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Comparative efficacy of ionophores and ZeeCox®, a phytogetic multistage anticoccidial against Chicken Coccidiosis

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ABSTRACT

The study was designed to investigate the comparative anti-coccidial efficacy of ZeeCox® (Phytogetic anti-coccidial formulation developed by Indian Herbs Specialities Ltd) at recommended dose levels, with commonly used ionophore anti-coccidial drugs such as salinomycin, lasalocid and anti-coccidial vaccine. A total of 324, day-old broiler chicks were reared under standard management practices. The chicks were randomly divided into nine groups with each group consisting of 36 birds. Body weight gain, Feed Conversion Ratio, intestine lesion score, oocyst per gram feces and European Performance Efficiency Factor (EPEF) of all experimental group birds were measured. All the chicks except Group 1 were inoculated orally with the total of 20,000 live sporulated oocysts of *Eimeria* species on 18th day of age. The overall performance such as Body weight gain, Feed Conversion Ratio and European Production Efficiency Factor (EPEF) of Group 5 (ZeeCox® 0.5g/kg treatment and Challenged) was better and comparable with Group 3 (Salinomycin treatment and Challenged) and Group 4 (Salinomycin & Lasalocid treatment and Challenged). It was concluded from the present study that ZeeCox® at 0.5g/kg of feed could be a potential alternative to the ionophore anticoccidial drugs used in the control of coccidiosis in broiler chicken.

Keywords: ZeeCox; Phytogetic anti-coccidial compound; Body weight gain; Feed conversion ratio (FCR); European Production Efficiency Factor (EPEF); Chicken coccidiosis.

INTRODUCTION

Coccidiosis is an important intestinal disease of poultry birds which causes considerable economic losses in broiler production. It is caused by protozoan parasites of the genus *Eimeria*. The most common *Eimeria* species are *Eimeria tenella*, which causes the caecal coccidiosis. *E. acervulina* and *E. maxima*, causing chronic intestinal coccidiosis. Poultry coccidiosis is associated with reduced growth rate, impaired feed conversion ratio and increased mortality [1].

Polyether ionophore anticoccidial drugs are being used as feed additive to prevent coccidiosis in broilers. Due to their regular use of ionophore coccidiostat in feed, drug-resistant *Eimeria* strains have been reported [2, 3]. The development of drug resistance in *Eimeria* parasite in the field, drug residue in the broiler meat, long withdrawal period of drugs prior to slaughter are major constraints of these drugs. Drug residue in the poultry products is also un-desirable from the consumer's perspective and could impact the export of poultry products [4].

Ionophore drugs allow chickens to be exposed to low levels of replicating *Eimeria* that induces immunity against the parasite which is of huge value to the bird when drugs are withdrawn prior to slaughter, or at the onset of egg production [5]. At present the ionophore anti-coccidial drugs dominate the market, representing more than 70% of drugs used in the poultry industry [6]. Although European Union is allowing the use of ionophores in food animal production, their status as antibiotics in countries such as the US is beginning to restrict their application. The FDA's proposed Veterinary Feed Directive (VFD) recommends that all antibiotics administered to food producing animals are only done so under the supervision and prescription of licensed veterinarians.

Although this strategy is cost-effective and successful, the selection of drug resistant parasites and public demand for residue free meat had encouraged development of alternative control strategies [7]. Application of vaccine to control poultry coccidiosis is an effective but expensive measure and in case of poor management the efficiency also reduced. Due to practical limitations on manufacturing capacity, vaccines can cost significantly more than anticoccidial drugs [8]. Vaccine-induced disease in birds is a significant drawback with the use of non-attenuated vaccines because the parasites are fully virulent.

Herbal products are characterized by having bioactive components such as phenolic acids, alkaloids, terpenes, tannins and flavonoids [9], with antioxidant properties and anticoccidial activity [10] comparable to that of synthetic drugs [11]. So, there is a strong need to develop and use some natural alternative agents to replace the existing coccidiostats.

Owing to the development of drug resistance against *Eimeria* and concerns about drug residues, attentions toward the alternative compounds including herbal medicines had been increased in recent past [12]. Peptasan® (Nuproxa, Switzerland), a commercial poly-herbal additive, improved production performance of broiler birds and represents a natural alternative for coccidiosis control in poultry. It was found to be effective against coccidiosis at 500 ppm concentration by reducing, oocysts excretion, intestinal lesions and mortality with the same efficacy as salinomycin (12 %) supplemented at 550 ppm [13]. Cocciban® (Indian Herbs Specialities Pvt. Ltd), herbal coccidiostat was effective against highly pathogenic *Eimeria* species by significantly reducing the oocyst counts and lesion score at a concentration of 1000g/ton of feed in comparison with Salinomycin (500g/ton) and Dinitolmide (500g/ton). Gross lesions and histopathology revealed that Cocciban® (1000 g/ton) group performed better in reducing the pathogenicity when compared to chemical anticoccidials [14]. HerbaCox® (Virazap, United States), a commercial herbal compound containing extracts from *Bombax malabaricum*, *Aegle marmelos*, *Anethum foeniculum*, *Resina salvia*, *Ferula asafoetida* and *Papaver somniferum* was found to be safe and effective compound for the treatment of *Eimeria stiedae* infection in rabbits which causes hepatic coccidiosis in rabbit [15]. Anticoccidial efficacy of Coxynil® (Growell India) a polyherbal preparation was tested against *Eimeria tenella* in broilers [16].

Natural dietary supplements may potentially be used as one of the novel approaches to treat coccidiosis due to their natural origin, wide dose range, no residues and lack of withdrawal period and stimulation of appetite [17, 18, 19, 20]. Recently the US-FDA declared restrictions on the use of antibiotics including ionophores in broiler bird production and other developed nations are also expected to follow USA [21]. Further, there is a growing interest among the poultry industry across the world on antibiotic free broiler production which will boost the demand for herbal coccidiostats in near future.

The present study was designed to investigate the comparative efficacy of the anti-coccidial efficacy of ZeeCox® (Phytogenic anti-coccidial formulation developed by Indian Herbs Specialities Ltd.) at the manufacturer recommended dose levels, in comparison with commonly used ionophore anti-coccidial drugs such as salinomycin/lasalocid and anti-coccidial vaccine based on growth performance, lesion score of intestine and oocyst shedding in broiler chicks by an experimental challenge study.

MATERIALS AND METHODS

Ethical approval

The Ethical Committee approval was obtained from Institutional Animal Ethics Committee of Tamil Nadu Veterinary and Animal Sciences University, Chennai. Institutional Animal Ethics Committee (IAEC) approval number: 95/SA/IAEC/2019.

Study period and location

The study was conducted from October 2019 to February 2020 at Translational Research Platform for Veterinary Biologicals, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai- 600051, India

Experimental design

There were 9 experimental bird groups and each was having 34 chicks.

Group-1: No infection control & ZeeCox 0.75g (Uninfected and treatment with ZeeCox@0.75g/kg of feed)

Group-2: Infection control (Challenged with oocyst and No Treatment)

Group-3: Salinomycin (Treatment with salinomycin at 1g/kg and Challenged with oocyst)

Group-4: Salinomycin & Lasalocid (Salinomycin (12%) at 1g/kg of feed (120 ppm of active salinomycin) during starter grower phase (till Day 18), Lasalocid (15%) at 1g/kg of feed (150 ppm active lasalocid) during finisher phase (till day 35) and Challenged with oocyst)

Group-5: ZeeCox® 0.5g (Treatment with ZeeCox@0.5g/kg of feed and Challenged with oocyst)

Group-6: ZeeCox® 0.75g (Treatment with ZeeCox@0.75g/kg of feed and Challenged with oocyst)

Group-7: ZeeCox® 1g (Treatment with ZeeCox@1g/kg of feed and Challenged with oocyst)

Group-8: Anti-coccidia vaccine (Vaccination with live attenuated *Eimeria* oocysts (at Day 5) and Challenged with oocyst)

Group-9: Anti-coccidia vaccine & ZeeCox® 0.75g (Vaccination with live attenuated *Eimeria* oocysts (at Day 5), treatment with ZeeCox®@0.75g/kg of feed and Challenged with oocyst)

Experimental birds

Cobb 400, commercial day-old broiler chicks (324 numbers) were reared under standard management practices in the large animal experimental shed located in Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai for the entire study.

Nine steel cages with floor space of 36 square feet (6ft. x 6ft.) were used in the experiment to ensure at least 1 square foot floor space per bird. The birds were given *ad-libitum* access to food and water during the experiment.

Drugs and vaccines

Salinomycin 12% (Coxrival, Quest Agrovets Services pvt.ltd, Hyderabad, Telangana) and Lasalocid 15% (Avatec, Zoetis Inc., Kalamazoo, MI) were the commercial ionophore compounds were

used in this study. Livocox® Q (Biopharm) containing the live attenuated *Eimeria* species oocysts of *Eimeria acervulina*, *E. tenella*, *E. maxima* and *E. necatrix* was used in the study. The Group 8 and 9 birds were vaccinated orally on day 5 through drinking water (0.4 ml per 200 ml water).

Parasite and dose

E. acervulina, *E. tenella*, *E. maxima* oocysts maintained at TRPVB, TANUVAS, Chennai were propagated. After obtaining sufficient number of oocysts, they were isolated by flotation using saturated NaCl solution and incubated with 2.5% potassium di-chromate solution for sporulation. The required concentrations of sporulated oocysts (20,000 mL⁻¹) were washed three times with phosphate buffered saline and by overnight shaking at 25°C. The sporulated oocysts were enumerated and stored at 4°C in 2% Potassium dichromate solution. The total of 20,000 live sporulated coccidial oocysts of *Eimeria* species were administered to each chick in all groups by esophageal intubation method on 18th day of age except Group 1 chicks.

Evaluation parameters

Weight gain, Feed Conversion Ratio (FCR), Oocyst output, Intestine lesion scores and European Production Efficiency Factor (EPEF) were the parameters measured. The weight gain and feed conversion ratio were recorded on weekly basis. Lesion scoring was done on a 0 to + 4 scales with descriptions of the gross pathologic changes for each score [22]. Oocyst output per gram (OPG) of faeces was measured from 7 DPC (Days Post Challenge) to 13 DPC (Days Post Challenge). Oocysts were counted microscopically using a McMaster counting chamber using a salt flotation method. The cumulative oocyst output was calculated by adding the oocyst output per gram per bird from 7 DPC to 13 DPC.

European performance efficiency factor (EPEF) was calculated using the following formula [23].

$$EPEF = \frac{\text{Body weight gain (Kg)} \times \% \text{ Viability}}{\text{Feed Conversion Ratio} \times \text{Trial duration in days}} \times 100$$

Table 1: Weight Gain differences and Mean FCR between groups across the weeks

Body weight gain	Week 1	Week 2	Week 3	Week 4	Week 5	Mean FCR
Group 1	74.78 ^c ±19.48	186.78 ^s ±44.06	479.24 ^f ±127.19	791.41 ^a ±160.59	1503.00 ^p ±284.37	1.92 ^a ±0.65
Group 2	54.47 ^{ab} ±12.79	130.28 ^{def} ±19.12	420.56 ^h ±58.43	646.41 ^k ±82.88	1383.03 ⁿ ±208.60	2.19 ^a ±0.96
Group 3	53.14 ^{ab} ±7.03	149.83 ^q ±29.92	457.15 ^h ±69.52	731.53 ^l ±99.33	1201.55 ^m ±181.85	2.05 ^a ±0.79
Group 4	52.11 ^{ab} ±14.94	132.28 ^{defq} ±29.58	466.62 ^h ±43.66	758.35 ^l ±86.54	1282.21 ⁿ ±186.41	1.95 ^a ±0.58
Group 5	60.06 ^b ±7.84	143.67 ^q ±30.48	707.18 ⁱ ±85.45	728.62 ^l ±82.91	1142.82 ^m ±166.58	1.87 ^a ±0.68
Group 6	57.94 ^b ±16.51	126.28 ^{de} ±19.67	585.91 ⁱ ±83.48	728.41 ^l ±83.13	1213.14 ^m ±183.09	2.15 ^a ±0.90
Group 7	49.44 ^a ±7.92	128.61 ^{def} ±29.44	459.71 ^h ±92.19	744.79 ^l ±131.61	1204.53 ^m ±181.64	2.17 ^a ±0.83
Group 8	55.11 ^{ab} ±12.65	127.72 ^{de} ±29.73	443.65 ^h ±75.69	732.12 ^l ±110.40	1183.75 ^m ±172.60	2.38 ^a ±1.19
Group 9	60.61 ^b ±7.48	121.44 ^d ±25.48	434.62 ^h ±69.38	755.18 ^l ±107.14	1134.68 ^m ±144.91	2.24 ^a ±0.92

The values not sharing a common superscript (a-s) differ significantly at p<0.05

Statistical analysis

Mean values for body weight changes, body weight gains, faecal oocyst shedding, were compared among groups by ANOVA for a complete randomized block design, using the general linear models and LSD test described by Steel and Torrie (1997) [24]. Small sample t-test is used to compare the mean lesion score of birds of different experimental groups. Statistical packages SPSS version 17 (IBM) was used to carry out the analysis.

RESULTS

Body weight gain

Mean body weight gain of Group 1 (No infection control & ZeeCox® 0.75g) chicks was significantly higher (p<0.05 and 0.01) than Group 2 (Infection control) chicks across all the weeks. There was no significant difference between Group 8 (Anti-coccidia vaccine) and Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g) chicks across all the weeks. During Week 4 and week 5, there was no significant difference between Group 5 (ZeeCox® 0.5g), Group 6 (ZeeCox® 0.75g) and Group 7 (ZeeCox® 1g) (Table 1).

There was no significant difference in mean body weight gain between Group 3 (Salinomycin) and Group 4 (Salinomycin & Lasalocid) chicks during the weeks of 1, 3, 4 and 5 (p>0.05). During week 1, 2 and 5, mean body weight gain of Group 1 (No infection control & ZeeCox® 0.75g) chicks was significantly higher than Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g) chicks at the level (p<0.01).

At week 1, 3 and 5, mean body weight gain of Group 5 (ZeeCox® 0.5g) chicks was significantly higher (p<0.01) than Group 4 (Salinomycin & Lasalocid) chicks. During week 1, 2, 3 and 5, mean body weight gain of Group 1 (No infection control & ZeeCox® 0.75g) was significantly higher (p<0.01) than Group 6 (ZeeCox® 0.75g) chicks.

Feed Conversion ratio

Group 1 (No infection & ZeeCox® 0.75g) and Group 5 (ZeeCox® 0.5g) chicks had better FCR 1.92 and 1.87 respectively, compared to other groups, but there was no significant difference between mean FCRs between the groups (Table 1).

Lesion score

Mean lesion score of chicks of different experimental groups with standard deviation and significance were displayed in Table 2.

Table 2: Mean lesion score of chicks of different experimental groups with standard deviation and significance

Groups	G1	G2	G3	G4	G5	G6	G7	G8	G9
Mean±SD	1.09±0.08	2.83±0.76	1.67±0.38	1.21±0.19	2.08±0.60	1.92±0.52	1.72±0.49	1.71±0.26	1.33±0.38
G1		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P = 0.0005
G2			P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
G3				P < 0.0001	P = 0.0069	P = 0.0293	P = 0.7044 ^{NS}	P = 0.6142 ^{NS}	P = 0.0002
G4					P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P = 0.1053 ^{NS}
G5						P = 0.4580 ^{NS}	P = 0.0261	P = 0.0094	P < 0.0001
G6							P = 0.1041 ^{NS}	P = 0.049	P < 0.0001
G7								P = 1.0000 ^{NS}	P = 0.0008
G8									P < 0.0001

NS- Non significant is highlighted

Lesser the lesion score denotes the higher the efficacy of treatment. Mean lesion score of Group 1 (No infection & ZeeCox® 0.75g) chicks was significantly low compared to all other Groups. Mean lesion score of Group 2 (Infection control) chicks was significantly high compared to all other Groups. Mean lesion score of Group 3 (Salinomycin) chicks was significantly high compared to Group 4 (Salinomycin & Lasalocid) suggesting salinomycin in combination with lasalocid was comparatively more effective in limiting the intestinal pathology of chicks challenged with Eimeria oocysts. Mean lesion score of Group 3 (Salinomycin) chicks was significantly low compared to Group 5 (ZeeCox® 0.5g) and Group 6 (ZeeCox® 0.75g) chicks. This result suggests that the effect of salinomycin was better compared to ZeeCox® (0.5g/kg to 0.75g/kg) in limiting the intestinal pathology due to coccidiosis. Mean lesion score of Group 4 (Salinomycin & Lasalocid) was significantly low compared to chicks of Group 5 (ZeeCox® 0.5g), Group 6 (ZeeCox® 0.75g), Group 7 (ZeeCox® 1g) and Group 8 (Anti-coccidia vaccine). This result suggests that the salinomycin and lasalocid treated birds had low level of intestinal lesion compared to ZeeCox® treated birds and Livacox vaccinated birds. No significant difference was found between the lesion score of chicks of Group 5 (ZeeCox® 0.5g) and Group 6 (ZeeCox® 0.75g).

Group 5 (ZeeCox® 0.5g) lesion score was significantly high compared to Group 7 (ZeeCox® 1g), Group 8 (Anti-coccidia vaccine) and Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g). This result suggests that ZeeCox® (1g/kg), Livacox vaccinated and Livacox vaccinated and ZeeCox® (0.75g/kg) treated groups showed better effect in limiting intestinal pathology compared to ZeeCox® (0.5g/kg) treated group. No significant difference was found between the lesion score of Group 6 (ZeeCox® 0.75g) and Group 7 (ZeeCox® 1g) chicks. No significant difference was found between the lesion score of Group 7 (ZeeCox® 1g) and Group 8 (Anti-coccidia vaccine). Mean lesion score of Group 7 (ZeeCox® 1g) chicks was significantly high compared to Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g) chicks.

Mean oocyst output

Mean oocyst output of Group 1 (No infection Control & ZeeCox® 0.75g) chicks was significantly lower ($p < 0.01$) than other groups. There was no significant difference between Groups 2, 3, 4, 5, 6, 7, 8 and 9 (Table.3).

Table 3: Mean oocyst output per gram per group

DPC	G1	G2	G3	G4	G5	G6	G7	G8	G9
7 DPC	0 ^d	94.68 ^{ab}	94.82 ^{ab}	98.18 ^{ab}	102.18 ^{ab}	109.47 ^{ab}	112.06 ^{ab}	102.88 ^{ab}	94.35 ^{ab}
10 DPC	0 ^d	121.56 ^a	95.26 ^a	92.32 ^a	101.15 ^a	106.56 ^a	77.59 ^a	134.91 ^a	113.38 ^a
11 DPC	0 ^d	131.79 ^c	170.76 ^c	124.24 ^c	190.41 ^c	175.35 ^c	154.03 ^c	165.32 ^c	140.41 ^c
13 DPC	0 ^d	116.00 ^b	112.79 ^b	101.35 ^b	83.41 ^b	106.65 ^b	105.85 ^b	109.32 ^b	109.50 ^b
Cumulative oocyst output per bird	0 ^d	116.01 ^b ±15.65	118.41 ^b ±35.89	104.02 ^b ±13.98	119.29 ^b ±48.19	124.51 ^b ±33.92	112.38 ^b ±31.56	128.11 ^b ±28.40	114.41 ^b ±19.18

The values not sharing a common superscript (a-d) differ significantly at $p < 0.05$

European production efficiency factor (EPEF)

The EPEF values of Group 1 (No infection control & ZeeCox® 0.75g), Group 2 (Infection control), Group 4 (Salinomycin & Lasalocid) and Group 5 (ZeeCox® 0.5g) were above the mean (163.12) and median (152.69) values. The highest EPEF values were found in Group 1 (269.38) and Group 5 (225.36) followed by Group 4 (210.78) and Group 2 (203.98) (Table 4) (Fig.1)

Table 4: European production efficiency factor (EPEF)

Groups	Body Weight gain (Kg) (5 th week)	Livability %	Age (Days)	FCR (5 th week)	EPEF
Group 1	1.503	100	35	1.95281	269.38
Group 2	1.383	88	35	2.088219	203.98
Group 3	1.201	97	35	2.670431	152.69
Group 4	1.282	97	35	2.064856	210.78
Group 5	1.142	94	35	1.667178	225.36
Group 6	1.213	85	35	3.352856	107.63
Group 7	1.204	88	35	3.205123	115.70
Group 8	1.183	85	35	4.201745	83.76
Group 9	1.134	91	35	3.655964	98.79

Mean±SD: 163.12±66.12 and Median = 152.69

EPEF values above mean and median values were highlighted

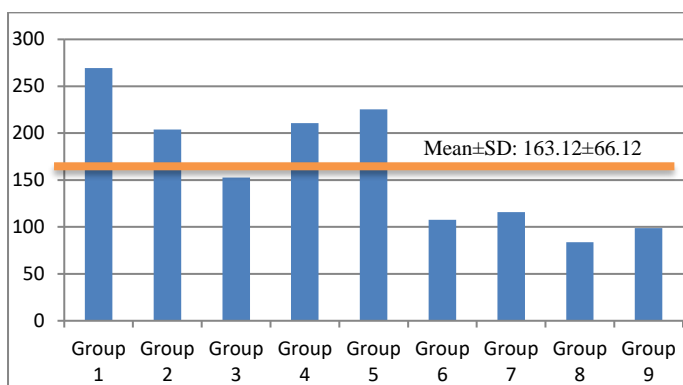


Figure 1: European production efficiency factor (EPEF) across the groups

DISCUSSION

In the context of increasing public awareness on drug residues in poultry meat and regulatory restrictions on the use of antibiotics including ionophores, the market for herbal based anti-coccidia drugs and growth promoters are expected to boom in the future. Though there are several herbal based anti-coccidia products available in the market with variable efficacy on oocyst output, lesion score and growth performance, very few products had been evaluated in comparison with commonly used ionophore compounds including the EPEF value. The mean body weight gain of Group 1 (No infection control & ZeeCox® 0.75g) was significantly higher than the Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g). This result suggests that in the absence of challenge the ZeeCox® treatment significantly improved the body weight gain. Significantly higher body mass gains and mild lesion score in birds medicated with herbal complex for *E. tenella* infection in chicken was also reported [25].

The mean body weight gain of Group 5 (ZeeCox® 0.5g) chicks was significantly higher than Group 4 (salinomycin & lasalocid) and

Group 3 (salinomycin). This result suggests that the ZeeCox® fed group performed better in body weight gain than ionophore fed groups. The above findings of the present study were akin to the report; the birds that received herbal complex showed better body mass gain than birds fed with salinomycin mixed feed for the same period [26]. However, the findings of the present investigation were not in agreement with the findings of Peek and co-workers that salinomycin enabled significantly higher body weight gains in broiler birds in comparison with phytochemicals/extracts and the fungal immunomodulatory protein [27].

There was no significant difference between the mean body weight gain of Group 5 (ZeeCox® 0.5g), Group 6 (ZeeCox® 0.75g) and Group 7 (ZeeCox® 1 g) birds. This result suggests that 0.5g/kg ZeeCox® as feed additive was enough to obtain the desired effect (body weight gain). Contradict to this result, while increasing the treatment doses across the different treatment groups, significant reduction of the oocyst count and significant increment of the mean weight gain were observed [28].

There was no significant difference between the mean body weight gain of Group 3 (salinomycin) and Group 4 (salinomycin and lasalocid). The findings of the present investigation were in agreement with the findings that, there was no significant difference between the mean body weight gains of salinomycin and lasalocid treated groups [29].

Group 1(1.92) and Group 5 (1.87) had better FCR compared to other groups, but there was no significant difference between mean FCR between the groups. Similarly, feed intake, body weight and feed conversion ratio (FCR) were significantly ($P < 0.05$) high in ginger and garlic supplemented birds in comparison with amprolium in broiler chickens challenged with *Eimeria* spp oocysts was reported [30].

Effect of salinomycin was better when compared to ZeeCox® (0.5g/kg to 0.75g/kg) in limiting the intestinal pathology due to coccidiosis. The findings of the present investigation were in agreement with the report, which the phytochemicals/extracts and the fungal immunomodulatory protein failed to reduce coccidiosis lesion scores and oocyst shedding, while salinomycin efficiently controlled the *E. acervulina* infection and enabled significantly higher body weight gains [27]. ZeeCox® (0.5g/kg) and ZeeCox® (0.75g/kg) treated birds showed no differences in the intestine lesion score and salinomycin and lasalocid treated group chicks (Group 4) showed better efficacy in limiting the intestinal pathology.

No performance differences between salinomycin treated birds and ZeeCox® (1g/kg) and Livacox vaccinated group in terms of limiting the intestinal pathology caused by *Eimeria* parasite or both were equally effective or comparable. This finding contradicts the results of another study, where the performance of salinomycin was significantly better ($p < 0.05$) when compared to a live attenuated vaccine, and trivalent live attenuated vaccine [31].

There was no significant difference between the mean oocyst output per bird of Groups 2, 3, 4, 5, 6, 7, 8 and 9. Similarly, oocyst shedding after 10 days of the infection was similar in the anticoccidial drug and natural products also reported [32].

The mean Broiler Performance Efficiency Factor in hot and cold climatic areas were 276.17 and 306.39 respectively, differed significantly ($P < 0.05$), which concluded that climate has definite

influence on performance of commercial broilers^[33]. For the entire rearing period the average performance efficiency factor of broiler was 224^[34]. As per this reference, Group 1 (269.38) and Group 5 (225.36) got above this value (224).

The overall lower EPEF values in the present experiment could be due to the use of starter feed for the entire study period, stress due to frequent weighing of birds and challenge experiment. The findings were in agreement with the findings of Chandrasekaran and co-workers that coccidial challenge significantly affected the body mass gain and feed conversion ratio of the chicks^[26].

The highest EPEF values were in Group 1 (269.38) and Group 5 (225.36) followed by Group 4 (210.78) and Group 2 (203.98). Similarly, the unchallenged chicks consumed more feed, gained more weight, converted feed more efficiently and as a result had higher feed efficiency factor as compared to challenged chicks^[32].

The overall performance such as Body weight gain, FCR and EPEF of Group 5 (Challenged and treated ZeeCox® 0.5g/kg) was better and comparable with Group 3 (salinomycin) and Group 4 (salinomycin and lasalocid).

CONCLUSION

- The overall performance such as Body weight gain, FCR and EPEF of Group 5 (ZeeCox® 0.5g) was better and comparable with Group 3 (salinomycin) and Group 4 (salinomycin & lasalocid).
- Effect of salinomycin was better compared to ZeeCox® (0.5g/kg feed and 0.75g/kg feed) in limiting the intestinal pathology due to coccidiosis.
- ZeeCox® (0.5g/kg) and ZeeCox® (0.75g/kg) treated birds showed no difference in the intestine lesion score.
- Group 7 (ZeeCox® 1g) and Group 9 (Anti-coccidia vaccine & ZeeCox® 0.75g) groups showed better effect in limiting intestinal pathology compared to Group 5 (ZeeCox 0.5g)
- It was concluded from the present study that ZeeCox® (Phytogenic anti-coccidial formulation to control coccidiosis) at a dose of 0.5g/kg could be an effective alternative for ionophore anticoccidial drugs, in the control the coccidiosis in broiler chicken.

Declaration of competing interest

The authors declare that they have no conflict of interest. One of the authors Dr. Shivi Maini works for Indian Herbs Specialities Ltd which funded this project.

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