Comparative Efficacy of Immucox Vaccine and Salinomycin in Control of Experimental Infection of Caecal *Eimeria* Strain in Chickens

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ABSTRACT

One hundred and twenty (120), one-day-old Ross chicks were used to compare Immucox; a coccidiosis vaccine; and salinomycin coccidiostat. Chicks were divided into four equal groups each in a separate pen cage. First group was not infected and not treated, second group fed ration without anticoccidial supplementation, and third group was vaccinated with immucox vaccine in drinking water at 7-day-old and fed ration without anticoccidial supplementation. While fourth group fed ration supplemented with salinomycin (60 ppm) along experimental period. The 2nd, 3rd and 4th groups were challenged with $1X10^5$ sporulated oocysts of caecal *Eimeria* strain at 28-day-old. Five litter samples were collected from different areas in each floor pen cage for counting of oocysts per gram of faeces. Clinical signs of chickens were recorded. On the 7th day PC, five chickens from each group were slaughtered for PM findings, lesion scores in the caeci and specimens of the caeca were taken after slaughtering and prepared for histological examination and numbers of developmental stages per Lieberkohn gland of caecum were counted from stained slides. The averages of body weight, gain, FCR and the mortality rates were recoded.

Post challenge (PC), the broiler chickens infected with caecal *Eimeria* strain and not treated showed clinical sings of coccidiosis. Immucox vaccinated chickens showed illness and asymmetry of growth in some individuals with creamy and reddish droppings. Meanwhile, broiler chickens fed ration supplemented with salinomycin showed mild signs. Overall mean of oocyst output per gram of faeces in salinomycin-treated broilers showed a reduction when compared with immucox-vaccinated ones. Reduction percentage of oocyst output in chickens treated with salinomycin was markedly higher than that of immucox vaccinated chickens.

On the 7th day PC, there was a significant decrease in the caecal lesion scores in salinomycin-treated broilers when compared with immucox vaccinated ones. Histopathologically, numerous developmental stages of caecal *Eimeria* strain were detected in the epithelial cells of Lieberkohn glands of the caeca in the positive control group (challenged and not treated). Salinomycin-treated chickens showed inflammatory reaction with very few developmental stages of *Eimeria* in the epithelial cells of Lieberkohn glands. Whereas, immucox-vaccinated ones showed severe lymphocytic infiltration with few developmental stages of caecal *Eimeria* strain. Broiler chickens vaccinated with immucox vaccine showed significant decreases in body weight and weight gain with unimproved feed conversion ratio (FCR) at 14, 21 and 28 days post vaccination if compared with salinomycin-treated birds. However, the body weight and weight gain of broiler chickens treated with salinomycin were increased with an improvement of FCR when compared with immucox-vaccinated broilers. Mortality rates were 3.33 % in salinomycin-treated chickens, 6.66 % in chickens vaccinated with immucox and 20 % in the positive control group. In conclusion, the present study showed that salinomycin is likely to remain the major method of coccidiosos control in broiler chickens to decrease the oocyst output, lesion scores and mortality rates with improvement of the growth performance.

INTRODUCTION

Chicken coccidiosis is an intestinal infection caused by the intracellular protozoan of the genus *Eimeria*. It is the major parasitic disease of poultry, with economic burden estimated to cost the industry more than $ 800 million in annual losses world-wide *(1)*. Modern intensive poultry

production is largely dependent upon chemoprophylaxis for control of coccidiosis *(2, 3)*; although there is a rising problem of drug resistant strain of *Eimeria*.

Live vaccines against coccidiosis in chickens have been used by poultry industry. Since the introduction of coccivac was in 1960, immucox in the mid of 1980's, paracox and livacox in the late of 1980's. Live coccidial vaccines provide the initial inoculums which subsequent acquire oocyst passed in the litter. *Eimeria* species exhibit different tissue and organ specificity in the infected host, so understanding the interplay between the host and the parasites in the intestine is crucial for the design of novel control approaches against coccidiosis *(4)*. The introduction of alternative prevention measures such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity may help to limit the use of anticoccidials in control of chicken coccidiosis *(5)*.

Vaccination with live oocysts may turn into a popular alternative to the use of coccidiostats in broilers, although cocci vaccination is frequently linked to temporary lower performance in young flocks. Chickens that were not cocci vaccinated and were fed two specific essential oil blends had better feed conversion ratio (FCR) and lesion scores than the unmedicated control in starter period. No beneficial effects on live performance were observed due to these specific essential oil blends in cocci-vaccinated broilers *(6)*. Immucox vaccine contained *E. acervulina, E. maxima, E. necatrix* and *E. tenella*. Immucox vaccine in roaster chickens resulted in faster body weight gain than monensin-treated group, while FCR and morality rates were not significantly differed between the vaccinated and monensin-treated birds *(7)*. The vaccinated flocks with immucox have higher average percent survival as compared to the salinomycin- or halofuginone-treated controls. The vaccinated birds were heavier than the medicated ones with an improvement of FCR compared to the medicated controls *(8)*.

Prevention of clinical signs and minimization of pathological changes in the intestine was achieved by vaccination of chickens with low-virulent strains of *E. acervulina, E. maxima* and *E. tenella (9)*. Meanwhile, gel-immunization of immucox vaccine enhanced the protection against coccidiosis *(10)*.

Polyether ionophorous antibiotics still achieve sufficient prevention and control of coccidiosis. Massive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis *(2, 3)*. In addition to chemotherapy, the use of live vaccines for control coccidiosis is also well established *(11)*.

Body weight of salinomycin-treated chickens was increased compared to untreated controls *(12)*. Salinomycin at 60 to 100 parts per million (ppm) showed a significant anticoccidial activity against infections with single species of *E. acervulina, E. necatrix,* and *E. brunetti* resulting in improved mortality, weight gain, feed conversion, dropping scores, and lesion scores *(13)*.

Histopathological examination of the chicks infected with *E. acervulina, E. maxima* and *E. tenella* and treated with salinomycin showed that the parasites were destroyed within host cells during asexual development. Most sporozoites were destroyed 30-72 hr after ingestion of the oocyst. The activity against asexual stages was so complete, meanwhile only a limited number of parasites survived to form gamonts, and then the oocyst shedding was reduced by 80-90% *(14)*.

Sixty ppm of salinomycin was highly efficacious based upon improved performance (weight gain and feed conversion ratio), lesion score, hematocrit, and serum optical density compared with monensin, halofuginone-medicated groups or unmedicated ones *(15)*. Salinomycin reduced the lesion scores significantly and improved the body weight and feed conversion in chickens, while the birds treated with salinomycin-monensin shuttle programs had similar lesion scores, body weight, and feed conversion to the salinomycin-treated ones *(16)*. Salinomycin was the most effective against field isolates of *E. acervulina* where various shuttle programs had been employed *(17)*.

Lesions were scored in birds to compare salinomycin-medicated birds after using low, medium, and high levels of inocula *(18)*. Oocyst output of birds medicated with salinomycin or halofuginone for 6 weeks was less than that of birds medicated for 4 or 5 weeks *(19)*. Salinomycin was better than lasalocid on performance of broiler chickens *(20)*.

The present study was designed to compare between coccidiosis vaccine (immucox) and coocidiostat (salinomycin) through economical (body weight, gain, FCR) and parasitological parameters (oocyst output) as well as lesion scores and mortality rate.

MATERIAL AND METHODS

Coccidial vaccine

Immucox vaccine; live coccidiosis vaccine; (Vetech Lab. Inc, 131, Malcolm Road, Guelf, Ontario, Canada). Immucox is a patent vaccine containing *E. acervulina, E. maxima, E. necatrix* and *E. tenella*. The vaccination of chicks was carried out according to the enclosed manufacture pamphlet.

Coccidiostat drug

Salinomycin; ionophorous compound; (Coxistac®, Pfizer comp.) was used in ration at a concentration of 60 ppm from 1st day to 6th week of age.

Birds and experimental design

One hundred and twenty (120), one-day-old Ross chicks (male and female) were purchased from Dakahlia Poultry Company and leg bangled. The chicks were divided into four equal groups (30 chicks per each). The birds were allocated in separate units of floor pen cages using the ranking method *(21)*. The birds were subjected to routine vaccination program against NDV, IB and IBD diseases and fed on commercial ration and boiled then cooled water *ad libitum*.

The first group was served as negative control (non-infected and non-treated). The second group was fed ration without supplementation of anticoccidial drugs, the third group was vaccinated with immucox vaccine in drinking water at 7-day-old and fed ration without supplementation of anticoccidial drugs. While the fourth group was fed ration supplemented with salinomycin (60 ppm) allover the experimental period. The last three groups (2nd, 3rd and 4th) were challenged with $1X10^5$ sporulated oocysts of caecal *Eimeria* strain at 28-day-old. Clinical signs were recorded. Five litter samples were collected from different areas in each floor pen cage four times PC within four days interval for counting of shedded oocysts per gram of faeces. The litter samples were soaked in 2.5 % potassium dichromate solution and then sieved in clean plastic containers till counting of the oocysts according to Long and Rowell *(22)*. On the 7th day PC, five chickens from each group were slaughtered and the lesions in the caeci were described and then scored according to Johnson and Reid *(23)*. Specimens of the caeca of all groups were taken immediately after slaughtering and fixed in 10% neutral buffered formalin. Paraffin sections were stained with H&E for examination according to Bancroft *et al (24)*. The numbers of developmental stages / Lieberkohn gland of caecum were counted from stained slides. The averages of body weight, gain, FCR were recoded every week and the mortality rates allover experiment was recoded *(25)*.

Statistical analysis

The obtained data were statistically analysed by Duncan multiple range test *(26)* and least significant difference (LSD) using a computer program *(27)*.

RESULTS

1.Clinical signs

The broiler chickens infected with caecal *Eimeria* strain and not treated (positive control) showed a decrease of the appetite, dullness, ruffled feather, diarrhea and bloody droppings. Immucox vaccinated chickens showed illness and asymmetry of growth in some individuals with creamy and reddish droppings just 1 week post vaccination as well as post challenge. Broiler chickens fed ration

supplemented with salinomycin showed mild signs.

2.Oocyst output in faeces

Post challenge, overall mean of oocyst output per gram of faeces in broiler chickens fed ration supplemented with salinomycin showed a significant reduction if compared with those immunized with immucox vaccine at $P \leq 0.05$ (Table, 1). Reduction percentage of oocyst output in chickens fed ration supplemented with salinomycin was markedly increased than that of immucox vaccinated chickens after challenge with caecal *Eimeria* strain (Table, 1).

Table 1. Oocyst output per gram faeces in chickens fed ration supplemented with salinomycin or vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of *caecal Eimeria* strain at 28-day old. (Mean ± SE, n=12)

Age (Days) \ Groups	Oocyst output per gram faeces ... no x 10^3 at groups				LSD
	(1)	(2)	(3)	(4)	
34	0.00 ^{d} ± 0.00	21.58 ^{a} ± 0.87	5.25 ^{c} ± 0.49	9.50 ^{b} ± 0.95	4.25 *
38	0.00 ^{d} ± 0.00	153.25 ^{a} ± 2.82	15.08 ^{c} ± 0.58	65.42 ^{b} ± 1.19	15.08 *
42	0.00 ^{c} ± 0.00	320.50 ^{a} ± 16.78	8.00 ^{c} ± 0.49	47.42 ^{b} ± 2.06	39.42 *
46	0.00 ^{b} ± 0.00	527.75^{a} ± 19.41	3.92 ^{b} ± 0.45	29.02 ^{b} ± 0.79	29.00 *
Overall mean	0.00 ^{b} ± 0.00	255.77 ^{a} ± 28.33	8.06 ^{b} ± 0.68	37.83 ^{b} ± 3.10	217.94 *
Production %	0	100	3.15	14.79	-----
Reduction %	0	0	96.85	85.21	-----

Data were analyzed by One Way ANOVA. (*) = Significant value.

LSD: Least significance difference among means at $P \leq 0.05$.

Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Group (1): Negative control (Not treated and not challenged).

Group (2): Positive control (Not treated and challenged).

Group (3): Fed ration supplemented with salinomycin and then challenged.

Group (4): Immunized with Immucox vaccine and then challenged.

3.Post mortem findings

The slaughtered chickens of coccidia challenge showed that the caecal pouch may become greatly enlarged and distended with clotted blood, visible caecal core become hardened and drier; eventually it is passed in the faeces. The infection can be seen from the serosal surface of the caeca as dark petechiae and foci that become coalesced. Broiler chickens fed ration supplemented with salinomycin showed mild lesions in the caecai when compared with that of immucox-vaccinated chickens.

4. Caecal lesion scores and Mortality rates

On the 7th day post challenge, there was a significant decrease in the caecal lesion scores in broiler chickens fed ration supplemented with salinomycin when compared with that of chickens immunized with immucox vaccine at $P \leq 0.05$ (Table, 2). Four chickens from the positive control (infected and non-treated) died in the 2nd week post vaccination and two birds post challenge. Two birds were died in the group vaccinated with immucox, one post vaccination and another post challenge. Meanwhile, only one bird was died in the salinomycin-treated group PC (Table, 2).

Table 2.Lesion scores and mortality rates in chickens fed ration supplemented with salinomycin or vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of *caecal Eimeria* strain at 28-day old. (Mean ± SE, n=10)

Parameters \ Groups		(1)	(2)	(3)	(4)	LSD
Lesion scoring on the 7th day Post challenge		0.00 ^{d}±0.00	3.50 ^{a}±0.17	1.10 ^{c}± 0.22	1.90 ^{b}±0.23	0.70 *
Mortality rate along experimental period	Dead	0	6	1	2	-----
	Total	30	30	30	30	-----
Mortality %		0.00 %	20.00 %	3.33 %	6.66 %	-----
Survival %		100 %	80 %	96.67 %	93.34 %	-----

Data were analyzed by One Way ANOVA. (*) = Significant value.
LSD: Least significance difference among means at $P \leq 0.05$.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.
Group (1): Negative control (Not treated and not challenged).
Group (2): Positive control (Not treated and challenged).
Group (3): Fed ration supplemented with salinomycin and then challenged.
Group (4): Immunized with Immucox vaccine and then challenged.

5.Growth performance

Broiler chickens vaccinated with immucox vaccine showed significant decreases in body weight and weight gain with unimproved feed conversion ratio (FCR) at 14, 21 and 28 days post vaccination when compared with that of salinomycin-treated birds. Post challenge, the body weight and weight gain of broiler chickens fed ration supplemented with salinomycin were significantly increased with an improvement of FCR when compared with that of immucox-vaccinated chickens at $P \leq 0.05$ (Table, 3).

Table 3.Growth performance parameters in chickens fed ration supplemented with salinomycin or vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of *caecal Eimeria* strain at 28-day old. (Mean ± SE, n=30)

Days PI	Growth performance parameters	Groups				LSD
		Group (1)	Group (2)	Group (3)	Group (4)	
One-day old	Body weight (gm)	39.93 ^{a} ± 0.33	39.82 ^{a} ± 0.34	39.97 ^{a} ± 0.33	40.07 ^{a} ± 0.35	NS
7	Body weight (gm)	172.40 ^{a} ± 1.30	171.73 ^{a} ± 1.41	171.80 ^{a} ± 1.43	172.3 ^{a} ± 1.29	NS
	Body gain (gm)	133.40 ^{a} ± 1.43	132.57 ^{a} ± 1.34	132.33 ^{a} ± 1.40	131.30 ^{a} ± 2.27	NS
	FCR	1.584 ^{a} ± 0.017	1.587 ^{a} ± 0.018	1.587 ^{a} ± 0.020	1.593 ^{a} ± 0.022	NS
14	Body weight (gm)	322.67 ^{a} ± 2.10	323.83 ^{a} ± 1.11	321.27 ^{a} ± 2.13	294.83 ^{b} ± 3.76	26.43*
	Body gain (gm)	149.43 ^{a} ± 1.67	150.73 ^{a} ± 1.54	148.87 ^{a} ± 1.80	129.60 ^{b} ± 2.42	19.77*
	FCR	1.758 ^{b} ± 0.022	1.775 ^{b} ± 0.030	1.776 ^{b} ± 0.043	2.185 ^{a} ± 0.037	0.390*
21	Body weight (gm)	636.00 ^{a} ± 1.78	631.87 ^{ab} ± 4.12	626.53 ^{b} ± 2.34	564.33 ^{c} ± 2.98	9.74*
	Body gain (gm)	313.70 ^{a} ± 2.12	312.53 ^{a} ± 2.48	310.92 ^{a} ± 1.87	268.17 ^{b} ± 3.07	9.63*
	FCR	1.929 ^{b} ± 0.018	1.937 ^{b} ± 0.017	1.958 ^{b} ± 0.020	2.539 ^{a} ± 0.015	0.401*
28	Body weight (gm)	1146.17 ^{a} ± 2.61	1145.05 ^{a} ± 1.69	1142.83 ^{a} ± 1.40	1110.83 ^{b} ± 2.70	5.17*
	Body gain (gm)	510.17 ^{a} ± 1.99	511.97 ^{a} ± 2.30	513.00 ^{a} ± 2.80	482.83 ^{b} ± 3.62	29.83*
	FCR	2.238 ^{b} ± 0.013	2.268 ^{b} ± 0.018	2.269 ^{b} ± 0.016	2.502 ^{a} ± 0.027	0.175*
35	Body weight (gm)	1572.33 ^{a} ± 12.34	1193.17 ^{c} ± 4.98	1311.83 ^{b} ± 3.41	1310.33 ^{b} ± 2.80	118.17*
	Body gain (gm)	425.17 ^{a} ± 12.04	47.50 ^{d} ± 5.55	201.50 ^{b} ± 3.25	173.78 ^{c} ± 4.12	27.63*
	FCR	2.390 ^{d} ± 0.020	3.559 ^{a} ± 0.056	2.565 ^{c} ± 0.022	2.846 ^{b} ± 0.030	0.175*
42	Body weight (gm)	2131.33 ^{a} ± 1.86	1453.00 ^{d} ± 3.67	1898.83 ^{b} ± 4.24	1860.33 ^{c} ± 4.78	38.50*
	Body gain (gm)	584.83 ^{a} ± 4.94	256.50 ^{d} ± 8.18	563.33 ^{b} ± 12.62	505.50 ^{c} ± 4.55	39.33*
	FCR	2.483 ^{d} ± 0.024	3.248 ^{a} ± 0.059	2.655 ^{c} ± 0.022	2.787 ^{b} ± 0.325	0.131*

Data were analyzed by One Way ANOVA. (*) = Significant value. NS = Non significant.
LSD: Least significance difference among means at $P \leq 0.05$. Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.
Group (1): Negative control (Not treated and not challenged).
Group (2): Positive control (Not treated and challenged).
Group (3): Fed ration supplemented with salinomycin and then challenged.
Group (4): Immunized with Immucox vaccine and then challenged.

6.Histopathological findings

The histopathological changes are recorded in Table (4).

Table 4. The histopathological findings in chickens fed ration supplemented with salinomycin or vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of *caecal Eimeria* strain at 28-day old.

Groups	Lesions of caeci on the 7th post challenge with 1 x 10^5 sporulated oocysts of caecal *Eimeria* strain at 28-day old.
(1)	- Normal histological structure. (Fig., 1).
(2)	- Numerous developmental stages and oocysts of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. - Severe lymphocytic infiltration in lamina propria with haemorrhages. (Fig., 2).
(3)	- Inflammatory reaction (lymphocytic infiltration) with very few developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. (Fig., 3).
(4)	- Severe inflammatory reaction (lymphocytic infiltration) with developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. (Fig., 4).

Group (1): Negative control (Not treated and not challenged).
Group (2): Positive control (Not treated and challenged).
Group (3): Fed ration supplemented with salinomycin and then challenged.
Group (4): Immunized with Immucox vaccine and then challenged.

7.Number of developmental stages

On the 7th day PC, the number of developmental stages per Lieberkohn gland of caecum was significantly decreased in broiler chickens fed ration supplemented with salinomycin when compared with that of chickens immunized with immucox vaccine at $P \leq 0.05$ (Table, 5).

Table 5.Number of developmental stages in chickens fed ration supplemented with salinomycin or vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of *caecal Eimeria* strain at 28-day old. (Mean ± SE, n=10)

Parameters \ Groups	(1)	(2)	(3)	(4)	LSD
Number of developmental stages / Lieberkohn gland on the 7th day PC	0.00 [d] ± 0.00	75.80 [a] ± 3.48	1.50 [c] ± 0.40	8.90 [b] ± 0.91	7.40 *
Reduction %	0	0	98.02	88.26	-----

Data were analyzed by One Way ANOVA. (*) = Significant value.
LSD: Least significance difference among means at $P \leq 0.05$.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.
Group (1): Negative control (Not treated and not challenged).
Group (2): Positive control (Not treated and challenged).
Group (3): Fed ration supplemented with salinomycin and then challenged.
Group (4): Immunized with Immucox vaccine and then challenged.

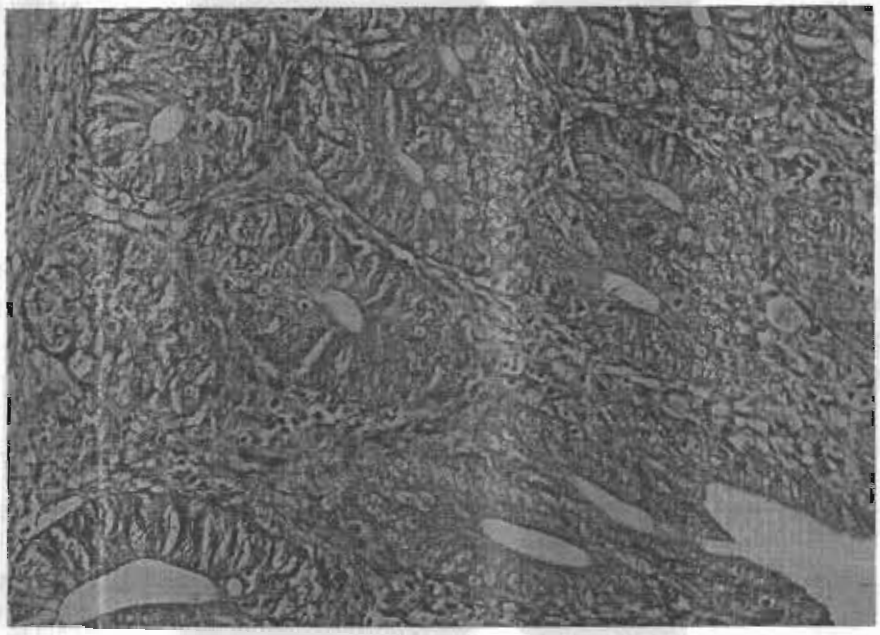

Fig. 1. Caecum section of negative control chicken fed ration neither supplemented with salinomycin nor vaccinated with immucox vaccine and not challenged with caecal *Eimeria* strain showing normal histological structure. (H & E, 400).

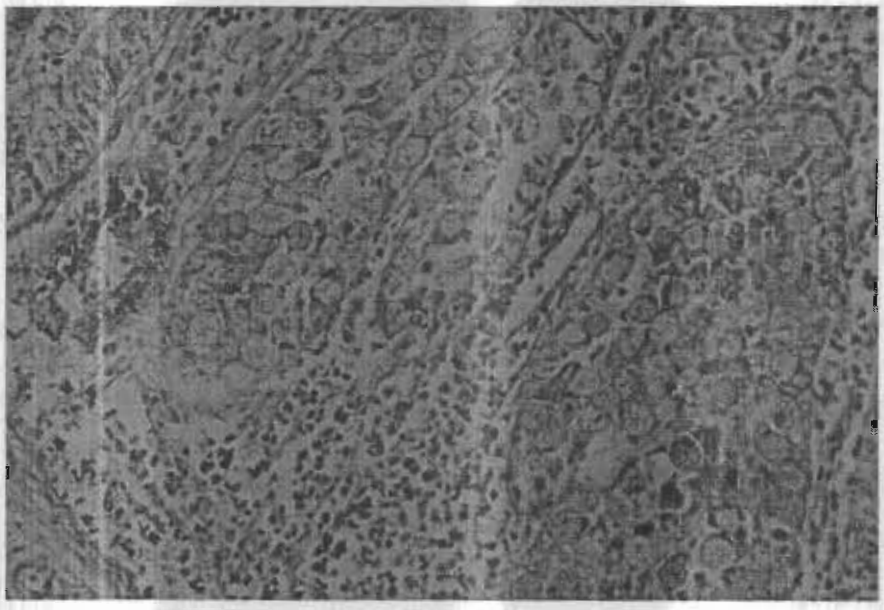

Fig. 2. Caecum section of positive control chicken fed ration neither supplemented with salinomycin nor vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of caecal *Eimeria* strain at 28 day-old showing numerous developmental stages of *Eimeria* strain in the epithelial cells of Lieberkohn glands, severe lymphocytic infiltration in lamina propria and haemmorrhage in blood vessels. (H & E, 400).

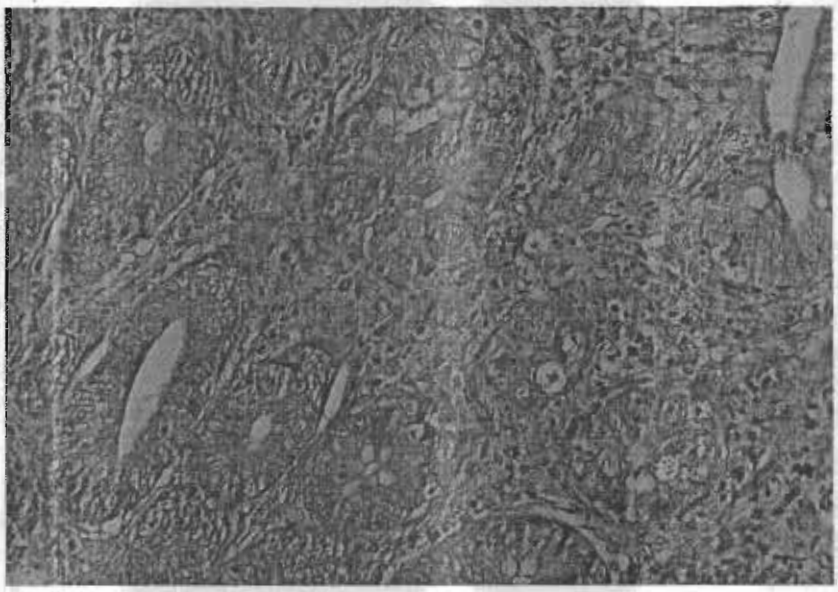

Fig. 3. Caecum section of chicken fed ration supplemented with salinomycin along the experimental period and challenged with 1 x 10^5 sporulated oocysts of caecal *Eimeria* strain at 28 day-old showing inflammatory reaction (lymphocytic infiltration) in lamina propria with very few developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. (H & E, 400).

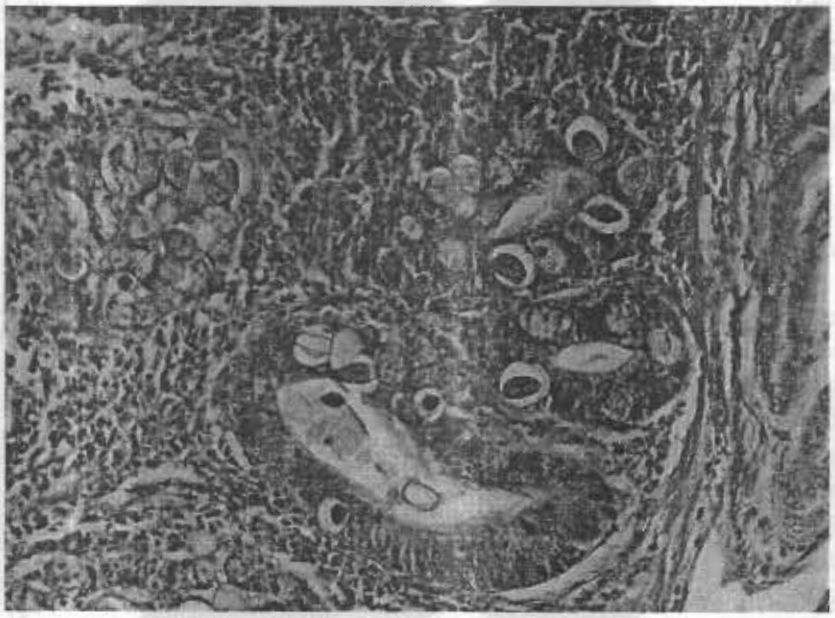

Fig. 4. Caecum section of chicken fed ration without supplementation of salinomycin but vaccinated with immucox vaccine and challenged with 1 x 10^5 sporulated oocysts of caecal *Eimeria* strain at 28 day-old showing severe inflammatory reaction (lymphocytic infiltration) in lamina propria with developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. (H & E, 400).

DISCUSSION

Coccidiosis is prevented and controlled mainly through the use of polyether ionophore antibiotics which still achieve sufficient control despite resistance being common. Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis *(2, 3)*, although there is a rising problem of drug resistant strains of *Eimeria*. In addition the use of live vaccines for control coccidiosis is also well established *(11)*.

The broiler chickens infected with caecal *Eimeria* strain and not treated (positive control) showed a decrease of the appetite, dullness, ruffled feather, diarrhea and bloody droppings. Immucox-vaccinated chickens showed illness and asymmetry of growth in some individuals with creamy and reddish droppings one week post vaccination and post challenge. This may be due to incomplete immunity which needs more time to be built. Where the caecal *Eimeria* strain requires 4-5 weeks to elicit complete protection post challenge as recorded by Rose and Long *(28)*. Broiler chickens fed ration supplemented with salinomycin showed mild signs. This may be due to complete protection in that short rearing time.

Post challenge, the oocyst output per gram of faeces in broiler chickens fed ration supplemented with salinomycin showed a significant reduction when compared with that of broiler chickens immunized with immucox vaccine. Reduction percentage of oocyst production in chickens treated with salinomycin was better than that of immucox-vaccinated ones after challenge with caecal *Eimeria* strain. These results are similar to previously reported findings *(7, 8, 14, 19, 29, 30)*.

On the 7th day PC, the slaughtered chickens of positive control showed that the caeca may become greatly enlarged and distended. Dark petechiae and coalesced foci can be seen from the serosal surface of the caeca. The caecal wall is often greatly thickened because of oedema and infiltration. These findings are nearly similar to that described by Mc Dougald and Reid *(31)*. However, the examined chickens treated with salinomycin showed lower caecal lesion scores when compared with that of chickens immunized with immucox. This result might be attributed to the salinomycin-treated broilers which inhibited the development of sporozoites or the first generation merogenic stages within the host tissues. Meanwhile, immucox vaccine requires more time to allow the trickle infection or repeated cycles for *Eimeria* species and furthermore eliciting complete immunity. These results are comparable with those reported by Rose *(32)* and Lee *(33)*.

In the present study, only one bird died in salinomycin-treated chickens (mortality % was 3.33 % and survival % was 96.67 %), but two birds died in immucox vaccinated group (mortality % was 6.66 % and survival % was 93.34 %. However, six chickens from the positive control (not-treated and challenged) died (mortality % was 20.00 % and survival % was 80.00 %). This attributed to anticoccidial effect and complete protection of salinomycin drug in comparison of immucox vaccine. However, Lee and Yovre reported that the vaccinated flocks with immucox have average percent survival of 96.9 % as compared to 96.7 % in the salinomycin- treated controls *(8)*.

The present study showed that the immucox vaccination is frequently linked to temporary lower performance in young chicks. The growth performances (body weight, weight gain and FCR) of broiler chickens were relatively improved by the use of salinomycin in control of caecal *Eimeria* strain as compared with immucox vaccine. Salinomycin improved FCR and gave a higher body weights and weight gains more than immucox vaccine. This may be due to complete immunity produced through treatment salinomycin against caecal *Eimeria* strain. Similar findings were reported by Johansen *et al (12)*. However, immucox vaccine produced incomplete immunity in broiler chickens. The reduction of body weight in the immucox-vaccinated chickens observed in the present study may be attributed to the fact that the cycling of the parasite partially suppress and weight gains because of the infection (live oocyst immunization) and the energy required these high production of chickens to initiate a protective immune response. These results are

comparable with that reported by Danforth *(29)*. Meanwhile, other authors showed that the vaccination with live oocysts of *Eimeria* elicited significant protection against coccidiosis and resulted in average body weight gains and feed efficiency similar to that obtained with conventional anticoccidial medication *(34, 35)*. Moreover, Danforth *et al.(10)* reported that the performance parameters (weight, weight gain and FCR) of gel immunization of 1-day-old roaster chicks in a floor pen experiment did not differ significantly from those recorded for medicated non immunized birds (medicated with maxiban / montiban shuttle). Meanwhile, immucox vaccine in roaster chickens resulted in faster body weight (3.2 %) than monensin-treated group, while FCR and morality rates were not significantly differed between the vaccinated and monensin-treated birds *(7)*. The vaccinated birds were heavier 2.15 Kg versus 2.11 Kg in the medicated ones, and also showed an average FCR of 2.04 versus 2.05 for the medicated controls *(8)*.

In the present study, the histopathological picture in the positive control group (not treated and challenged) showed that the numerous developmental stages of caecal *Eimeria* strain were detected in the epithelial cells of Lieberkohn glands of the caeca. The funds of the caecal mucosa are completely occluded with mature oocysts. Similar findings were reported by Long and Reid *(36)* and Conway and Mckenzie *(37)*. Post challenge, the broiler chickens fed ration supplemented with salinomycin showed inflammatory reaction (lymphocytic infiltration) with very few developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands. This may be due to complete protection caused by salinomycin prophylactic measures which may be destroyed the most of sporozoites and merozoites consequently the most of sexual stages were not developed. Similar results were reported by Chappel *(14)*. Meanwhile the immucox vaccination did not produce complete immunity against the early developmental stages; hence the sexual stages (gametes and oocysts) were developed. So a severe inflammatory reaction (lymphocytic infiltration) with few developmental stages of caecal *Eimeria* strain in the epithelial cells of Lieberkohn glands was detected in immucox-vaccinated chickens. This may be attributed to incomplete protection of immucox vaccine in the short rearing time for broiler chicken industry. These results are comparable with that obtained by Simovart *(9)*. On the 7^{th} day PC, the numbers of developmental stages per Lieberkohn gland of caecum were significantly decreased in broiler chickens fed ration supplemented with salinomycin when compared with that of chickens immunized with immucox vaccine .

In conclusion, the present study showed that salinomycin is likely to remain the major method of coccidiosos control in broiler chickens to decerase the oocyst output, lesion scoring, and number of developmental stages per Lieberkohn gland and mortality with improvement of growth performance in comparison with immucox vaccination. Furthermore, the immucox vaccination may be highly effective in the long time rearing birds (replacement flocks) for laying to elicit complete immunity against coccidiosis especially species that causing bloody diarrhea (*E. tenella, E. necatrix* and *E. brunetti*) which require 4-5 cycles to consist complete specific immunity.

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REFERENCES

1. ***Williams, RB (1998):*** Epidemiology aspects of the use of live anticoccidial vaccines for chickens. Int. J. Parasitol., 28: 1089-1098.

2. ***Chapman HD (1999):*** Anticoccidial drug and their effects upon the development of immunity to *Eimeria* infection in poultry. Avian Pathol., 28: 521-535.

3. ***Allen PC and Fetter RH (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. Review, 15: 58-65.

4.Dalloul R A and Lillehoj H S (2005): Recent advances in immunomodulation and vaccination strategies against coocidiosis. Avian Dis., 49: 1-8.

5.Kituandu A and Juranova R (2006): Progress in control measures for chicken coccidiosis. Acta Vet. Brno, 75: 265-276.

6.Oviedo-Rondon E O, Clemente-Hernandez H, Williams P and Losa R (2005): Responses of coccidia-vaccinated broilers to essential oil blends supplementation up to forty- nine days of age. J. Appl. Poult. Res., 14: 657-664.

7.Lee EH (1987): Vaccination against coccidiosis in commercial roaster chickens. Canad. Vet. J., 28(4): 434-436.

8.Lee EH and Yvore P (ed) (1989): Control of coccidiosis in broiler chickens by vaccination. Field trial comparison between Immucox (Coccidiosis vaccine) and halofuginoe and salinomycin program in Texas, USA. Coccidia and intestinal coccidiomorphs. Proceedings of the 5th international coccidiosis conference. Tours (France), 17-20 October, 1989. pp: 661-665.

9.Simovart Kh E, Shatteshneider T and Parre Yu (1993): Experimental immuno-prophylaxis of coccidiosis in chickens. Biologija, No.1: 212-215.

10.Danforth HD, Lee EH, Martin A and Dekich M (1997): Evaluation of a gel-immunization technique used with two different Immucox vaccination formulations in battery and floor pen trails with broiler chickens. Parasitol. Res., 83 (5): 445-451.

11.Williams RB (2002): Anticoccidial vaccines for broiler chickens: Pathways to success. Avian pathol., 31: 317-353.

12.Johansen CH, Bjerrum L and Pedersen K (2007): Impact of Salinomycin on the intestinal microflora of broiler chickens. Acta Vet. Scand, 49 (1):30.

13.Danforth H D, Ruff MD, Reid MW and Miller RL (1977): Anticoccidial activity of Salinomycin in battery raised broiler chickens. Poult. Sci., 56: 926-932.

14.Chappel LR (1979): The site of action of anticoccidial salinomycin (Coccistac) ®.J. Parasitol, 65 (1): 137-143.

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

16.McDougald, L. R. and Keshavarz, K. (1981): Anticoccidial efficacy of Salinomycin (AHR- 3096C) and compatibility with Roxarson in floor- pen experiments with broilers. Poult. Sci., 60: 2416-2422.

17.Kawazoe U, Chapman HD and Margaret S (1991): Sensitivity of field isolates of *E. acervulina* to Salinomycin, Maduramycin, and a mixture of clopidol and methyl benzoquate in the chicken. Avian Pathol.. 20: 439 - 446.

18.Conway DP, Dayton AD and McKenzie ME (1999): Comparative testing of anticoccidialis in broiler chickens: The role of coccidial lesion scores. Poult. Sci., 78 (4): 529-35.

19.Chapman HD, Marsler P and LaVorgnat MW (2004): The effect of Salinomycin and Roxarsone on the performance of broilers when included in the feed for four, five, or six weeks and infected with Eimeria species during the starter or grower phase of production. Poult. Sci., 83: 761-764.

20.Ebrahimnezhad Y and Pourreza J (2005): Effect of Ionophorous anticoccidials drugs, Salinomycin and Lasalocid, on the performance of broiler chicks and relationship of these drugs to supplementary Methionin. Int. J. Poult. Sci., 4 (11): 911-916.

21.Gardiner JL and Wehr EE (1950): Selecting experimental groups of chicks by weight. Proc. Helminthol. Soc. Washington, 17: 25-26.

22.Long PL and Rowell JG (1975): Sampling broiler house litter for coccidial oocysts. British Poult. Sci., 16: 583-592.

23.Johnson J and Reid WM (1970): Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments with chickens. Exper. Parasitol., 28: 30-36.

24.Bancroft GD, Steven SA and Turnal N (1996): Theory and practice of histopathological technique, 4th Ed., Churchill, Livingstone, Edinburgh, London, Melbourne and New York.

25.Sainsbury D (1984): System of management in "Poultry health and management". 2nd ed., Granda Publishing (TD), 8 Grafton St., London. WIX 3LA

26.Duncan DB (1955): Multiple Range and Multiple F-test., Biometrics (11): 1-42.

27.SPSS for Windows, Version: 11 (19 September, 2001): Copyright © SPSS Inc. 1989-2001. All rights reserved.

28.Rose ME and Long PL (1962): Immunology, 5: 79-92.

29.Danforth HD (1997): Use of live oocyst-based vaccines in avian coccidial control. Proceeding of VIIth international coccidiosis conference and European Union cost 820 workshops. 1-5 September, Keble college, Oxford, UK. P. 95-96.

30.Williams RB (1997): Live anticoccidial vaccines for breeding chickens: current and future impacts on epidemiology. Proceeding of VIIth international coccidiosis conference and European Union cost 820 workshops. 1-5 September, Keble college, Oxford, UK. P. 98-100.

31.Mc Dougald LR and Reid WM (1991): Coccidiosis in Diseases of poultry. Pages: 780-797, 7th edition. Calneck, B. W. (ed) Iowa State University Press, Ames, IA.

32.Rose ME (1985): Immune responses to *Eimeria* infection. In Mc Dougald, L.R.; Joyner, L.P.; Long, P.L. (eds). Researches in avian coccidiosis. University of Georgia, Athens. pp. 449-469.

33.Lee EH (1993): Live coccidiosis vaccine and a field immune variant of *Eimeria maxima*: A case report: In Coccidiosis conference. University of Guelph. Ontario. pp. 118-121.

34.Shirley MW and Bedrink P (1997): Live attenuated vaccines against avian coccidisis: Success with precocious and egg adapted lines of *Eimeria*. Parasitol. Today, 13: 481-484.

35.Danforth HD (1999): Use of live oocyst vaccines in the control of avian coccidiosis: Experimental studies and trials. Int. J. Parasitol., 28: 1099-1109.

36.Long PL and Reid WM (1982): A guide for the dianosis of coccidiosis in chickens. University of Georgia, College of Agriculture Research Report 404.

37.Conway DP and McKenzie ME (1991): Poultry coccidiosis. Diagnostic and testing procedures. The 2nd edition.

الملخص العربى

الكفاءة المقارنة للقاح الإميوكوكس والسالينوميسين للسيطرة على العدوى المعملية للأيميريا الأعورية فى الدجاج

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فى هذه الدراسة إستخدمت مائة وعشرون (120) كتكوتا عمر يوم من سلالة روص للمقارنة بين لقاح الإميوكوكس ومضاد الكوكسيديا سالينومايسين. وقسمت الكتاكيت إلى أربعة مجموعات متساوية (30 كتكوت لكل مجموعة). إعتبرت المجموعة الأولى مجموعة ضابطة سالبة (غيرمصابة وغير معالجة). والمجموعة الثانية تغذت على علف متزن وبدون إضافة أى مضاد للكوكسيديا عليه. المجموعة الثالثة تم تحصينها بلقاح الإميوكوكس فى مياه الشرب عند عمر أسبوع وتغذت على علف متزن وبدون إضافة أى مضاد للكوكسيديا عليه. بينما المجموعة الرابعة تغذت على علف متزن ومضافا إليه مضاد الكوكسيديا (سالينومايسين) بجرعة 60 جزء فى المليون طوال فترة التجربة. المجموعات الثلاث الأخيرة (الثانية, الثالثة والرابعة) تم تحديها مباشرة داخل الحوصلة بجرعة $10x1^5$ حويصلة متجرثمة من عترة الأيميريا الأعورية لكل كتكوت عند عمر 28 يوم. قد تم تسجيل الأعراض على الطيور طول فترة التجربة وتم تجميع خمسة عينات من السبلة من أماكن مختلفة لكل حظيرة (pen), كما تم تحديد عدد حويصلات الأيميريا فى زرق الفراخ (لكل 1 جم زرق) أربعة مرات إبتداء من اليوم السادس بعد التحدى وكل أربعة أيام بعد ذلك. وعند اليوم السابع من التحدى تم ذبح خمسة طيورمن كل مجموعة لعمل الصفة التشريحية ولتحديد درجة الإصابة فى الأعورين, وقد تم أخذ عينات نسيجية بعد الذبح مباشرة من الأعورين وذلك لعمل الدراسات الهستوباثولوجية عليها وتسجيل عدد الأطوار المختلفة للطفيل لكل غدة ليبركوهن . وأيضا تم تسجيل الأوزان والأوزان المكتسبة ومعدل تحويل العلف للفراخ إسبوعيا.

وتبين من النتائج أن المجموعة الضابطة الإيجابية (غير معالجة وتم إجراء التحدى لها بعترة الأيميريا الأعورية) قد أوضحت قلة الشهية, الإكتئاب, الريش المنفوش, إسهال وبراز مدمم. وبدا الأعور متضخم وممتلىء بالدم المتجلط مع وجود حبل أعورى بداخله. والطيور المحصنة بلقاح الأميوكوكس قد أوضحت إعياءات وعدم تجانس النمو فى بعض الأفراد مع ملاحظة البراز الكريمى والمدمم عند أسبوع بعد التحصين وكذلك بعد التحدى. أما الطيور المعالجة بالسالينومايسين قد أوضحت أعراض وصفة تشريحية بسيطة عند مقارنتها بتلك المحصنة بلقاح الأميوكوكس بعد التحدى. وكانت نسبة النفوق3,33 % فى المجموعة المعالجة بالسالينومايسين, 6,66 % فى المحصنة بلقاح الأميوكوكس, 20 % فى المجموعة الضابطة الإيجابية.

بعد التحدى, قد قل معنويا كل من عدد الحويصلات لكل 1 جم زرق ودرجة الإصابة فى الأعورين فى الطيور المعالجة بالسالينومايسين مقارنة بنظيرتها فى المحصنة بلقاح الأميوكوكس. كما بينت النتائج أن الطيور المحصنة بلقاح الأميوكوكس قد أوضحت قلة الأوزان والأوزان المكتسبة مع عدم تحسن معدل التحويل الغذائى عند مقارنتها بتلك المعالجة بالسالينومايسين عند 14, 21, 28 يوم بعد التحصين. ولكن بعد التحدى قد إزدادت الأوزان والأوزان المكتسبة مع التحسن الملحوظ فى معدل التحويل الغذائى فى الطيور المعالجة بالسالينومايسين مقارنة بنظائرها فى الطيور المحصنة بلقاح الأميوكوكس. وقد أوضحت الدراسة الهستوباثولوجية وجود عديد من المراحل التطورية لعترة الأيميريا الأعورية داخل الخلايا المبطنة لغدد ليبركوهن فى الأعورين فى طيور المجموعة الضابطة الإيجابية (تم تحديها وغير معالجة). بينما أوضحت المجموعتين المحصنة بلقاح الإميوكوكس والمعالجة بالسالينومايسين تفاعل إلتهابى (Lymphocytic infiltration) مع تواجد أعداد قليلة جدا من المراحل التطورية من الأيميريا الأعورية فى الخلايا المبطنة لغدد ليبركوهن فى المجموعة المعالجة بالسالينومايسين, وتواجد مراحل تطورية متوسطة العدد من الأيميريا الأعورية فى الخلايا المبطنة لغدد ليبركوهن فى المجموعة المحصنة بلقاح الإميوكوكس, وتواجد مراحل هائلة العدد من الأيميريا الأعورية فى الخلايا المبطنة لغدد ليبركوهن فى المجموعة الضابطة الإيجابية.

وخلاصة هذه الدراسة: أن السالينومايسين يتبقى كطريقة مفضلة للسيطرة على الكوكسيديا الأعورية فى دجاج التسمين عند مقارنته بلقاح الإميوكوكس حيث أنه أفضل فى تقليل عدد الحويصلات فى الزرق و درجة الإصابة فى الأعورين مما يؤدى إلى تحسين أداء النمو فى الطيور.