Evaluation of new biological product and salinomycin anticoccidial on performance, behaviour, and *eimeria* count in broiler chickens

El Iraqi G., Kassem, and Melegy M., Tarek [1]
Department of Veterinary Hygiene and Management and [1] Department of Nutrition and clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

Abstract
This study was conducted to evaluate the efficacy of Saltose (Biological product) and Salinomycin (Ionophore product) on coccidia mixed infection in Broiler. 90 broiler chicks were divided into three experimental groups, three replicates (10 birds each). Group A was fed on basal diet, group B fed on basal diet with 0.5 gm Salinomycin 12 % / kg feed and group C was fed on basal diet with 0.5gm Saltose /kg feed. At 20 days old, all groups were challenged with 175000 mixed oocyst (*Eimeria* tenella, *Eimeria* maxima, and *Eimeria* acervulina*)* per ml oral gavage. Data collected before challenge and 7 days post challenge. The following behaviour patterns were measured; feeding, drinking, leg and wing stretching, body scratching, preening and resting, also the final body weight, total feed intake, food conversion ratio and mortality rate were recorded. Moreover the estimation of *Eimeria* oocyst count and intestinal lesions were scored. Results obtained revealed that the behaviour patterns after challenge were significantly differ at $P<0.05$, as Group C showed higher feeding $17 \pm 0.03\%$ followed by group B, $12 \pm 0.01\%$. Also resting behaviour was higher in group A 61 ± 1.38 % followed by B $49.5 \pm 3.4\%$ and C, $34 \pm 1.76\%$. Group C showed the highest average body weight 1810 ± 5.7 gm, following by group B 1710 ± 2.67 gm. The count of *Eimeria* oocyst in group A was 12×10^3 /gm before challenge and 6×10^5/gm after challenge, while in group B was 5×10^2/gm before challenge and 7×10^3/gm after challenge and in group C the count was 2.5×10^2/gm before challenge and 6×10^2/gm after challenge. From the obtained results it was concluded that biological control in Coccidiosis infection more efficient than ionophore control.

Key words: Broiler, Salinomycin, Coccidiosis, biological, Behaviour.

Introduction

Avian coccidiosis caused by intracellular protozoa of the genus *Eimeria*. The most important species affecting broiler chickens were *Eimeria acervulina, E. maxima, and E. tenella* ***(Conway and McKenzie, 2007).***Coccidiosis continues to be one of the most economically critical diseases of the industry exacting economic losses of around 800 million dollars a year; the bulk of these costs come from performance losses related to infection and the cost of anticoccidial drugs to control the disease ***(Allen and Fetterer, 2002).***

The coccidiosis control programmes depend on chemotherapy either synthetic chemicals anticoccidials or ionophores. Development of drug resistance in the field and the withdrawal period for these drugs prior to slaughter necessitate the exploration for alternative methods for controlling coccidiosis ***(Chandrakesan et al, 2009).*** Salinomycin, an ionophoric coccidiostats widely used in chicken feed ***(Johansen et al, 2007),*** is a compound that acts by transporting alkali metal ions, resulting in altered ionic gradients and disturbed physiological process in coccidia ***(Pressman, 1976).***

By 2006, the use of all antibiotic growth promoters was prohibited in the European Union. Also the use of ionophores coccidiostats is expected to be banned in the near future ***(Van et al, 2004).*** New Japanese biological feed additive saltose used efficiently for prophylactic control of coccidiosis in broiler ***(PIC-BIO, 2013)*** and ***(El Iraqi et al, 2014).*** So the objective of this study was comparative evaluation to the efficacy of biological product (saltose) and coccidiostat (Salinomycin) on broiler behaviour, performance *Eimeria* count and Intestinal scoring lesion in broiler challenged with mixed *Eimeria* oocyst.

Materials and methods

Birds and Housing:-

This study was conduct at the experimental unit at Veterinary Serum and Vaccine Research Institute, Abbassia, 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, Gumboro at 12 days old and lasota at 18 and 28 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 ***(Steven and John, 2008).*** All diets used were formulated to meet the nutrient requirement of the broiler chickens according to ***NRC (1994)*** and illustrated in table (1).

Experimental design:

The chicks were divided into three experimental groups (30/group); three replicate, (10 chicks for each). Group A; feed on the basal diet without any additives, group B fed on basal diet fortified with Salinomycin 12 %, 0.5 gm / kg feed according to ***Wafaa (2010),*** and group C fed on basal diet fortified with 0.5gm/kg Saltose(probiotic plus coccidiocidal) produced by Poultry Industry Consultant Company (PIC-BIO, Inc.), Tokyo, Japan, according to ***Pic-Bio (2013).***

Challenge of chicks with Eimeria oocyst

At 20 days old the chicks were challenged with mixed *Eimeria* sporulated oocyst with infective dose one ml oral gavage distilled water contains 175000 mixed oocyst (*E*. tenella, *E*. maxima and *E*. acervulina ***(Abbas et al, 2001 and Collier et, al., 2003).***

Measurements:-

Behavioral measurements: the behaviour patterns were observed and measured three weeks before challenge and one week after challenge, the following behaviour patterns were measured; feeding, drinking, wing and leg stretching, preening, Leg scratching and resting. All patterns were measured according to ***Altmann (1974),*** through daily instantaneous sampling for 10 minutes /2 times daily/pen; observation session was divided into short intervals and recorded whether the behaviour pattern was occurred, the results were expressed as the percentage of birds performing the behaviour ***(Reiter and Bessei, 2000).***

Productive Performance: Final Body weight was calculated at the end of experimental period, feed intake (gm/bird), feed conversion ratio and Mortality percentage.

Eimeria* oocyst count:** Total 18 samples were collected from each group, two times, 9 samples at 15 days before challenge, and 9 samples at 7 days post challenge, for estimation of oocyst per gram of faeces by Mc Master Technique ***(Peek and Landman, 2003).

Lesion scoring*:* Three birds per each group were sacrificed on day 7 post challenge to determine the lesions score as described by ***Johnson and Mreid (1970).*** The lesions caused by the three species of *Eimeria* were monitored separately.

Statistical Analysis: the data statistically were analyzed with general linear model method (GLM) in SAS Software. The comparison of means was done with Duncan, 1955, and in order to get regression equation and correlation coefficients. The level of statistical significance was pre-set at $p \leq 0.05$.

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

Results

Behavioral measurements: as illustrated in table (2), the behaviour patterns of broiler before challenge showed that, there were no statistical significance differences between different groups as $P > 0.05$, in feeding behaviour, group A, B and C were 12 ± 0.73 %, 13 ± 0.72 % and 15 ± 0.23 % respectively, while, drinking behaviour in group A was 5.4 ± 0.69 %, group B was 6.7 ± 0.0 % and group C was5.3 ± 0.77%. Also in Leg wing stretching, and preening there was no statistical significance differences between different groups as $P > 0.05$.

While in resting behaviour there was significant difference at $P \leq 0.05$, as group B showed higher resting behaviour 43.8 ± 4.5 %, followed by group C 33 ± 0.95% and group A , 29 ± 0.0%.

After challenge the broiler behaviour patterns showed statistical significance differences at $P \leq 0.05$. Although the infection with coccidia was occurred, Group C given the higher feeding frequency 17 ± 0.03% followed by group B, 12 ± 0.01% and group A, 10 ± 0.14%. Also drinking behaviour was higher in group C 6.7 ± 1.21 % while in group A and B, 4.7 ± 0.46% and 4 ± 0.17% 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 behaviour was higher in group A showed 61 ± 1.38 % followed by group B 49.5 ± 3.4% and group C, 34 ± 1.76%.

Productive Performance: Performance of broilers as presented in table 3; there was a highly significance difference between the productive performance parameters at $P \leq 0.05$. Group C showing the highest average body weight 1810 ± 5.7 gm, following by group B 1710 ± 2.67 gm, and group A 1671 ± 4.06 gm. While the feed intake recorded were 3250± 4.6, 3050± 2.83 and 3116± 4.03 in group A, group B, and group C. Lower food conversion rate achieved in group C 1.72 ± 0.04, followed by group B 1.78 ± 0.03, and group A was 1.94 ± 0.007. Also the mortality rate showing a highly significance difference between different groups, group C showed lower mortality rate 2.5± 0.0 %, followed by group B 12.5± 0.0 %, and group A 15± 0.0 %.

Eimeria* oocyst count:* there was a great difference between different groups in oocyst count, in group A the oocyst count was 12×10^{3} /gm before challenge and 6×10^{5}/gm after challenge, while in group B was 5×10^{2}/gm before challenge and 7×10^{3}/gm after challenge and in group C the count was 2.5×10^{2}/gm before challenge and 6×10^{2}/gm after challenge, this reflect on the oocyst concentration and *Eimeria* oocyte index as illustrated in table 4.

Scoring Lesion*:* The lesions of coccidia were recorded, as group A showed scoring lesion ++++ in *E. Tenella* and *E.acervulina*, +++ in *E.maxima* while group B showed +++ in *E. Tenella* and ++ in *E. maxima* and *E.acervulina* so finally group C showed scoring lesion + in both *E. Tenella* and *E.acervulina* and *E.maxima was* zero lesion as presented in table 5.

Table 2: *Effect of Biological and Ionophore product on Broiler behaviour patterns measured as percentage before and after coccidia challenge.*

	Before challenge			After Challenge		
Parameter	Group A	Group B	Group C	Group A	Group B	Group C
Feeding Behaviour	12 ± 0.73 ^{a}	13 ± 0.72 ^{a}	15 ± 0.23 ^{a}	10 ± 0.14^{c}	12 ± 0.01^{b}	17 ± 0.03^{a}
Drinking Behaviour	5.4 ± 0.69 ^{a}	6.7 ± 0.0 ^{a}	5.3 ± 0.77 ^{a}	4.7 ± 0.46 ^{a}	4 ± 0.17 ^{a}	6.7 ± 1.21 ^{b}
Leg and wing stretching	7 ± 0.23 ^{a}	4 ± 0.58 ^{a}	4.6 ± 0.23^{a}	2.5 ± 0.11^{ab}	3 ± 0.4 ^{a}	1.26 ± 0.14^{b}
Leg scratching	6.6 ± 0.49^{ab}	8 ± 0.95 ^{a}	5.4 ± 0.23 ^{b}	2.3 ± 0.01^{a}	2.7 ± 0.14^{a}	1.9 ± 0.03^{b}
Preening	8.8 ± 0.25 ^{a}	7.5 ± 0.0 ^{a}	9 ± 0.95 ^{a}	5 ± 0.54^{a}	4.6 ± 0.11^{a}	3.4 ± 0.14^{b}
Resting behaviour	29 ± 0.0 ^{b}	43.8 ± 4.5 ^{a}	33 ± 0.95 ^{b}	61 ± 1.38^{b}	49.5 ± 3.4 ^{b}	34 ± 1.76^{a}

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

Table 3: *Effect of Biological and Ionophore product on Broiler performance after coccidia challenge.*

Parameter	Group A	Group B	Group C
Final body weight	1671± 4.06 ^{b}	1710 ± 2.67 ^{ab}	1810 ± 5.7 ^{a}
Food intake	3250 ± 4.6 ^{a}	3050 ± 2.83 ^{a}	3116 ± 4.03 ^{a}
Food conversion ratio	1.94 ± 0.007 ^{a}	1.78 ± 0.03 ^{b}	1.7 ± 0.04 ^{b}
Mortality	15 ± 0.0 ^{a}	12.5 ± 0.00 ^{b}	2.5 ± 0.0^{c}

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

Table 4: *Effect of Biological and Ionophore product on oocyst count and concentration after coccidia challenge.*

Parameter	15 days old Before coccidia challenge.			7 days After coccidia challenge.		
	Group A	Group B	Group C	Group A	Group B	Group C
Eimeria oocyte concentration	+++	+	-	++++	++	+
Eimeria oocyte index	3	1	0	4	2	1
Eimeria oocyte count /g faeces	12×10^{3}	5×10^{2}	2.5×10^{2}	6×10^{5}	7×10^{3}	6×10^{2}

Table 5: *Effect of Biological and Ionophore product on coccidia lesion score.*

Group	*E. Tenella*	*E. Maxima*	*E. Aceruvolina*
Group A	++++	+++	++++
Group B	+++	++	++
Group C	+	0	+

Discussion

In this study the behaviour of broiler before challenge for first 15 days, there was no significance difference although group C recorded the higher feeding behaviour compared with group A and B. This slight improvement may be related to the effect of saltose as probiotic via containing five species of beneficial bacteria that increase palatability and digestibility of dietary nutrients; these findings are in accordance with ***Naglaa (2013).*** while Salinomycin group showing lower feeding behaviour this confirm the previous result of ***Yvore et al (1980)*** who declared that Salinomycin reduced broiler feed intake.

After the challenge with *Eimeria* oocyst, also saltose group recorded a higher feeding and drinking activity followed by salinomycin group, these is related to the efficacy of saltose and salinomycin on coccidia infection according to ***Pic-Bio (2013)*** ; ***El Iraqi et al (2014)*** and ***Johansen et al (2007).*** Also higher resting behaviour after challenge recorded in control group followed by salinomycin group and the lower resting behaviour recorded in saltose group; this may be related to the sever signs of coccidia in control group, as sick bird appeared ruffled feathers, huddling, depression according to ***Conway and McKenzie (2007)*** and ***McDougald and Reid. (1991).*** Resting behaviour less clear in saltose group and salinomycin, due to the ability of both to minimize the infection with coccidia and decrease the severity of signs.

Saltose has a significant effect on the productive performance of broiler compared with salinomycin and control, It showed the higher final body weight, the lower feed intake, lower food conversion ratio and minimum mortality rate after challenge, this related to the beneficial effect of saltose as biological probiotic 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 and Cell Wall Lyaze 3,700 U/g that able to control the coccidial infection so minimizing mortality rate according to ***PIC-BIO (2013)*** and ***(El Iraqi et al (2014)***. The significant reduction in body weight of broilers fed on Salinomycin, growth reduction was related to reduced feed intake according ***Morrison et al (1979)*** and ***Hossein et al (2010)*** concluded that salinomycin adverse effects on chicken performance and intestinal morphology, especially villus dimensions and absorptive surface.

Concerning to the oocyst count per gram faeces of birds from the different groups was observed from day 7 post challenge. There was significant difference between the groups. However, lower count was observed in Saltose group 6×10^2 oocyst also lower *Eimeria* oocyte index and concentration as compared to other groups. This is related to the potent effect of saltose on coccidian microorganism ***PIC-BIO (2013)*** and ***El Iraqi et al (2014).*** Also salinomycin group showed reduced number of oocyst after challenge 7×10^3 this related to efficacy of salinomycin as ionophoric coccidiostat. Finally lesion scores recorded in different treatment group, control group showed complete ballooning and distension of intestine and caecum packed with caseous cores, while Salinomycin treated group showed Moderate ballooning of caecal loops, but in saltose group there was few petechial haemorrhage in the cecal wall with the presence of normal contents.

Conclusion

Based upon findings of this study, it can be concluded that supplying broiler with new biological feed additives Saltose, can improve performance, and behavioural pattern of infected birds in addition increasing bird ability for recovery from coccidia and minimize weight losses; so it can be used as feed additives for treatment of coccidia mixed infection as an alternative to Ionophore anticoccidials.

Acknowldgement

This study was supported by PIC-BIO, Inc. Company, Gosaku Bld., 1-29-2 Nishigotanda, Shinagawa-ku, Tokyo, 141-0031, Japan and Elyoser medicine trading Company, Egypt.

References

Abbas R. Zahid M., Shokat H., Zafar I., Muhammad N.,Muhammad K., Muhammad A., and Arfan Y. (2011): Anticoccidial activity of hydrochloric acid against Eimeria tenella in broiler chcikens. Pesquisa Veterinaria Brasileira.31 (5)425-429.

Allen, P. C., and R. H. Fetterer. (2002): Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin. Microbiol. Rev. 15:58-65.

Altman, J. (1974): Observational study of behaviour: sampling methods. Behaviour. 49: 227–267.

Chandrakesan Periasamy, Kathirvel Muralidharan, Vijayakumar Dinesh, Kumar, Gurusamy, Ponnudurai, Tirunelveli Jeyagopal Harikrishnan and Kandasmy Senthil Veland Natarajan Rani (2009): Efficacy of a herbal complex against caecal coccidiosis in broiler chickens. Veterinarski arhiv. 79: 2: 199-203

Collier, C.T., Van Der Klis, J.D., Deplancke, B., Anderson, D.B. and Gaskins,H.R. (2003): Effects of tylosin on bacterial mucolysis, Clostridium perfringens colonization, and intestinal barrier function in a chick model of necrotic enteritis. Antimicrobial Agents Chemotherapy, 47: 3311-3317.

Conway, D. P. and McKenzie M. E. (2007): Poultry coccidiosis diagnosis and testing procedures, pp.41-45. 3rd ed. Blackwell publishing.

Duncan, D. B. (1955): Multiple range and muliple F tests. Biometrics. 11: 1- 42.

El Iraqi K.G., Melegy T.M. and Hassan A.O. (2014): Evaluation of New Biological Product Saltose for Controlling Coccidia and Clostridia in Broiler Chickens. Global Veterinaria 12 (2): 257-263, 2014

Hossein Hassanpour, Shahab Bahadoran, Sanaz Koosha, Elaheh Askari and Sima Homai (2010): Effect of Diclazuril, Semduramicin, Salinomycin and Maduramycin as Preventive Anticoccidial Drugs on Chicken Intestinal Morphology. Global Veterinaria 5 (1): 01-05, 2010

Johansen, C. H., L. Bjerrum and K. Pedersen (2007): Impact of salinomycin on the intestinal microflora of broiler chickens. Acta Vet. Scand., 49(1): 30-38.

Johnson, J., and Mreid W. (1970): Anticoccidial drugs, lesion scoring techniques in battery and floor pen experiments with chickens. Exp. Parasitol. 28, 30-36

McDougald, L. R., and W. M. Reid. (1991): Coccidiosis. In Diseases of Poultry, 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., 780–97. Ames, IA: Iowa State Univ. Press.

Morrison, W. D., Ferguson, A. E. and Leeson, S.(1979): Efficacy of salinomycin and stenoral against various species of *Eimeria* and effect on chick performance. Poult. Sci. 58: 1 160-1 166.

Naglaa, M. Abdel- Azeem (2013): Do probiotics affect the behavior of turkey poults?,Journal of Veterinary Medicine and Animal Health, 5(5), 144-148.

NRC (National Research Council) (1994): Nutrient requirements of Poultry, pp. 19-26.7th ed., National academy press, Washington. D.C.

Peek, H. W., and W. J. Landman. (2003): Higher incidence of Eimeria spp. field isolates sensitive for diclazuril and monensin associated with the use of live coccidiosis vaccination with paracox-5 in broiler farms. Avian Dis. 50:434-439.

P**IC-BIO (2013): (Poultry Industry Consultant Biotechnology Company)** Saltose. September 20. Available from www.bio-live.com/en/static/pdf/saltose_A4.pdf

Pressman, B. C. (1976): Biological applications of inophores. Ann. Rev. Biochem., 45: 501-529.

Reiter, K. and W. Bessei (2000): Effect of stocking density of broilers on temperature in litter and at bird level. Archv. Fuer. Geflugelkunde, 64: 204-206.

Steven, L. and John D. S. (2008): Feeding program for Broiler chickens. In: Commercial Poultry Nutrition, 3rd ed. pp.229-296. British Library.

Van Immerseel F, De Buck J, Pasmans F, Huyghebaert G, Haesebrouck F, Ducatelle R. (2004): Clostridium perfringens in poultry: an emerging threat for animal and public health. Avian Pathol, 33:537-549.

Wafaa, A. A. (2010): Comparative Evaluation on the effect of coccidiostats and symbiotic preparations on prevention of Clostridium perfringens in Broiler chickens, Glob. vet. 5(6): 324-333.

Yvore, P., Raynaud, J. P., Conan, L.and Naciri, M. (1980): Evaluation of the efficacy of salinomycin in the control of coccidiosis in chicks. Poult. Sci. 59:2412-2416.