth promoter. The misuse and incorrect application of antibiotics increases the risk of antibiotic residues in edible tissues of the chicken (Marazuela and Bogialli, 2009). Low and continuous exposure to antibiotic residues produce potential threats of direct toxicity in humans, alteration of microflora with possible development of resistant strains, that leads to failure of antibiotic therapies (Nisha, 2008). Therefore, the demand by consumers for antibiotic residue free chicken meat is growing very fast. This has led to the emergence of organic broiler farming where use of antibiotics in any form for prevention of disease or improving the productivity of birds is not allowed (Nagarajan *et al*., 2017). In this scenario there has been a significant increase in number of studies focused on searching for alternatives to antibiotics, having similar antimicrobial and growth promoting effects, without causing any potential side effects to the birds fed on it. Probiotics, prebiotics, organic acids, enzymes and phytobiotics have been widely studied as potential alternatives to antibiotics in feed. Numerous studies on phytobiotics have demonstrated their positive effects in terms of antimicrobial, antioxidative and anti-inflammatory activity and it has been stated that the primary mode of action of phytogenic feed additive is controlling potential pathogens and beneficially modulating the intestinal microbiota (Murugesan *et al*., 2015). A healthy gut is extremely important for enhanced broiler performance and production of hygienic chicken meat. However, very limited research exists on the use of a mixture of indigenous herbs as phytobiotics, it is in this context that this study was executed to evaluate two different phytobiotic mixtures on production performance and gut health in broilers.

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MATERIALS AND METHODS

The study of six weeks duration was carried out between the months of July and August, 2019, at Poultry Farm Complex, Department of Poultry Science, Veterinary College

and Research Institute, Namakkal, Tamil Nadu, which falls in the North-Western agro climatic zone of the state (Anonymous, 2019).

Experimental birds and experimental design

One hundred and fifty, one-day-old Vencobb broiler (feather sexed) chicks belonging to the same hatch were wing banded, weighed and distributed randomly into five treatment groups, each group had three replicates, with 10 birds per replicate.

The treatments adopted for this study were as follows:

Treatments	No. of replicates	No. of birds/ replicate	Total
T_1- Control - No phytobiotic mixture supplementation	3	10	30
T_2- Phytobiotic mixture (I) - Level I (1 Kg/tonne of feed)	3	10	30
T_3 - Phytobiotic mixture (I) -Level II (2 Kg/tonne of feed)	3	10	30
T_4 - Phytobiotic mixture (II) -Level I (1 Kg/tonne of feed)	3	10	30
T_5 - Phytobiotic mixture (II) -Level II (2 Kg/tonne of feed)	3	10	30
Total number of birds			150

The following was the composition of phytobiotic mixtures used in the study.

1. Phytobiotic mixture (I) - *Syzygium aromaticum* buds + *Mentha spicata* leaves + *Emblica officinalis* fruits in 1:1:1 ratio.
2. Phytobiotic mixture (II) - *Emblica officinalis* fruits + *Murraya kenigii* leaves + *Syzygium aromaticum* buds in 1:1:1 ratio.

Management system

The experimental birds were reared in a deep litter system. All management practices adopted except for the feed offered, were similar to all treatments.

Experimental feed

Feed ingredients, procured locally were used for the formulation of experimental feed. Experimental broiler pre starter (0-2 weeks), starter (3-4 weeks) and finisher (5-6 weeks) feeds were formulated as per Vencobb 400 specification. The phytobiotic mixtures (ground form) at their respective levels as per the treatments were mixed with the feed. The ingredients and nutrient composition of experimental broiler pre starter, starter and finisher ration is presented in Table 1.

Production performance

The birds were weighed individually every week till the end of the study period (6th week) in a calibrated electronic weighing balance of 10 g accuracy to document their body weight and calculate weight gain (g). Every day the left-over feed was weighed to record the accurate feed intake (g). Feed consumption was calculated after correction of feed wastage. The feed conversion ratio per kilogram weight gain was calculated.

Gut health

At the end of the experimental period six birds per treatment were randomly selected and slaughtered and the gut contents and portion of ileum of the birds were collected to study the microbial profile of gut contents and gut histomorphology.

Microbial profile of gut contents

To study the microbial profile, the ileum (from the duodenum and Meckel's diverticulum) of each bird was accurately removed and samples of the intestinal contents were collected and diluted with sterile saline solution and different medias (Mac-Conkey agar (MAC), De Man Rogosa and Sharpe agar (MRS) and *C. perfringens* agar were used respectively for the enumeration of *E.coli, Lactobaccilli* spp. and *C. perfringens*. The plates were incubated at 37°C for overnight. Finally the grown colonies were counted using colony counter.

Histomorphology of ileum

A piece of the mid portion of the ileum was collected and washed with normal water and stored in 10 per cent formalin. The tissues were processed as per the method of Bancroft and Stevens (1996). The sections were examined by Carl Zeiss Image analyser using soft ware KS 300.

RESULTS AND DISCUSSION

Production performance

The production performance and gut health characters of birds in the various treatments are presented in the Table 2. At the end of sixth week, significantly ($P<0.05$) highest body weight (2668.47 g) and body weight gain (2616.28 g) was observed in the birds of T_5 - supplemented with phytobiotic mixture II (*E.officinalis* fruits + *M.koenigii* leaves + *S.aromaticum* buds) at level II (2 Kg/ tonne of feed). The cumulative feed intake was significantly ($P<0.05$) lowest in T_1 (4013.70 g) and in T_2 compared to other treatment groups. T_3 and T_5 groups documented significantly ($P<0.05$) highest cumulative feed intake. No significant variations ($P>0.05$) were observed between treatments with respect to feed conversion ratio of birds, though numerically lowest FCR was observed in T_5. Nuan *et al*., (2016) also reported that the birds fed diets supplemented with *Murraya koenigii* powder had a higher weight gain ($P<0.05$; ADG), improved feed conversion ratio ($P<0.05$; FCR) and lower mortality ($P<0.05$) rates compared to the birds in the control group.

Gut health

Microbial profile of gut contents

Both the phytobiotic mixtures significantly increased *Lactobacilli* spp. count compared to control in the gut contents of birds. Significantly ($P < 0.05$) highest (60.00) *Lactobacillus* spp. count was observed in T_5 - birds

supplemented with phytobiotic mixture II (*E. officinalis* fruits + *M. koenigii* leaves + *S. aromaticum* buds) at level II. Supplementing selected phytobiotic mixture II significantly reduced *E.coli* count compared to control in the gut contents of birds. Significantly (P<0.05) lowest *E.coli* (31.83 cfu /g) and *C. perfringens* (450.00 cfu /g) count were observed in birds supplemented with phytobiotic mixture II at level II. Supplementing selected phytobiotic mixture significantly reduced *C. perfringens* count compared to control in the gut contents of birds.

Savage *et al.* (1996) also reported that 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. The plant extracts are known to beneficially modulate the composition of the microflora in the ileum and caeca by increasing the number of *Lactobacilli* spp. and reducing harmful bacteria, such as potential pathogenic coli forms and *C. perfringens* (Vidanarachchi *et al*., 2010). In another study, colonization of beneficial bacteria such as *Lactobacilli* spp. and *Bifidobacterium* spp. and reduction in *Clostridium* spp. load were comparatively better in polyherbal product supplemented groups which was attributed to the presence of bioactive compounds in the herbal product (Vasanthakumar *et al*., 2012). The mechanism of action of phytochemicals is not clearly understood, but may depend upon the composition of the active ingredients in the product being used. The beneficial effects of phytochemicals are attributed to their antimicrobial and antioxidant properties. In addition, the inclusion of phytochemicals in the diets alters and stabilizes the intestinal microbiota and reduces microbial toxic metabolites in the gut, owing to their direct antimicrobial properties on various pathogenic bacteria, which results in relief from intestinal challenge and immune stress, thus improving performance (Kim *et al*., 2015).

Histomorphology of ileum

Supplementing phytobiotic mixtures I or II at either dose (level I or II) in broiler diets significantly (P<0.05) increased villus height, of the birds. Significantly (P<0.05) highest (1159.87 µm and 353.56 µm) villus height and crypt depth were observed in birds of T_5, supplemented with phytobiotic mixture II (*E. officinalis* fruits + *M. koenigii* leaves + *S. aromaticum* buds) at level II. Significantly (P<0.05) highest (1128.43 µm and 246.76 µm) villus height and crypt depth ratio were

Table 1: Ingredients and nutrient composition of experimental broiler pre starter, starter and finisher ration.

Name of the Ingredient	Inclusion level (%)		
	Pre starter	Starter	Finisher
Maize (%)	53.45	55.38	57.39
Soybean meal (CP 45 %)	38.97	35.4	31.89
Rice Bran oil (%)	3.40	5.29	6.90
Dicalcium phosphate (kg)	2.05	1.90	1.83
Calcite (kg)	1.05	1.10	1.05
Lysine (g/100 Kg)	0.20	0.12	0.15
DL Methionine (kg)	0.27	0.26	0.24
Threonine (kg)	0.03	0.00	0.00
Salt (kg)	0.35	0.35	0.35
Trace mineral mixture[1] and vitamin supplements[2,3] (kg)	0.11	0.10	0.10
Toxin binder and coccidiostat [4] (kg)	0.12	0.10	0.10
Total	100.00	100.00	100.00
	Nutrient composition (%DMB)*		
Energy (kcal/Kg)	3000	3125	3250
Crude protein (%)	22.50	21.00	19.50
Calcium (%)	0.94	0.92	0.88
Available phosphorus (%)	0.45	0.42	0.40
Crude fibre (%)	3.98	3.92	3.96
Total lysine (%)	1.42	1.25	1.14
Total methionine (%)	0.62	0.58	0.54
Energy/Protein ratio	133	149	167

*Calculated values.

Trace mineral mixture was added at the level per kg feed, supplied manganese-81 mg, zinc -78 mg, iron-30 mg, iodine-3 mg and copper -3 mg and cobalt- 1.5 mg.

Vitamin AB_2D_3K was added at the level per kg feed supplied, vitamin A-16500 IU, B2-10 mg, D3-3200 IU and vitamin K-2 mg.

Vitamin B complex added at the level per kg feed supplied, Thiamine 2.8 mg, Pyridoxine 5.6 mg, Niacin 42 mg, Cyanocobalamine 28 µg, vitamin E 28 mg, Calcium D pantothenate 28 mg and Folic acid 2.8 mg, Calcium 30.1 mg.

Coccidiostat was added at the level of 125 mg of Di-nitro-ortho-toluamide per kg feed.

Table 2: Effect of supplementing different phytobiotic mixtures on production parameters, gut microbiome and histomorphology of ileum of broilers (Mean*±S.E).

Parameters	T_1 Control	T_2	T_3	T_4	T_5
Production parameters					
Hatch weight (g)^{NS}	51.55 ±0.58	52.92±0.74	53.30±0.69	52.80±0.65	52.18±0.74
Body weight at 6th wk (g)	2469.03^{b}±67.68	2514.60^{ab}±50.61	2629.03^{ab}±65.95	2528.87^{ab}±66.00	2668.47^{a}±61.06
Body weight gain (g)	2417.48^{b}± 67.62	2461.68^{ab}±50.42	2575.73^{ab}±65.87	2476.07^{ab}±65.82	2616.28^{a}±61.01
Feed intake (g)	4013.70^{c}±12.46	4017.33^{c}±10.52	4137.00^{a} ±2.84	4042.87^{b}±7.35	4150.53^{a}±8.58
Feed conversion ratio^{NS}	1.70±0.04	1.65±0.03	1.63±0.04	1.66±0.04	1.61±0.04
Gut microbiome					
Lactobacilli (10^4 cfu/g)	11.33^{d} ±2.15	20.00^{c}±2.72	25.33^{c} ±1.40	42.33^{b}±1.26	60.00^{a}±2.59
E.Coli (cfu/g)	763.33^{a} ±54.87	248.33^{b}±37.98	82.00^{c} ±3.74	66.83^{c}±3.10	31.83^{c}±2.67
Clostridium perfringens (cfu/g)	5833.33^{a} ±378.30	5333.33^{a}±105.41	4500.00^{b}±238.05	2183.33^{c}±83.33	450.00^{d}±23.81
Histomorphology of ileum					
Villi height (µm)	311.90^{c} ±16.68	1123.72^{a}±56.22	1128.43^{a}±51.13	919.29^{b}±33.71	1159.87^{a}±22.90
Crypt depth	239.23^{b}±18.80	249.34^{b}±12.40	246.76^{b}±14.04	347.86^{a}±35.39	353.56^{a}±19.42
Villi height and crypt depth ratio	1.36^{c}±0.16	4.59^{a}±0.40	4.66^{a}±0.33	2.75^{b}±0.23	3.35^{b}±0.28

*Mean of 30 observations.

Mean values bearing different alphabetical superscripts within column differ significantly (P<0.05).

NS-Means do not vary significantly.

Phytobiotic mixture (I) - *Syzygium aromaticum* buds + *Mentha spicata* leaves + *Emblica officinalis* fruits Level I - 1 kg/tonne of feed. Level II - 2 kg/tonne of feed.

Phytobiotic mixture (II) - *Emblica officinalis* fruits + *Murraya koenigii* leaves + *Syzygium aromaticum* buds Level I - 1 kg/tonne of feed, Level II - 2 kg/tonne of feed.

observed in birds of T_2 and T_3. Rajput *et al.* (2013) had reported that villus height was significantly (P<0.05) increased in duodenum, jejunum and ileum in curcumin supplemented broilers. In another study, basal diet supplemented with amla fruit powder at the rate of 0.25 per cent, 0.50 per cent, 0.75 per cent and 1 per cent, respectively increased the villi height and significant (P<0.05) increment was observed in one per cent supplementation (Dalal *et al*., 2018). Increased intestinal villi height and the ratio of villi height to crypt depth is an indication of the vast area for nutrient absorption and higher absorption function (Sieo *et al*., 2005). Higher villi height, low crypt depth and high villi height/crypt depth ratio are desirable parameters for better absorption of nutrients (Xu *et al*., 2003).

CONCLUSION

Phytobiotic mixture comprising powder of *E. officinalis* fruits + *M. koenigii* leaves + *S. aromaticum* buds in 1:1:1 ratio at 2 kg/tonne of feed recorded significantly (P<0.05) highest weight gain, highest *Lactobacillus* count, significantly (P<0.05) lowest *E.coli* and *C. perfringens* count in gut contents and significantly (P<0.05) highest villi height and crypt depth and hence this phytobiotic mixture is recommended as an alternate feed additive.

Conflict of interest: None.

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