Global Veterinaria 12 (2): 257-263, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.02.82162

Evaluation of New Biological Product Saltose for Controlling Coccidia and Clostridia in Broiler Chickens

[1]K.G. El Iraqi, [2]T.M. Melegy and [3]A.O. Hassan

[1]Department of Veterinary Hygiene and Management,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[2]Department of Nutrition and clinical Nutrition,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[3]Department of Microbiology, mycology and immunology,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Saltose is one of the biological feed additives used for controlling coccidia and *clostridia,* in broiler, their effect on performance, behaviour and gut microbiology was evaluated in comparison with Salinomycin and Avilamycine. A total of 2040 one day old Cobb chicks, were divided into four groups, each consisting of 6 replicates. In group A, the chicks were fed on commercial basal diet, group B was fed on the same diet enriched with 0.5gm Salinomycin /kg feed, group C fed on the same diet enriched with 0.5gm Salinomycin /kg and 0.3 gm Avilamycine/ kg feed and group D fed on the same diet enriched with 0.5gm Saltose /kg feed. Data collected during 5 weeks experimental period were; behavioural parameters including feeding, drinking, leg and wing stretch, Leg scratch, preening and resting behaviour also broiler performance including feed intake, final body weight, feed conversion rate and mortality rate, finally gut microbiology was examined. The result obtained showing significant differences at $P \leq 0.05$ as Saltose and salinomycin plus avilamycine improve the animal feeding and drinking behaviour. Also Saltose achieved a great significant effect at $P \leq 0.05$ on broiler performance, *Lactobacillus acidophilus, total anaerobic count,* Clostridia*, E.coli* and *Eimeria* oocyst count. These results suggested that supplying broiler with Saltose as biological product or combination of salinomycin and avilamycine as chemical product, improve Boiler behaviour, productive performance and minimize incidence of *clostridium perferengense* and *coccidiosis* in the broiler flocks.

Key words: Saltose · Behaviour · Avilamycine · Salinomycin · Broiler

INTRODUCTION

Clostridium perfringens is a widespread spore-forming, Gram-positive, anaerobic, microorganism. It is recognized as an enteritic bacterial pathogen in humans, poultry, other farm animals and wildlife worldwide [1, 2]. It is often found in the intestinal tract of healthy birds but it can cause necrotic enteritis (NE) in many species of poultry and especially in broiler and turkey flocks [3].

The disease caused by *Clostridium perfringens* in poultry is called necrotic enteritis and presented in an acute or subclinical form; the acute form leads to increased mortality in the last weeks of the rearing period. The subclinical form is characterized by damage to the intestinal mucosa that decreases digestion, absorption and reduces weight gains also associated with hepatitis and cholangiohepatitis. The damage to intestinal epithelium caused by coccidia is a major predisposing factor for necrotic enteritis, allowing *Clostridium perfringens* to replicate rapidly and produce toxin, so necrotic enteritis and coccidiosis are often linked as one set of similar symptoms [4].

Coccidiosis, caused by intracellular protozoan parasite of the genus *Eimeria,* is a major parasitic disease within the intensive poultry production system. *Eimeria* species are highly host specific, with six species having an economic impact in chickens [5, 6]. In broilers,

Corresponding Author: Kassem G. El Iraqi, Department of Veterinary Hygiene and Management,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

coccidiosis control should not only address the prevention of clinical disease and mortality but also mild and subclinical infections, as even minor intestinal lesions can interfere with growth, feed conversion and therefore profitability [7].

Medication by anticlostridial and anticoccidial drugs in chicken as feed additives had been started about 30 years ago and numerous products were introduced, which are readily available and in use [8]. The intensive use of anticoccidials and anticlostridial drugs has led to the development of microbial resistance [9], in addition to the public concern of chemical residues in meat and pollution of the environment has led to restricted regulations against their in food in Europe [10, 11]. So, the main objective of the current study was to evaluate the effects of new biological product; Saltose used as anticoccidial and anticlostridial in comparison with Salinomycin used as anticoccidial and Avilamycine used as anticlostridial, on the behaviour patterns, productive performance and intestinal microbiology and parasitology in broiler.

MATERIALS AND METHODS

Birds and Housing: A total of 2040 one day-old Cobb chicks were used in this study. All chicks were housed in a broiler house belonging to Poultry Management Research Center, Faculty of Veterinary Medicine, Cairo University, Egypt. On arrival, chicks were randomly housed in experimental pens with stocking density 15/m^2. Continuous lighting was provided throughout the experiment; during brooding at one day old the ambient temperature was 35°C and gradually decreased to reach 25°C on day 21 and then kept constant. Feed and water were provided ad libitum via trough feeders and bell drinker. The birds were vaccinated against Newcastle disease and infectious bronchitis (I.B.) on day 6 of age given Hitchiner and I.B. and given lasota vaccine at day 18 and 28 of age, also vaccinated against Gumboro on day 12 of age.

Experimental Design: Chicks were divided into four groups, one control and three test groups 510 chicks each, each group divided into six replicates 85 chicks each. Group A, the chicks were fed on commercial basal diet, group B was fed on the same diet enriched with 0.5gm Salinomycin /kg feed(as anti coccidial drug), group C fed on the same diet enriched with 0.5gm Salinomycin /kg and 0.3 gm Avilamycine/ kg feed(as anti clostridial drug) and group D fed on the same diet enriched with 0.5gm Saltose /kg feed(as antibacterial product) (Table 1). The chicks fed starter diet from 1-15 days old and then fed grower diet from 16- 25 days and finally fed on finisher from 25 days to 35 days [12]. All diets used were formulated to meet the nutrient requirement of the broilers according to recommendations of the national research council [13]. The experimental diet composition and nutrient value of basal diet used were illustrated in table 2.

Measurements

Behavioral Measurements: All behavioral parameters were measured according to method of Altman [17] through; Daily instantaneous sampling for 10 minutes /2 times daily/pen; observation session was divided into short intervals and recorded the behaviour pattern occurred [18]. The behavioral parameters observed and measured includes feeding behaviour, drinking behaviour, wing and leg stretching, preening, body scratching and resting behaviour table 3.

Productive Performance: Final Body weight (gram/bird): The average final body weight was calculated at the end of experimental period. Feed intake (gram/bird) was calculated for each group at the end of the experiment, as follow the residual amount of feed was weighed and subtracted from the known weight of feed at the beginning of experiment. Feed conversion ratio (gram feed/gram gain) was calculated at the end of experiment as the amount of feed consumed per unit of body gain. Mortality rate (%), the accumulated mortality rate was calculated by subtracting the number of live

Table 1: The experimental groups treated with different feed additives

Groups	Treatment
Group A (control)	Chicks fed on basal diet without any feed additives
Group B (test)	Chicks fed on basal diet enriched with 0.5 gm Salinomycin sodium 12% / kg feed as anti coccidial drug [14].
Group C (test)	Chicks fed on basal diet enriched with 0.5 gm Salinomycin sodium 12%/ kg as anti coccidial plus 0.3 gm Avilamycine /kg as anti clostridial drug [14, 15].
Group D (test)	Chicks fed on basal diet enriched with Saltose®* powder 0.5 gm in starter and 0.25 gm/kg in grower and finisher [16].

* Saltose®: Patent product produced by Poultry Industry Consultant Company (PIC-BIO, Inc.) Gosaku Bld., 1-29-2 Nishigotanda, Shinagawa-ku, Tokyo, 141-0031, Japan and distributed by Elyoser Medicine trading Company, Egypt. Saltose composed of Cell Wall Lyaze (patent new enzyme) 3,700 U/g, *Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus* 1.8 x 10^9 cfu/g, *Enterococcus faecalis, Enterococcus faecium* 2.5 x 10^8 cfu/g, Protease, Lipase, Cellulase, Amylase 12,000 U/g and Beta-Xylanase 350 U/g [16].

Table 2: Composition and nutritive value of starter, grower and finisher diet used

Ingredients	Starter	Grower	Finisher
Yellow Corn	61.4	68.3	73.4
Soya bean meal (46%)	29.9	20.3	15.5
Full fat soy (35%)	1	1	3.2
Corn gluten meal (62%)	3.5	6.1	3.7
DL-Methionine	0.26	0.27	0.3
L-Lysine HCL	0.265	0.450	0.470
L-Threonine	0.085	0.085	0.1
Mono calcium phosphate	1.17	1.13	0.75
Lime stone	1.63	1.575	1.5
Salt	0.24	0.24	0.23
Sodium bicarbonate	0.2	0.2	0.2
Choline Chloride (60%)	0.05	0.05	0.05
Premix	0.3	0.3	0.3
Calculated chemical composition (%			
Crude Protein (%)	21.6	19.5	17.07
Metabolisable Energy (Kcal/kg)	3050	3150	3200
Calcium (%)	0.1	0.95	0.9
Phosphorus (total) (%)	0.64	0.6	0.57
Available Phosphorus (%)	0.5	0.48	0.46
Methionine%	0.63	0.63	0.61
Lysine%	1.3	1.21	1.12
Threonine%	0.95	0.85	0.77
Sodium%	0.17	0.17	0.17

Table 3: Description of each behaviour pattern measured during experiment

Behaviour	Description
Feeding (eating)	Head extended towards available feed resources while beak in or above the feeder and appear to be ingesting feed.
Drinking	Beak in contact with water in or above the drinker and appear to be drinking water.
Wing/leg stretching	Extending one wing and one leg at the same side of the body.
Preening	Beak related behaviour that beak touches the plumage of the bird itself.
Body Scratching	Scratching parts of the body especially head and neck with feet.
Resting	Head rested on something (Litter or another bird) while sitting

birds at the end of the experiment from the total number of birds at the beginning of the experiment and the product is multiplied by 100 to obtain the percentage of mortality rate.

Gut Microbiology and Parasitology: Cloacal swabs were taken at 15 and at 25 days old, three swabs for each replicate, means 18 swabs for each treatment, these swabs examined for, total *Lactobacillus* count, total anaerobic count, total *clostridia* count and total *E.coli* count [19]. Total 48 samples from the chick's droplets were collected from several representative areas of the chicken house, 6 samples from each treatment, two times at 15 and 25 days old from the top layer of material. Oocyst detection was performed according to the standard method. The Sporulated oocyst were counted per 1.0 ml of solution using the hemocytometer [20, 21].

Statistical Analysis: The data were analyzed statistically with general linear model method (GLM) in SAS Software [22]. The comparison of means was done with Duncan method [23] and in order to get regression equation and correlation coefficients, significance difference at $P \leq 0.05$.

RESULTS

Behavioral measurements: A significant reduction in the feeding behaviour at $P \leq 0.05$ was recorded as group B showed lower feeding frequency 10.37±0.73 followed by group D 11.2±0.47, the higher feeding frequency recorded in group A and group C 13.75±0.32 and 13.66 ±0.44 respectively. The higher drinking frequency recorded in control group, followed by group C 4±0.25and 3.72±0.3 respectively, while lower drinking behaviour recorded in group B followed by group D, but without significance difference, no significant difference observed between different groups in resting behaviour patterns (Table 4).

Productive Performance: Performance traits of broiler chickens including body weight, feed intake, feed conversion ratio and mortality rate were presented in

Table 4: Effect of Saltose, Salinomycin and Salinomycin plus Avilamycine on Behaviour patterns

	Group A	Group B	Group C	Group D
Feeding behaviour	13.75±0.32[a]	10.37±0.73[b]	13.66±0.44[a]	11.2±0.47[b]
Drinking behaviour	4±0.25[a]	2.96±0.3[a]	3.72±0.3[ab]	3.1±0.19[bc]
Wing and leg stretch	1.6±0.92[a]	1.6±0.2[a]	1.54±0.18[a]	1.7±0.12[a]
Leg scratch	1.8±0.18[a]	1.56±0.23[ab]	1.22±0.07[b]	1.48±0.19[ab]
Preening	6.9±0.15[a]	5.95±0.18[b]	6.25±0.3[ab]	5.8±0.38[b]
Rest	33±0.94[a]	38.4±1.4[a]	36.7±1.8[a]	37.7±2.1[a]

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

Table 5: Effect of Saltose, Salinomycin and Salinomycin plus Avilamycine on Broiler Performance

	Group A	Group B	Group C	Group D
Final Body weight	1616.7±52.1[b]	1645±38.1[b]	1748 ±27.7[ab]	1815±66.8[a]
Average feed intake	3154.6±30.5[a]	3065.4±16.5[b]	2967±27.9[c]	3026±20.1[bc]
Food Conversion rate	1.96±0.07[a]	1.919±0.15[a]	1.7±0.02[a]	1.67±0.06[a]
Mortality rate	13.7±1.94[a]	8.17±1.9[b]	7.5±1.17[b]	5.16±0.9[b]

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

Table 6: Effect of Saltose, Salinomycin and Salinomycin plus Avilamycine on Broiler Gut microbiology

	Group A		Group B		Group C		Group D	
Parameters CFU/gm	15 days old	25 days old	15 days old	25 days old	15 days old	25 days old	15 days old	25 days old
Total Lactobacillus count	22×10^6	29×10^5	23×10^4	26×10^3	28×10^4	23×10^3	27×10^6	21×10^7
Total anaerobic count	19×10^4	29×10^4	18×10^4	15×10^4	25×10^3	22×10^3	12×10^3	17×10^3
Total clostridia count	27×10^3	23×10^3	14×10^3	22×10^3	25×10^2	21×10^2	19×10^2	15×10^2
Total E.coli count	25×10^5	11×10^6	14×10^4	12×10^4	15×10^3	25×10^3	27×10^3	17×10^3

Table 7: Effect of Saltose, Salinomycin and Salinomycin plus Avilamycine on Coccidiosis in broiler chickens

	Group A		Group B		Group C		Group D	
Parameters	15 days old	25 days old	15 days old	25 days old	15 days old	25 days old	15 days old	25 days old
Eimeria oocyte concentration	+++	++++	+	+	+	+	-	-
Eimeria oocyte count /ml	12×10^3	6×10^4	5×10^2	7×10^2	4×10^2	5×10^2	5	5

table 5; there is a highly significance difference between the productive performance parameters at $P \leq 0.05$. Group D showed the highest average body weight 1815±66.8 gm, followed by group C 1748±27.7 gm, group B 1645±37.1 gm and the lower final body weight recorded in group A (the control group)1616.7±52.1 gm. The feed intake recorded were 3154.6±30.5 gm, 3065.4±16.5 gm, 3026±20.1 gm and 2967±27.9 gm in group A, group B, group D and group C respectively,. Lower food conversion rate achieved in group D 1.67±0.06, followed by group C 1.7±0.02, group B was 1.919±0.15 and higher conversion rate was in group A 1.96 ±0.07. Group D showed low mortality rate 5.16 ±0.9%, while the higher mortality rate recorded was 13.7±1.94% in group A.

Microbiology and Parasitology: The effect of different feed additive on the gut microbiology at 15 and 25 days old (Table 6). Group D showing some beneficial modification on normal flora by increasing count of *lactobacillus* species. It was 27×10^6 CFU/gm at 15 days old and became 21×10^7 CFU/gm at 25 days old. While in group A, *lactobacillus* species was 22×10^6 CFU/gm at 15 days old and became 29×10^5 CFU/gm at 25 days old. In group B and group C the *lactobacillus* count at 15 days old was 23×10^4 CFU/gm and 28×10^4 CFU/gm and became 26×10^3 CFU/gm and 23×10^3 CFU/gm respectively at 25 days old.

Total anaerobic count in group D at 15 and 25 days old was 12×10^3 CFU/gm and 17×10^3 CFU/gm respectively, while total clostridia count was 12×10^2 CFU/gm and 15×10^2 CFU/gm. Also *E coli* count was 27×10^3 CFU/gm and 17×10^3 CFU/gm at 15 and 25 days old. In group B total anaerobic count at 15 and 25 days old was 18×10^4 CFU/gm and 15×10^4 CFU/gm respectively while total *clostridia* count was 14×10^3 CFU/gm and 22×10^3 CFU/gm, also in group C the total anaerobic count and total *clostridia* count reduced than in group A. While *E coli* count was 14-12×10^4 CFU/gm and 15-25×10^3 CFU/gm in group B and group C respectively.

The effect of different feed additives on coccidiosis table 7, in group D the number of Eimeria oocyst was decreased to be 5 Sporulated oocyst/ml. Group A showed higher incidence of coccidiosis, the number of *Eimeria* oocyst was increased from 12 $\times 10^3$ /ml to 6 $\times 10^4$/ml at 15 and 25 days old respectively, while in group B the number of *Eimeria* oocyst increased from 5 $\times 10^2$ /ml to 7 $\times 10^2$/ml also in group C the number of Eimeria oocyst was increased from 4 $\times 10^2$ /ml to 5 $\times 10^2$/ml at 15 days and 25 days old respectively.

DISCUSSION

High-intensity rearing systems, particularly in the poultry industry, have resulted in a dependence on anti-clostridia and anticoccidial feed additives to provide prophylactic control against necrotic enteritis caused by *Clostridium perfringens* and protozoa infections caused by pathogenic species of Eimeria [24].

It is not allowed to routinely use any type of antibiotics or chemotherapeutics in organic farming. This means that anti clostridial drugs and anticoccidials used to prevent outbreaks of Necrotic enteritis and the parasitic disease coccidiosis *(Eimeria species),* cannot be used routinely in conventional poultry husbandry [25].

Different feed additives showed no significance difference in wing and leg stretch, leg scratch, preening and resting behaviour, these behaviour patterns used as behaviour indicator to the broiler welfare [26], this means that the feed additives for prophylactic control against necrotic enteritis and coccidiosis doesn't affect the broiler welfare.

The Saltose positive effect on the productive performance of broiler related to their composition that containing probiotics; three bacillus species and two enterococcus species and five enzymes that have positive effect on the performance of broiler [27, 28].

Salinomycin and Avilamycine reduce count of lactic acid bacteria in broiler, while Saltose has beneficial effect on the lactic acid bacteria which constitute the main normal flora in poultry gastro intestinal tract [29, 30]. Combination of Salinomycin and Avilamycine decreased in the *clostridia* count in broiler chickens and not effected on the gram negative bacteria [31] but Saltose has antibacterial effect against anaerobic bacteria special clostridia and gram negative bacteria *E coli*. The effect of Salinomycin and combination of Salinomycin and Avilamycine on *Eimeria* was related to effect of Salinomycin as chemical ionophoric coccidiostats, has great effect to control infection with coccidia [32, 33]. While the effect of Saltose on *Eimeria* was related to great effect of cell wall lyaze enzyme on lysise of the outer cell wall of Eimeria different stages resulting misshape and loss of infective characters [16].

CONCLUSSION

Based upon findings of this study, it can be concluded that supplying broiler with biological feed additives Saltose or combination of Salinomycin and Avilamycine improved broiler behaviour, productive performance and minimized incidence of infection with *Clostridium perfringens* and *coccidiosis* in the broiler flock.

ACKNOWLDGEMENTS

This study was supported by the Poultry Management Research Center, Faculty of Veterinary Medicine, Cairo University, Elyoser medicine trading Company, Egypt and PIC-BIO, Inc. Company, Gosaku Bld., 1-29-2 Nishigotanda, Shinagawa-ku, Tokyo, 141-0031, Japan.

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