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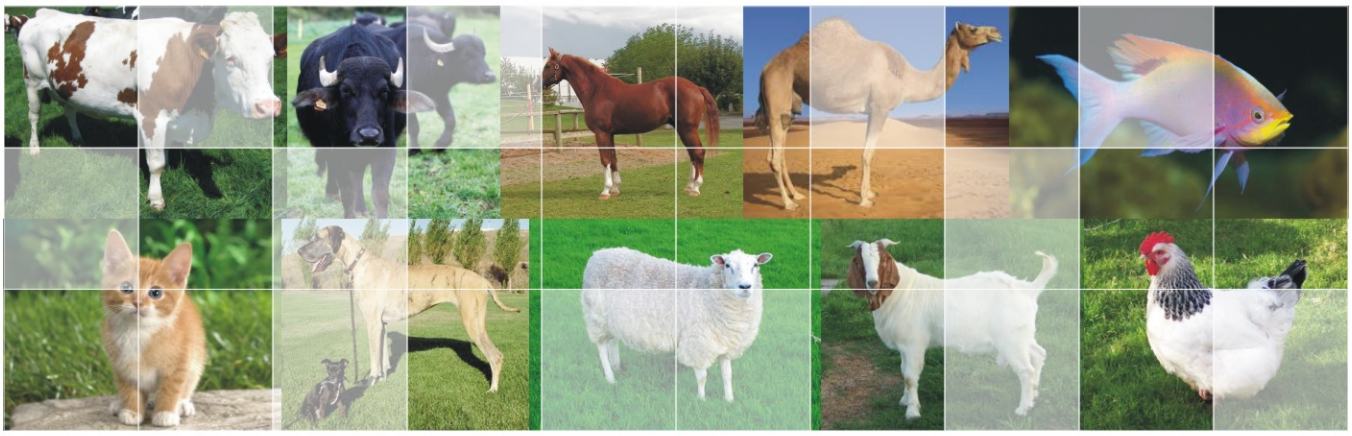

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Non Antibiotic Improve Performance and Minimizing Shedding in *Clostridium perfringens* Infected Broiler

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Abstract: This study was conducted to evaluate the efficacy of non-antibiotic biological product Saltose on *Clostridium perfringens* challenged broilers, compared with antibiotic growth promoter represented by Avilamycin. 90, one day old broiler chicks were divided into 3 experimental groups 30 chicks each, three replicates for group 10 for each. Group A was control group fed on basal diet, Group B fed on basal diet with 0.3 gm Avilamycin / kg feed and group C was fed on basal diet with 0.5gm Saltose/kg feed in starter grower and finisher. All groups were challenged with *Clostridium perfringens* (1mL of 1 x 10^8 CFU/mL orally). Efficacy of non-antibiotic product saltose was tested in laboratory culture of *Clostridium perfringens* followed by experimental challenge. Data collected before challenge and one week post challenge were; the behavior patterns including feeding, drinking, leg and wing stretching, body scratching, preening and resting. The performance parameters include final body weight, total feed intake, food conversion ratio and mortality rate, finally the estimation *Clostridium perfringens in* culture and in cloacal swab. The behaviour parameters before challenge were not significantly differ at $P>0.05$, but after challenge there were great significance difference at $P\leq0.05$. Also final body weight, feed conversion ratio and mortality rate were significantly differ at $P\leq0.05$, The count of *Clostridium perfringens* in culture broth and in cloacal swaps were reduced after adding Non antibiotic saltose in comparison with Avilamycin and control group.

Key words: Broiler · *Clostridium perfringens* · Antibiotic · Non Antibiotic · Saltose · Avilamycin

INTRODUCTION

Pathogenic organisms (e.g. *Clostridium perfringens*) inhibit the small intestines of broiler chickens as part of their micro flora dynamics. Normally, populations of these organisms do not reach levels where overt disease is recognized. Some of these bacteria however produce toxic metabolites and microscopic lesions that have the ability to interfere with broiler growth [1].

Clostridium perfringens is frequently found in the intestinal tract of healthy broilers, usually at low levels ($<10^4$ CFU/g) and is spread in the poultry production and processing environment through faeces [2]. Necrotic enteritis occurs as outbreaks characterized by depression, ruffled feathers, diarrhea, huddling, anorexia and frequently high mortality [3].

Antibiotic growth promoters (AGP) were routinely administered to animal feeds in Europe and the rest of the world as a means to improve the performance of poultry whilst at the same time, reducing the incidence and severity of disease [4]. The long term and extensive use of antibiotics for medical and veterinary purposes may eventually result in the selection for the survival of resistant bacterial species [5].

Avilamycin is one kind of nutritional antibiotics and animal feed with avilamycin supplement can prevent disease, improve nutrients metabolism and growth performance [6].

European Union decided in 1999 to ban four commonly used growth promoters namely: virginiamycin, spiramycin, tylosin and zinc bacitracin [1]. Flavomycin, avilamycin and salinomycin were discontinued from animal feeds from the January 2006 [7].

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Development of alternative products is therefore necessary to eliminate the dependence on antibiotic growth promoter to maintain performance while maintaining the same productivity [8].

Saltose is new patent non antibiotic biological product has positive effect on productive performance and minimized incidence of infection with *Clostridium perfringens* [9, 10]

The aim of our study was to evaluate the efficacy of non-antibiotic biological product Saltose in *Clostridium perfringens* challenged broilers, compared with antibiotic growth promoter represented by Avilamycin.

MATERIALS AND METHODS

Birds and Housing: A total of 90 one day-old Cobb chicks were used in this study. On arrival, chicks were randomly housed in small experimental pens with stocking density 10/m^2. Feed and water were provided ad- libitum via trough feeders and bell drinker. The birds were vaccinated with Hitchiner and infectious bronchitis (I.B.) at 6 days old, lasota at 18 and 28 days old and Gumboro at 12 days old. The chicks fed starter diet from 1-18 days old and grower diet from 19- 30 days and finally fed on finisher from 31 days to 35 days [11]. All diets used were formulated to meet the nutrient requirement of the broiler chickens according to NRC [12] and illustrated in Table 1.

Experimental Design:

Laboratory Test of non Antibiotic Product Saltose on Clostridium Perfringens Culture: Field strains of *Clostridium perfringens* were inoculated directly into cooked meat broth medium (Oxoid) and were incubated anaerobically in anaerobic Gas pack jar for 24 h at 37°C; and then harvested by centrifugation for one hour at 3000 rpm. Gram's stained smears were done from sediment and examined microscopically to check purity. Sediment was washed three times in sterile physiological saline solution to be standardized against Brown's opacity tubes to have a final concentration of $2.4x10^8$ organisms/ml.

The tested Non antibiotic product (Saltose) was diluted using 300 ppm hard water solution on the day of use. The hard water solution was prepared according to Bloder [13] as following:

- 986 ml bi-distilled water
- 6ml solution A (19.84 g anhydrous $MgCl_2$) + (46.24 anhydrous $CaCl_2$)/ L
- 8ml solution B (35.02 g $NaHCO_3$/L)

Yeast Extract: 3% yeast extract powder solution was prepared by adding 3g yeast extract to 100ml bi- distilled water.

Challenge of Chicks with Clostridium Perfringens: The chicks were divided into 3 groups; 30 birds for each, 3 replicate for each group, 10 chicks for each replicate. chicks were divided into three treatment groups, group A represent control one, feed on the basal diet without any additives, group B in which chicks feed on basal diet fortified with Avilamycin0.3 gm / kg feed according to Tuelo and Botlhoko [7] and group 3 fed on basal diet fortified with Saltose, 0.5gm/kg feed in starter grower and finisher according to El Iraqi *et al*. [10].Saltose is Patent product produced by Poultry Industry Consultant

Table 1: Composition and nutritive value of starter and grower diet used

	Components	Starter Kg/100 kg	Grower Kg/100 kg	Finisher Kg/100 kg
Ingredients use in the diet	Yellow Corn	60.28	64.3	58.7
	Soya bean meal (47%)	34.25	29.28	33.4
	Safflower oil	1.51	2.49	3.5
	Na bicarbonate	0.18	0.07	0.05
	Sodium chloride	0.33	0.33	0.33
	DL-Methionine	0.16	0.15	1.7
	Lysine	0.18	0.21	1
	Di calcium phosphate	1.49	1.52	1.8
	Lime stone	1.33	1.34	1.5
	Premix	0.3	0.3	0.3
Chemical analysis of diet	Metabolisable Energy (Kcal/kg)	2988	3083	32000
	Crude Protein (%)	21	19	18
	Crude Fat (%)	4.83	5.79	6.5
	Fiber (%)	3.7	3.42	3.4
	Calcium (%)	0.9	0.9	0.9
	Phosphorus (total) (%)	0.73	0.7	0.7
	P. Available (%)	0.40	0.4	0.4

Company (PIC-BIO, Inc.), Tokyo, Japan; it was composed of Cell Wall Lyaze 3700 U/g, *Bacillus licheniformis, Bacillus subtilis and Bacillus pumilus* 1.8 x 10^9 cfu/g, *Enterococcus faecalis and Enterococcus faecium* 2.5 x 10^8 cfu/g., Protease, Lipase, Cellulase, Amylase 12,000 U/g and Beta-Xylanase 350 U/g [9]. At 20 days old the chicks were challenged with *Clostridium perfringens* infective dose contain1 x 10^8 CFU/mL orally according to Tuelo and Botlhoko [7].

Measurements

Behavioral Measurements: The behavioral patterns were observed and measured throughout the experiment before challenge and after challenge to know if the birds behavior changed with *Clostridium perfringens* infection or not; the behavior parameters includes; feeding, drinking, wing and leg stretching, preening, Leg scratching and resting. All behavior were measured according to Altman [14], through daily instantaneous sampling for 10 minutes /2 times daily/pen; observation session was divided into short intervals and recorded whether the behavior pattern was occurred, the results were expressed as the percentage of birds performing the behavior [15].

Productive Performance: Final body weight (gram/bird), Feed intake (gram/bird), Feed conversion ratio and mortality rate (%).

Microbiological Measurements

Clostridium Perfringens Count in Culture Broth: Test was performed by thoroughly mixing 0.2 ml of the suspension with 0.8 ml of tested agent previously prepared with sterile hard water to reach 0.5 % concentration; the test was performed using 3 % yeast extract as an organic load. Samples (0.1 ml) were taken from the reaction mixture after 5, 15, 30, 60,120 minutes and 10- fold dilutions then seeded on blood agar and incubated anaerobically at 35°C for 48 hours. Colonies were counted and expressed as colony-forming units (CFU)/ml.

Clostridium Perfringens Count in Cloacal Swabs: Total 27cloacal swabs were collected from groups, 9 samples from each group, two times, at 15 days and 20 days old and one at 7days post challenge, for detection the count *Clostridium perfringens* before challenge and after incubation period post challenge according to method described by Health Protection Agency [16].

Statistical Analysis: The data statistically were analyzed using SAS Software. The comparison of means was done with Duncan [17] and in order to get regression equation and correlation coefficients. The level of statistical significance was pre-set at $p \leq 0.05$.

RESULTS

Behavioral Measurements: The behavior patterns of broiler before challenge showed that, there were no statistical significance differences between different groups as $P > 0.05$, in feeding behavior, group A, B and C were 18.3 ± 0.00 %, 19.17 ± 0.4% and 20.82 ± 1.44 % respectively, while, drinking behavior in group A was 11.67 ± 0.69 %, group B was 11 ± 1.9% and group C was 14 ± 0.28 %. Also in Leg and wing stretching, Leg scratching and preening there were no statistical significance differences between different groups as $P > 0.05$. While in resting behavior there was significant difference at $P \leq 0.05$, as group C showed higher resting behavior 42.5 ± 2.4 %, followed by group B 42.5 ± 2.4% and group A, 32.5 ± 2.42 % (Table 2).

The Behavior patterns of broiler after challenge showed statistical significance differences between different groups at $P \leq 0.05$. Although the infection with clostridia was occurred, Group C given the higher feeding frequency 16.22 ± 0.26 % followed by group B, 15.16 ± 0.95 % and group A, 15.58 ± 1.34 %. Also drinking behavior was higher in group C10.75 ± 1.35% followed by group B and C 9.29 ± 0.16% and 8.15 ± 0.43% respectively. Also there were statistical significance differences between different groups in the leg and wing stretching, Leg scratching and preening at $P \leq 0.05$. Finally the resting behavior was higher in group B 67.03 ± 3.6 % followed by group A 62.6±4.8% and group C, 61.03±3.9 % (Table 2).

Productive Performance: Performance traits of broilers including final body weight, feed intake, feed conversion ratio and mortality rate as presented in Table 3; Although all groups were challenged with *Clostridium perfringens,* there was a highly significance difference between the productive performance parameters at $P \leq 0.05$. Group C showing the highest final body weight 1636 ± 3.6 gm, following by group B 1450± 3.44 gm and group A 1350± 2.86 gm. While the feed intake recorded were 2916.67± 6.09, 2916 ± 2.83 and 2943± 4.03 gm in group A, group B and group C. Lower food conversion ratio achieved in group C 1.8 ± 0.04, followed by group B 2.01± 0.01 and

Table 2: Effect of non-antibiotic saltose and Avilamycin on Broiler behavior pattern measured as percentage before and after *Clostridium perfringens* challenge

	Before challenge			After Challenge		
Parameter	Group A	Group B	Group C	Group A	Group B	Group C
Feeding Behavior	18.3 ± 0.00[a]	19.17 ± 0.4[a]	20.82 ± 1.44[a]	15.58 ± 1.34[a]	15.16 ± 0.95[a]	16.22 ± 0.26[a]
Drinking Behavior	11.67± 0.96[a]	11 ± 1.9[a]	14 ± 0.28[a]	8.15 ± 0.43[a]	9.29 ± 0.16[ab]	10.75 ± 1.35[b]
Leg and wing stretching	9.16 ± 0.49[a]	9.2 ± 1.4[a]	13.16 ± 1.09[a]	5.03 ± 0.14[ab]	5.47 ± 0.07[a]	6.5 ± 1.03[a]
Leg scratching	10.8 ± 1.4[a]	8.3 ± 1.93[a]	9.16 ± 0.49[a]	7.0 ± 0.75[a]	4.16 ± 0.66[b]	4.4 ± 0.72[b]
Preening	11.7 ± 0.0[a]	9.96 ± 0.95[a]	10.8 ± 1.44[a]	11.2 ± 1.47[a]	5.8 ± 0.51[b]	7.7 ± 0.52[b]
Resting Behavior	32.5 ± 2.42[a]	42.5 ± 2.4[a]	42.5 ± 2.4[B]	62.6 ± 4.8[ab]	67.03 ± 3.6[a]	61.03 ± 3.9[b]

[a,b,c]Means with the different indices between groups are significantly different at p<0.05

Table 3: Effect of non-antibiotic saltose and Avilamycin on Broiler performance challenged with *Clostridium perfringens*

Parameter	Group A	Group B	Group C
Final body weight(gm)	1350± 3.86[b]	1450 ± 3.44[b]	1636 ± 3.6[a]
Food intake(gm)	2916.67 ± 6.09[a]	2916 ± 2.83[a]	2943 ± 4.03[a]
Food conversion ratio	2.16 ± 0.05[a]	2.01 ± 0.01[ab]	1.8 ± 0.04[b]
Mortality %	30 ± 0.0[a]	10 ± 0.00[b]	5 ± 0.0[c]

[a,b,c]Means with the different indices between groups are significantly different at p<0.05

Table 4: *Clostridium perfringens* count (CFU/ml) in culture Broth after 5, 15, 30, 60,120 minutes

Time	Count	Log	Log reduction
Zero	2.4 x 10^8	8.38	
5 minutes	2 x 10^6	6.30	2.08
15 minutes	1 x 10^6	6.00	2.38
30 minutes	1 x 10^5	5.00	3.38
60 minutes	4.7 x 10^4	4.67	3.71
120 minutes	4.2 x 10^4	4.62	3.76

Table 5: Effect of non-antibiotic saltose and Avilamycin on *Clostridium perfringens* count (CFU/gm) before and after challenge

		Treatment groups		
Parameter	Period	Group A	Group B	Group C
Before challenge	15 days old	5x10^2	Less than 10	Less than 10
	20 days old	7x10^3	2x10^2	Lless than 10
After challenge	7 days post challenge	23×10^{10}	14×10^7	27×10^4

group A was 2.16 ± 0.05. Also the mortality rate showing a highly significance difference between different groups, group C showed lower mortality rate 5 ± 0.0 %, followed by group B 10 ± 0.0 % and group A 30 ± 0.0 %.

Microbiological Measurements

Clostridium Perfringens Count in Culture Broth: There were great decreases in the count of *Clostridium perfringens* after different Contact times, as in Table 4, the test start with 2.4 x 10^8 CFU/ml, 5 minutes Contact with Non antibiotic saltose the count reduced 2.08 log and became 2 x 10^6CFU/ml, then after 15 minutes Incubation the count reduced 2.38 log and become 1 x 10^6 CFU/ml, the count still reduced after 30 minute, 60 minutes until reduced 3.76 log and became 4.2 x 10^4 CFU/ml after 120 minutes contact with non-antibiotic saltose.

Clostridium Perfringens Count in Cloacal Swabs: As illustrated in Table 5, there was a great difference between different groups in *Clostridium perfringens* count. Before challenge, at 15 days old, in group A the *Clostridium perfringens* count was 5×10^2/gm, while in group B and C the *Clostridium perfringens* count was less than 10 cfu/gm. At 20 days old the

Clostridium perfringens count was 7×10^3cfu/gm, 2×10^2cfu/gm and less than 10cfu/gm respectively in group A, B and C. After 7 days post challenge with 1×10^8cfu/ml *Clostridium perfringens Perfringens* the count was23×10^{10}cfu/gm in group A, 14×10^7cfu/gm in group B and 27×10^4cfu/gm in group C.

DISCUSSION

Concerning to the behavior results obtained before challenge for first 15 days, there was no significance difference between different groups these findings are in agreement with those of El Iraqi *et al*. [10] After the challenge with *Clostridium perfringens*, group C recorded a higher feeding and drinking activity followed by group B and group A, these is related to the efficacy of Non antibiotic saltose on controlling *Clostridium perfringens* infection according to Pic-Bio and El Iraqi *et al.* [10]. Also the resting behavior after challenge was higher in control group followed by group B and the lower resting behavior record in group C, this was related to the new patent product saltose enable the birds to overcome the symptoms *Clostridium perfringens*. Resting behavior less clear in group C, due to the ability of saltose to minimize the infection and decrease the severity of signs PIC-BIO.

There was a significant effect to Non antibiotic product saltose on the productive performance of broiler, although the challenge with *Clostridium perfringens*, group C showed the higher final body weight, the lower feed intake, lower food conversion ratio and minimum mortality rate this may related to the beneficial effect of Non antibiotic product that containing five beneficial bacteria *Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus* 1.8 x 10^9 cfu/g, *Enterococcus faecalis and Enterococcus faecium* 2.5 x 10^8 cfu/g, in addition to five enzymes Protease, Lipase, Cellulase, Amylase 12,000 U/g, Beta-Xylanase 350 U/g that increase palatability and digestibility of dietary nutrients, PIC-BIO [9] and El Iraqi *et al*. [10].

Concerning to the *Clostridium perfringens* count per ml/broth The reduction of the clostridium count was faster during the first few minutes than the rest of the exposure time, it could be attributed to the presence of both spores and vegetative forms in the suspension used, the vegetative bacilli may be affected more rapidly by the Non antibiotic saltose than the spores which may need more exposure time. After challenge with *Clostridium perfringens,* Antibiotic growth promoter Avilamycin showed good effect in prophylactic control of clostridium, but not able facing challenge, while Non antibiotic saltose showed excellent effect in prophylactic and control of *Clostridium perfringens.*

CONCLUSSION

Based upon findings of the study, it can be concluded that supplying broilers with Nan antibiotic feed additives Saltose was alternative to antibiotics growth promoter Avilamycin for controlling Necrotic enteritis caused by *Clostridium perfringens* and it is a step for future vision toward organic poultry production.

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