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Research Article

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Evaluation of Endotoxin Binder as Growth Promoter in Broiler Production

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ABSTRACT

Antibiotic growth promoter used in broilers not only mitigate the harmful pathogens colonising in gastrointestinal tract they also increase endotoxin release in gut lumen from killing Gram-negative bacteria. The current research was carried out to evaluate the effect of endotoxin binder (EB) alone and along with oxytetracycline antibiotic (AB) as growth promoter and to assess its techno-economics. Two hundred day-old Vencobb 400 broiler chicks were divided into five groups with four replicates of 10 chicks in each. Experimental groups fed with basal diet (BS) prepared as per BIS (IS 1374: 2007) recommendations. The birds in G1 fed with BD + 2.5 kg of EB/ton of feed, G2 fed with BD + 0.5 kg of AB /ton of feed, G3 with BD+ 2.0 kg of EB /ton, G4 with BD + 1.5 kg of EB /ton + 0.5 kg of AB /ton and G5 with BD + 1.0 kg of EB /ton + 0.5 kg of AB /ton. The present study results revealed G3 and G5 group birds gained significantly higher ($p<0.01$) body weight than G1 and G2 birds and cumulative feed intake in all group birds are similar at 42^nd^ day of its age. Cumulative feed conversion ratio calculated at 42^nd^ day, G3 was better ($P<0.05$) than G2 and G4, whereas G1 and G5 was similar to that of G3. The profit per kg live weight in G3 calculated was 1.22 times higher than G5 and 3.37 times higher than G2 group birds. The findings revealed better growth performance and better profit in G3 and G5 groups. It concluded that supplementation of endotoxin binder at 2 kg per ton of feed could be considered as alternative to antibiotic growth promoter for broilers.

Keywords: *Broilers, Growth promoter, Endotoxin binder, Clinoptilolite, HSCAS.*

INTRODUCTION

In Indian broiler industry, use of growth promoters are accepted and usually included as feed additive. Antibiotic are also used in poultry diets as growth promoter to mitigate the harmful effects of pathogens that colonise in the gastrointestinal tract. Antibiotic growth promoter not only change gut microflora they also increase plausible release of lipopolysaccharides in the gut lumen that results from killing of Gram-negative bacteria. Poultry are regularly being exposed to endotoxins either through water, feed, air or through endotoxin released by Gram-negative bacteria present in the intestinal flora (*Escherichia coli*).

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Endotoxins are part of the outer membrane of cell wall of all Gram-negative bacteria (*Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, etc.) released by shedding or through bacterial lysis (Rietschel et al. (1994), Silverman & Ostro, (1999). Endotoxin in the lumen cause inflammation of intestinal epithelium and increases para-cellular permeability that cause altered intestinal integrity leading to impaired absorption and utilization of nutrients which affects the growth performance of bird (Ghareeb et al., 2016).

The toxin binders widely used as growth promoter are hydrophilic molecules (activated charcoal, bentonites and alumino-silicates) which need not bind endotoxin or lipopolysaccharide or lipid in which causes pathogenic effect. The aluminosilicate molecules with different minerals combination effect different pore size with different binding ability (Alam et al., 2004). Clinoptilolite (Hydrated sodium potassium calcium aluminosilicate) and HSCAS (Hydrated sodium calcium aluminosilicate) are used as toxin binder, growth promoter in diets of poultry and swine (Afaf et al., (2011), Mallek et al. (2012). Clinoptilolite and HSCAS with their differed structure has ability to disintegrate the chemical and physical form of high molecular weight substances, contaminants, toxins, entering in to their pore and have adsorbent capacity in animal production with ability to bind endotoxin (Jin, et al. (2017), Wu et al. (2015).

The present study was planned to ascertain the effect of supplementation of endotoxin binder (mixture of CLI and HSCAS) alone and along with oxytetracycline antibiotic as growth promoter in broiler ration and to assess its techno-economics.

MATERIALS AND METHODS

The feeding trial was conducted in Poultry Farm, Instructional Livestock Farm Complex (ILFC), Pookode and chemical analysis was carried out in Department of Animal Nutrition at College of Veterinary and Animal Sciences, Pookode, Wayanad.

ENDOTOXIN BINDER PREPARATION

Clinoptilolite (CLI), Hydrated sodium calcium alumina silicates (HSCAS), calcite powder, Lithothamn was purchased from local market in Bangalore and 80 per cent CLI, 10 per cent HSCAS, 9 per cent calcite powder and 1 per cent lithothamn was mixed together and the mixture was sieved using a sieve of BSS 22 (710 microns), resultant fine powder was used in the ration.

EXPERIMENTAL SHED

The experiment shed was prepared using routine disinfection and fumigation. The birds were reared under deep litter (wood shavings) system with incandescent bulb brooding for till they attained three weeks of age. Thereafter, light was provided only during night hours. Each replicate was housed in separate pen in the same shed with *ad libitum* assess to experimental feed and clean water. All the experimental chicks were vaccinated as per standard.

EXPERIMENTAL DESIGN

Two hundred, Vencobb-400 commercial strain broiler chicks were purchased from local hatchery. Then the chicks were weighed individually, wing banded and randomly distributed to five groups *viz* G1, G2, G3, G4 and G5 with four replicates of ten chicks in each replicate. Experimental groups fed with basal diet (BS) prepared as per BIS[17] recommendations. The birds in G1 fed with BD + EB at 2.5 kg/ton of feed, G2 fed with BD + AB at 0.5 kg/ton of feed, G3 with BD+ EB at 2.0 kg/ton, G4 with BD + EB at 1.5 kg/ton + AB at 0.5 kg/ton and G5 with BD + EB at 1.0 kg/ton + AB at 0.5 kg/ton.

The ingredient composition of experimental rations of broiler pre-starter, starter and finisher and calculated values of metabolisable energy, crude protein, calcium and phosphorous are presented in Table 1. The broiler pre-starter mash ration was fed to the birds from day-one to 7th day; starter ration was given from 8th day to 21st day; from 22nd day to 42nd day broiler finisher ration was provided.

GROWTH PERFORMANCE

The feed consumed at the end of every day was recorded and weekly and cumulative feed intake of birds were calculated. The live body weight of all birds were recorded at weekly interval and weekly and cumulative body weight gain was calculated. The feed conversion ratio for every week and cumulative till 6th week was calculated by dividing the feed consumption by weight gain.

CHEMICAL COMPOSITION ANALYSIS

The experimental rations proximate principles analysed as per AOAC (2016), acid insoluble ash as per BIS (IS: 14826: 2000), calcium and phosphorus as per Talapatra et al. (1940).

PROFIT CALCULATION

The sales price of live bird, chicks cost and other overhead cost, broiler cumulative feed intake during pre-starter, starter and finisher feed consumed and cumulative body weight was considered to calculate profit per bird and profit per kg live weight.

STATISTICAL ANALYSIS

The data recorded during the experiment was analysed using statistical software SPSS version 24.0.

Table 1: Ingredient composition of pre-starter, starter and finisher broiler rations

Ingredient	GROUPS														
	Pre-starter (0- 7th day)					Starter (8th -20th day)					Finisher (21st -42nd day)				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Maize	54.4	54.4	54.4	54.4	54.4	57.2	57.2	57	57.2	57.2	59.3	59.3	59.8	59.3	59.3
SBM^	38.75	38.95	38	38.8	38.85	35.1	35.25	35.3	35.1	35.15	31.65	31.85	30.9	31.7	31.75
Vegetable oil	2.5	2.5	3.3	2.5	2.5	3.35	3.4	3.4	3.4	3.4	4.65	4.6	4.9	4.65	4.65
OTC **	0	0.05	0	0.05	0.05	0	0.05	0	0.05	0.05	0	0.05	0	0.05	0.05
EB #	0.25	0	0.2	0.15	0.1	0.25	0	0.2	0.15	0.1	0.25	0	0.2	0.15	0.1
DCP^^	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.65	1.7	1.7	1.65	1.65
Shell grit	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
FA*	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated Value															
ME (kcal/kg)	3038.	3043.	3088.	3039.	3040.	310	3117.	3112.	3113.	3114.	3201.	3202.	3220.	3203	3204.
CP (%)	23.5	23.59	23.14	23.52	23.54	22	22.07	22.07	22	22.02	20.53	20.63	20.21	20.5	20.58
Ca (%)	1	1	0.99	1	1	0.98	0.98	0.98	0.98	0.98	0.98	0.99	0.99	0.98	0.98
Total P (%)	0.7	0.7	0.69	0.7	0.7	0.68	0.68	0.68	0.68	0.68	0.67	0.68	0.67	0.67	0.67

*Feed additive

^^DCP

RESULTS AND DISCUSSION

At 2nd week, there was a significantly highest feed intake recorded in G4 birds (EB at 1.5 kg/ton and AB at 0.5 kg/ton) of this study. However, by the end of 42nd day, cumulative feed intake was similar between groups irrespective of the treatment (Table 2). Similar to our findings, Wu et al. (2015) who studied the effect of supplementing 2 per cent CLI (natural and modified) against broiler chicken challenged with endotoxin (lipopolysaccharide at 250 μg/kg body weight) observed no significant difference in their feed intake between groups and also against control. In contrary, Wawrzyniak et al. (2017) observed supplementation of CLI at 2 or 3 per cent group birds shown increased feed intake (4136.4 and 4016.8g, respectively) compared to birds with no CLI supplementation (3923 g). Similarly, increased feed intake ($p<0.01$) of broiler birds were observed in a 35 days feeding trial upon adding HSCAS (5 g/ kg ration) (2790 g) to diet of aflatoxin challenged groups (2600 g) Assar et al. (2018).

Table 2: Cumulative feed intake of broilers (g/bird)

Age (Week)	Groups					SEM	p-value
	G1	G2	G3	G4	G5		
I	150.45	143.39	150.90	150.82	146.85	1.21	0.197 ^{ns}
II	500.34 ^{ab}	479.03 ^{c}	509.46^{ab}	513.44 ^{a}	490.23 ^{bc}	3.90	0.013**
III	1118.01	1031.54	1104.65	1130.21	1080.40	12.13	0.059 ^{ns}
IV	1906.43	1804.62	1923.06	1972.51	1949.90	23.50	0.189 ^{ns}
V	2950.70	2841.27	2968.37	3101.80	3079.45	35.06	0.104 ^{ns}
VI	4014.95	3919.96	4063.30	4237.19	4150.17	43.67	0.173 ^{ns}

$^{a\ b\ c}$ Mean ± SE values with different superscripts within a row differ significantly
** Significant at 0.01 level; ^{ns}-Non-significant

Weekly cumulative body weight gain (g)

Mean weekly body weight gain of birds were significantly higher in G3 and G5 group birds up to 4th week of its age compared to other groups. However, there was no significance was recorded during 5th week of age between the groups. While, G1 birds which received higher concentration of EB (2.5 kg per ton) shown lowest cumulative body weight gain but at EB at 2.0 kg per ton shown significantly better BWG during this study period (Table 3.). Like that, supplementation of AB alone at 0.5 kg/ton of feed also shown same result as that of G1. The supplementation of EB and AB (G4 and G5) combination also shown better cumulative body weight gain ($p < 0.01$) compared against G1 and G2 groups.

Afaf et al. (2011). corroborates with our findings supplementation of CLI at 2 per cent level in broiler birds as growth promoter increased the body weight and body weight gain and other researcher Mallek et al. (2012) reported addition of 1 per cent CLI also significantly improved ($p < 0.01$) the final body weight (2.44 kg) compared to control with no CLI addition (2.17 kg).

Assar et al. (2018) who supplemented HSCAS (5 g/kg ration) to diet of aflatoxin challenged broiler birds as toxin binder observed a significantly increased body weight gain. However, Singh & Mandal (2018) who supplemented HSCAS (0.25 and 0.50 per cent) as feed additive to basal diet had no significant effect on body weight gain over control with no HSCAS at 42nd day.

Wu et al. (2015). supplemented 2 per cent CLI (natural or modified) to lipopolysaccharide challenged birds for 21 days. They observed no significant difference in average body weight gain between groups and against control group. Like that, Zamora et al. (2017) observed a no significant in mean body weight at 42nd day in oxytetracycline (0.25g per kg) supplemented group (2651.73 g) compared to control group (2604.3 g).

Table 3: Cumulative body weight gain of broilers (g/bird)

Age (Week)	Group					SEM	p-value
	G1	G2	G3	G4	G5		
I	139.36 ^{b}	139.88 ^{b}	151.60 ^{a}	151.57 ^{a}	146.09^{ab}	1.72	0.044*
II	412.12	370.12	404.86	405.77	392.63	5.12	0.078 ^{ns}
III	782.24 ^{a}	695.90 ^{b}	774.42 ^{a}	769.10 ^{a}	805.61 ^{a}	9.52	0.004**
IV	1192.41^{b}	1161.05^{b}	1241.10^{b}	1222.88^{b}	1322.59^{a}	12.29	0.001**
V	1793.68	1752.64	1857.38	1834.45	1890.08	17.79	0.124 ^{ns}
VI	2120.32^{b}	2068.47^{b}	2293.20^{a}	2196.44^{ab}	2291.11^{a}	23.21	0.004**

$^{a\ b\ c}$ Mean ± SE values with different superscript within a row differ significantly
* Significant at 0.05 level; ** Significant at 0.01 level; ^{ns}-Non-significant

Feed conversion ratio

In the present study, groups supplemented with 2.0 per cent EB alone (G3) had significantly better cumulative FCR (1.79) compared to G2 group with 0.5 per cent OTC alone (1.96) and G4 (1.97). However, feed conversion efficiency of G1 (1.90) and G5 (1.83) was statistically similar to G3 (Table 4 and Figure 1).

Afaf et al. (2011) who reported better cumulative FCR was recorded, as dose of CLI was increased from 1.0 to 2.0 per cent in broiler birds. Similarly, Nikolakakis et al. noticed significantly (P < 0.05) better feed conversion efficiency in birds fed with basal diets containing 2 and 3 per cent of clinoptilolite.

In contrary, Wu et al. (2013) who mentioned supplementation of NCLI or MCLI in broiler diets, found no significant difference in their feed conversion efficiency compared to control group at 42nd day. Like that, supplementation of zeolite (clinoptilolite, bentonite montmorillonite) and activated charcoal mixture (Prvulovic et al., 2007); HSCAS at 0.25 and 0.5 per cent (Singh, & Mandal, 2018) and oxytetracycline at 0.25 g per kg (Zamora et al., 2017) had not improved the feed conversion efficiency of broiler birds while compared against control.

Casarin et al. (2005) noticed improved feed conversion efficiency upon addition of HSCAS at 0.25 per cent to mixed basal diet containing mycotoxin. Li et al. (2015) also observed improved feed conversion efficiency in LPS challenged group upon probiotic (*Bacillus amyloliquefaciens*) supplementation at 0.5 and 1.0 g/kg diet (1.69 and 1.54, respectively) were LPS challenged group had a 1.76 FCR.

Table 4: Cumulative FCR of broilers

Age (Week)	Group					SEM	p-value
	G1	G2	G3	G4	G5		
I	1.07	1.05	1.01	1.02	1.03	0.01	0.500 ns
II	1.21 b	1.38 a	1.28 ab	1.30 ab	1.23 b	0.02	0.046*
III	1.47 ab	1.59 a	1.44 ab	1.55 a	1.34 b	0.02	0.016*
IV	1.64 a	1.61 ab	1.57 ab	1.66 a	1.48 b	0.02	0.049*
V	1.68	1.67	1.62	1.74	1.64	0.02	0.336 ns
VI	1.90 ab	1.96 a	1.79 b	1.97 a	1.83 ab	0.02	0.046*

a b c Mean ± SE values with different superscripts within a row differ significantly

* Significant at 0.05 level; ns-Non-significant

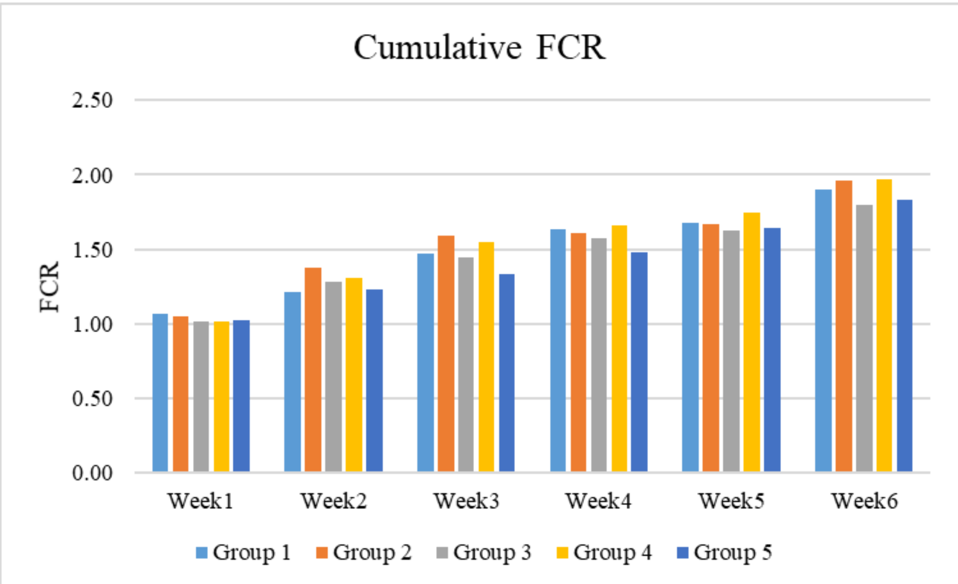

Fig. 1: Cumulative FCR of broilers

CONCLUSION

It this study it was evident that addition of EB (2 kg per ton of feed) alone (G3) and EB (1.0 kg per ton) along with AB (0.5 kg per ton) (G5) had a better growth performance with a better body weight and feed conversion ratio. This results could be due to the ability of EB to protect the intestinal structures responsible for absorption of nutrients from ameliorative effect of antibiotic stimulated endotoxin release from gram negative bacteria in gut flora. However, to growing concern on antibiotic resistance EB alone at 2kg/ton could be considered as a better alternative for antibiotic growth promoters in broiler production.

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