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Evaluation of lysozyme as potential alternative for replacement of antibiotic as feed supplement for growth performance in broiler birds

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ABSTRACT

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An experiment was conducted on 360 broiler chicks to study the impact of lysozyme as potential alternative for replacement of antibiotic as feed supplement for growth performance. The day old chicks were distributed in six treatment groups on similar body weight basis with three replicates in each. The birds under control group received basal diet. The two treatment groups received basal diet supplemented with antibiotic flavomycin @ 100 g/ton of feed and lysozyme @ 250 g/ton of feed. One treatment group was challenged with *Clostridium Perfringens* on 17, 18 and 19th day of age without any supplementation. Remaining two groups were challenged with *Clostridium Perfringens* with the supplementation of antibiotic flavomycin @ 100 g/ton and lysozyme @ 250 g/ton of feed. At the end of the experiment, the feed intake and feed conversion ratio, body weight of broiler chicken were significantly ($P<0.05$) improved due to addition of dietary lysozyme compared to non-supplemented control group birds. The non-supplemented but *Clostridium perfringens* challenged birds showed significantly lower body weight gain compared to treatment group supplemented with lysozyme. Intestinal microbiology assays shows that supplementation of lysozyme and antibiotic gives more beneficial effect on gut bacterial count at early age, whereas the age of the bird progress the effect nullified. In can be concluded that addition of Lysozyme (@ 250 gm per Ton of feed) can be good alternative for antibiotic in commercial broiler diet for higher live body weight, gut health and overall performance without affecting performance of broiler birds.

Keywords: Antibiotic, Broiler, *Clostridium perfringens*, Lysozyme, Performance

INTRODUCTION

Rapid growth in the poultry sector is achieved due to advancements in breeding, nutrition, management practices and use of newer technologies. At the same time, poultry industry facing challenges such as animal welfare, food safety, environmental impact, restrictions on use of antimicrobials etc. Hence, it is a need of time to focus on use of nutraceuticals to modulate intestinal microbiota and the immune system as an alternative to therapeutics instead of controlling enteric diseases with therapeutic compounds (Ferket, 2009). The World Health Organization declared that antibiotic use in animal feeds is a public health concern due to the development of resistant bacteria and possible transfer of resistance to humans. The European Union banned the use of routinely used antibiotics for controlling the incidence of Necrotic enteritis, a sporadic disease routinely infects the flocks of chicken and results in significant economic losses (Sluis, 2000; Dahiya *et al.*, 2006).

Now the time has come for us to adopt new management approaches, strict biosecurity measures or use of the other wide range of nutraceuticals available in the market including acidifiers, prebiotics, probiotics, organic acids, essential oils, enzymes, osmoregulatory, nucleotides, zinc oxide, etc. in order to grow broiler chickens without the use of antibiotics as feed supplements. Lysozyme is one of the alternatives which has the potential to replace antibiotics as feed supplements. An enzyme lysozyme with an antibacterial property was discovered which is chemically a 1, 4-β-N-acetyl muraminidase and commercially obtained from egg white. Antimicrobial properties of lysozyme are due to its property of breaking the bond between N-acetylmuramic acid (NAMA) and N-acetyl-D-glucosamine (NAG) in peptidoglycan of bacterial cell wall. Recent studies (Gong *et al.*, 2016; Latief *et al.*, 2017) show that lysozyme could be used as a feed additive to achieve desired growth performance and control enteric pathogens without the use of antibiotic. Therefore, present experiment was carried out to testify the effect of dietary addition of lysozyme with or without *Clostridium perfringens* challenge on broiler growth performance, gut health and immune status.

MATERIALS AND METHODS

Experimental design and feeding

The experiment was conducted on 360, day-old commercial broiler chicks for a period of 42 days. The experimental chicks were randomly distributed into six treatment groups each having 3 replicates of 20 chicks. During the experiment the birds were not fed antibiotics as prophylactic or treatment measures. Commercial broiler mash diets were prepared without any antibiotic and coccidiostat.

The birds under control group received basal diet. The two treatment groups received basal diet

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supplemented with antibiotic flavomycin @ 100 g/ton of feed and lysozyme @ 250 g/ton of feed. One treatment group was challenged with *Clostridium perfringens* on 17, 18 and 19th day of age without any supplementation. Remaining two groups were challenged with *Clostridium perfringens* with the supplementation of antibiotic flavomycin @ 100 g/ton and lysozyme @ 250 g/ton of feed.

The birds of the challenged group were inoculated orally with 1.5 ml of stock solution of *Clostridium perfringens* of 10^9 cfu/ml. Prior administration of the oral gavages the feed was restricted four hours before to minimize regurgitation and to induce successful challenge. Throughout the experiment uniform management practices were followed for all the birds. The alpha toxin producing strains of *Clostridium perfringens* were obtained from Microbial Type Culture Collection (MTCC), Chandigarh.

Broiler performance

Body weight gain and the feed intake of chicks allotted in replicates were recorded at weekly interval up to six weeks of age. The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately, taking mortality into consideration, if any. The economics of broiler production was worked out by considering the total cost of production which included the feed cost, chicks, labour, medicines, vaccines and the overhead costs. The profit per bird was calculated by subtracting the cost of production per bird from the price fetched per bird after selling it in the local market on live weight basis.

Bacterial enumeration

On 24 and 42nd day of age six birds per treatment were sacrificed and intestinal content from jejunum were collected aseptically in a sterile bag and stored at refrigeration temperature until analysis. One gram of faecal material from intestine was diluted with 9 ml normal saline solution, further 3 fold serial dilutions was carried out and 1 ml from each dilution was plated in duplicate by using *Clostridium perfringens* agar (Himedia Laboratories, India). Then the plates were sealed with paraffin and incubated at 37^{f} C for 48 hours anaerobically by using anaerobic gas pack and anaero bag system. After 48 hours the colonies were counted and *Clostridium perfringens* counts were found per gram of sample by using Miles and Mishra technique as described by Quin *et al.* (1994). The results obtained were expressed as $\log_{10}$ cfu/gram of the sample.

Immunological parameter

Haemagglutination test (HA) and haemagglutination inhibition test (HI) was performed to observe the titer against commercial strain of NDV used as an antigen. The results were expressed as $\log_2$ of the highest serial dilution showing complete inhibition of 4HA unit of the test antigen.

Statistical analysis

All the data collected during the experiment was statistically analyzed using SPSS (17.0) in complete randomized block design. The significant differences were separated by Duncan's multiple range test (1955). Replicates were used as experimental unit for analysis of all the parameters.

RESULT AND DISCUSSION

Live body weight (BW) and body weight gain (BW gain)

The results of the present experiment indicated that the effect of lysozyme was more prominent at the later stage (29-42 day) of production. In context to 0-42 d performance, broiler birds received supplementation of lysozyme had highest live body weight (1744.89 g) among all the treatment groups and control group had second highest live body weight (1722.71 g) compared to any of the treatments groups (Table 1). However, broiler birds challenged with *Clostridium perfringens* recorded significantly ($P<0.05$) lower live body weight (1494.67 g) compared to any of the control, antibiotic and lysozyme fed groups. BW gain of broiler birds were significantly ($P<0.05$) improved due to supplementation of dietary lysozyme (@ 250g/ton of feed) compared to control birds. Moreover, significant BW gain was improved in the broiler chicken challenged with *Clostridium perfringens* supplemented with lysozyme as compared to the birds challenged devoid of lysozyme supplementation. The positive response to dietary supplementation of lysozyme were recorded by Liu *et al.* (2010) by feeding exogenous lysozyme @ 40mg/kg diet in poultry without *Clostridium perfringens challenge* resulted in significant increase in BW gain in Arbour acre broiler chickens. Similarly, Latief *et al.* (2017), fed lysozyme @ 90g per ton of basal diet and observed significantly ($P<0.05$) improved body weight gain in Ross 308 broiler chickens. In 2016, Krimpen *et al.* recorded that lysozyme @ 40 mg/kg diet increased ($P<0.05$) body weight gain in male Ross 308 broiler compared to the control birds. The birds received dietary lysozyme and challenged with *Clostridium perfringens* shown better live body weight of 1598.83g as compared to the birds challenged with *Clostridium perfringens* and received dietary antibiotic (1547.32g). During challenge condition the lysozyme acted well and gave higher live body weight or gain in weight compared to antibiotic and non-antibiotic group. Few scientists have recorded contrast finding Torki *et al.* (2018) reported a decrease in body weight gain of male Ross 308 broilers fed the diets supplemented with lysozyme @ 40 mg/kg compared to the control group. Dietary supplementation @40, 100, and 200 ppm lysozyme had no significant ($P>0.05$) effects on Di-Gao broiler chickens body weight and weight gain (Xia *et al.* 2019).

Table 1: Growth performance in response to *Clostridium perfringens* challenge and lysozyme supplementation in broiler chicken

Parameters	Control (Basal diet)	Control + flavomycin	Control + Lysozyme	Control +*Cl. perfringens* challenge	Control flavomycin + *Cl. perfringens* challenge	Control lysozyme *Cl. perfringens* + challenge	SEM	P value
0-14 d								
Live BW, g	379.96	363.15	375.84	367.48	354.78	349.78	4.57	0.390
Final BW gain, g	337.17	320.71	332.28	324.32	311.62	306.94	4.56	0.399
Feed intake, g	385.72	374.92	382.53	363.58	376.12	385.11	3.65	0.546
FCR	1.14	1.17	1.15	1.12	1.21	1.27	0.02	0.483
15-28 d								
Live BW, g	1043.51	1043.44	1036.54	980.91	988.31	993.33	9.97	0.175
Final BW gain, g	663.55	680.29	660.70	613.43	633.52	643.55	10.59	0.570
Feed intake, g	1051.15	1021.83	1051.32	1028.13	996.21	932.27	17.71	0.412
FCR	1.58	1.50	1.59	1.68	1.57	1.47	0.03	0.634
29-42 d								
Live BW, g	1722.71^{a}	1669.46^{ab}	1744.89^{a}	1494.67^{c}	1547.32^{bc}	1598.83^{bc}	27.90	P<0.05
Final BW gain, g	679.20^{a}	632.92^{ab}	701.45^{a}	506.36^{b}	566.41^{b}	605.50^{b}	21.30	P<0.05
Feed intake, g	1390.34	1219.57	1394.87	1155.25	1172.63	1230.11	31.78	0.061
FCR	2.07	1.94	1.99	2.28	2.07	2.03	0.04	0.114
0-42 d								
Live BW, g	1722.71^{a}	1669.46^{ab}	1744.89^{a}	1494.67^{c}	1547.32^{bc}	1598.83^{bc}	27.90	P<0.05
Final BW gain, g	1679.92^{a}	1625.90^{ab}	1702.45^{a}	1451.50^{c}	1504.16^{bc}	1555.99^{bc}	27.92	P<0.05
Feed intake, g	2827.21^{a}	2653.42^{bc}	2791.62^{ab}	2527.58^{c}	2564.35^{c}	2547.49^{c}	33.07	P<0.01
FCR	1.68	1.63	1.64	1.74	1.70	1.64	0.02	0.278
Mortality %	8	7	6	17	12	10	8	7

*Means bearing different superscript in a row a, b and c differ significantly (P<0.05).
SEM is standard error of difference between mean values
P value is probable significance value

Feed intake and feed conversion ratio

During the entire study period feed intake (g) was not significantly affected in different growth phases except in the overall performance period (0-42 d). The birds received basal diet plus antibiotics had significantly (P<0.05) lower feed consumption (2653.42 g) than the control (2827.21 g), whereas the feed intake difference was non-significant among basal diet plus antibiotic (2653.42 g) and basal diet plus lysozyme (2791.62 g) fed birds. The feed intake difference was absolutely non-significant among the birds challenged with *Clostridium perfringens* and treated with antibiotic and lysozyme. There was non-significant difference in feed conversion ratio among all the treatment groups at any stage of production. Similar to these finding Qayyum *et al.* (2012) recorded no significant (P>0.05) change in feed conversion ratio of broiler chickens supplemented with Lisovit® at a dose rate of 100 and 200 mg/kg body weight. Xia *et al.* (2019) noted that dietary supplementation @40, 100, and 200 ppm lysozyme had no significant (P>0.05) effects on feed consumption and feed efficiency in broilers. In contrast to our study, Humphrey *et al.* (2002) recorded no significant (P>0.05) effect on feed consumption due to addition of lysozyme @ 5 and 10% in the diet of broiler chicken whereas feed conversion ratio was significantly (P<0.05) improved. The Arbor acre broiler birds supplementation with 40mg exogenous lysozyme/kg diet for birds challenged with *C. perfringens* significantly (P<0.05) improved the feed conversion ratio (Liu *et al.*, 2010). The difference in feed intake, feed conversion ratio among the birds fed on antibiotic and lysozyme was non-significant. Hence, the antibiotic can be altered with lysozyme in broiler diet to have better feed conversion ratio.

Mortality percentage

In non-challenged groups mostly the mortality was associated with heat stress, but in challenged groups heat stress and infection exaggerated the mortality percentage. Birds challenged with *Clostridium perfringens* without any supplementation recorded the highest mortality percentage and at the same time supplemented with lysozyme and antibiotic recovered the mortality rate.

Gut health and humoral immune response

CFU count: Intestinal microbiology assays resulted that supplementation of lysozyme and antibiotic gives more beneficial effect (P<0.05) on gut bacterial count at the age of 24 d, whereas the age of the bird progresses the effect may remain the same till liquidation of the flock (Table.2). The supplementation of the lysozyme significantly reduces the *Clostridium perfringens* bacteria

Table 2: *Clostridium perfringens* counts (CFU/g) and Hemagglutination inhibitor (HI) titer (log2) in broiler chicken at 24 and 42 d age

Days	Control (Basal diet)	Control + flavomycin	Control + Lysozyme	Control + *Cl. perfringens* challenge	Control flavomycin + *Cl. perfringens* challenge	Control lysozyme *Cl. perfringens* + challenge	SEM	P value
				CFU/g				
24 d	3.40^{ab}	2.96^{bc}	1.63^{c}	4.80^{ab}	4.61^{ab}	3.95^{a}	0.31	P<0.05
42 d	5.13	5.33	5.44	5.49	5.26	5.11	0.05	0.203
				HA & HI				
24 d	4.14	4.30	4.83	4.91	4.84	5.00	0.04	0.118
42 d	4.84	4.84	4.87	4.84	4.90	4.94	0.03	0.643

*Means bearing different superscript in a row a, b and c differ significantly (P<0.05).
SEM is standard error of difference between mean values
P value is probable significance value

in infected condition as compared to supplementation of the antibiotic in feed. Moreover, in non-challenged groups' supplementation of the lysozyme gave lesser value of CFU as compared to control birds. Similarly, Liu *et al.* (2010) noted that 40mg exogenous lysozyme/kg diet supplementation significantly (P<0.05) decreased *Clostridium perfringens* colonization in Arbor Acres broiler chickens. The *Clostridial* count in exogenous dietary lysozyme fed birds (@ 90g per ton of basal diet) was lowest at 21 and 35 days of age compared with other groups in Ross 308 broiler chickens (Latief *et al*., 2017).

There was no significant difference observed (P>0.05) in haemagglutination inhibition (HI) titer against the NCDV due to addition of the lysozyme or antibiotic with or without *Clostridium perfringens* challenge in broiler chicken on any of 24 and 42nd day of age.

Production economics

The minimum cost of production was recorded in lysozyme supplemented birds on the other side minimum profits were generated through the sale of birds which were exposed to *Clostridium perfringens.*

CONCLUSION

In present study it can be concluded that supplementation of Lysozyme (@ 250 gm per Ton of feed) can be a good alternative for antibiotic in commercial broiler diet for higher live body weight, gut health and overall performance without affecting performance of broiler birds.

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