

## Evaluation of salinomycin for an extended period in laying replacement hens

**Running title:** Salinomycin in replacement hens for 14 weeks

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## **Abstract**

The clinical efficacy of salinomycin premix (Pisacox®) administered in feed to replacement pullets to control coccidiosis was evaluated by dosing during 4 or 14 weeks. The productive performance, signs, and lesions associated with coccidiosis and direct oocyst counts were analyzed in 3 000 Bovans White pullets distributed in 3 groups of 1 000 birds having the same diet: control group (Ctrl) without coccidiostat; Sal<sub>4</sub> group with the administration of salinomycin for 4 weeks; and Sal<sub>14</sub> group that received salinomycin for 14 weeks. Predictably, in both Sal<sub>4</sub> and Sal<sub>14</sub> treatments, lower oocyst counts than in the control were observed. However, it was more evident with Sal<sub>14</sub> towards the end of the trial, whose oocyst count was < 5 000 g of feces. This response can be associated with better productivity variables. The coccidial-type intestinal lesions detected in control were classified as severe (2.55), while Sal<sub>4</sub> and Sal<sub>14</sub> presented values of 1.0 and 0 (mild lesions and normal appearance, respectively). The accumulated mortality associated with coccidiosis was statistically higher in the Ccontrol group (9.8 %) (P < 0.001), 1.33 % for Sal<sub>4</sub>, and 0.2 % for Sal<sub>14</sub> (P < 0.05, between these two groups). In conclusion, using salinomycin for 4 weeks successfully controls clinical coccidiosis. However, the lower rate of coccidiosis, the improvement in productive variables, and the lower accumulated mortality in Sal<sub>14</sub> allow for the proposal of including the treatment with salinomycin for 14 weeks in replacement pullets.

**Keywords:** Replacement pullets; Salinomycin; Treatment duration; *Eimeria* spp.; Coccidiosis.

## **Study contribution**

Given the importance of egg production for human nutrition and the impact of coccidiosis on the health of replacement hens during their early life stages, the antiparasitic and productive effects of supplementing them with salinomycin for 14 weeks instead of 4 were tested. It was determined that productive variables increased, the coccidia load was better controlled, and no adverse reactions occurred with the 14-week dose scheme. Since salinomycin does not influence the problem of antibacterial resistance neither in human nor in veterinary medicine, the adoption of a 14-week salinomycin dosing regimen for this type of poultry is proposed.

## **Introduction**

Coccidiosis is a common parasitic/protozoan disease affecting poultry caused by *Eimeria* spp. microorganisms that invade the intestinal lining and cause significant health problems and economic losses. The most common species are *E. tenella*, *E. maxima*, *E. acervulina*, and *E. necatrix*, each affecting different parts of the intestines, resulting in varying severity of symptoms. For example, *E. tenella* is often associated with severe outbreaks due to its effect on the cecum. Common symptoms include diarrhea, sometimes bloody, decreased feed intake, and poor weight gain. If laying chickens are affected, reduced egg production, pale combs and wattles, and lethargy due to anemia become apparent.<sup>(1)</sup> The disease is especially problematic in young birds, particularly before developing immunity, and poor biosecurity often increases the risk of coccidiosis, i.e. humid and warm conditions, poor litter management, and overcrowded housing favor the spread of oocysts, the infectious stage of *Eimeria* spp. In addition, inadequate protocols or excessive use of anticoccidial drugs can contribute to increased protozoan

resistance, leading to outbreaks.<sup>(2)</sup> Also, subclinical infections contribute to an increase in the economic impact of the disease.

Fecal sampling and microscopic identification, quantification of oocysts, and postmortem examination of affected chicken allow diagnosis. When raising young replacement laying hens, coccidiosis prevention and control can be achieved, to a certain degree, with vaccination. However, their success is still partial and variable.<sup>(3)</sup> Complete treatment to remove oocysts is not advisable because hens fail to develop immunity. The most common and successful approach is the prophylactic use of anticoccidial drugs, among which ionophores are often preferred, as they have been linked to developing a good immune response in laying hens.<sup>(4-6)</sup> Because outbreaks of coccidiosis or a considerable load of these protozoa can lead to decreased feed conversion, reduced growth rates, increased mortality, and a decrease in egg production, it becomes critical to control this disease in young replacement laying hens.<sup>(7)</sup>

One of the most widely used drugs for controlling coccidiosis is salinomycin, an ionophore antibiotic.<sup>(8)</sup> This drug acts against *Eimeria* species by fatally disrupting ion transport across the cell membranes, and it is particularly effective in controlling coccidiosis and avoiding the clinical and subclinical presentation of the disease.<sup>(9, 10)</sup> Considering the above, this study aimed to assess the efficacy of salinomycin for the control of acute and subclinical coccidiosis, evaluating its use either for 28 days (4 weeks), as it is often done in broilers and is sufficient to counteract the development of clinical coccidiosis, or for 14 weeks in replacement hens whose productive life is almost a year-long, starting customary at 18 weeks of age.

## **Materials and methods**

### *Ethical statement*

Since the animals were handled according to farm standards and only fecal samples were taken, an animal handling ethics opinion was not necessary.

### *Study location*

This study was carried out on the farm “El Tiron” (Parque Industrial San Francisco, Aguascalientes, México), on a replacement pullet farm.

### *Animals*

In all 3 000 pullets of the Bovans White lineage were included in this trial and were all randomly assigned to three groups of 1 000 chickens per group, divided by chicken wire mesh.

### *Experimental design*

Two treatments with the salinomycin (Pisacox<sup>®</sup>, PiSA Salud Animal, México) were administered, and the control was left without treatment; hence groups were: Sal<sub>4</sub> group, supplemented with salinomycin for 4 weeks, at a dose of 500 g of the commercial premix (60 ppm of salinomycin) per ton of feed. The dose ranged from 6 to 6.3 mg/kg at the beginning and end of the test period that started in week 2 and ended in week 6; the Sal<sub>14</sub> group, supplemented with salinomycin as for the Sal<sub>4</sub> group, but lasting 14 weeks. Given the flock's consumption over the 98 days of treatment in the Sal<sub>14</sub> group, the dose ranged from 6 to 6.3 mg/kg at the beginning and end of the test period, and also starting in week 2 and ending in week 16. The control group was not supplemented with any drug.

For this test, the birds were housed on a concrete floor with a bed of shavings, rice husks, and straw (1:1:1). The feeding was based on the birds' development in different phases (pre-starter from week 0–3, Initiation from week 3–6, growth from week 6–12, and

development from week 12–17). The feed and drinking water were administered *ad libitum*. No antibacterial or growth promoter was used.

Coccidia inoculation was not utilized to facilitate the infestation of the “natural” outbreak. Hence, part of the bedding litter from the previous cycle (approximately 50 %) was left since sporulated (infectious) oocysts are undoubtedly present. When ingested, the sporozoites are released and enter asexual and sexual development cycles, producing thousands of new oocysts in the intestines, and the cycle is repeated. The parameters evaluated were mortality (in number and percentage, only related to lesions in intestines, caecum, and rectum);<sup>(2)</sup> viability/morbidity percentages; feed intake; initial weight; weight gain, and flock uniformity on weeks 4, 10, and 14.

#### *Sample collection and examination*

With coproparasitoscopic examinations in fresh droppings using the flotation technique in 20 birds per group, the anticoccidial efficacy was evaluated every 7 days up to week 16. Then, further assessment followed for 5 weeks. Fecal samples were collected randomly from the pullets using saline solution-moistened swabs. The swabs were carefully inserted into the cloacal cavity of each pullet to collect the fresh fecal samples. Samples were placed in separated test tubes, labeled, and transported to the laboratory in ice to be examined through the flotation technique as described by Urquhart et al.<sup>(11)</sup> Additionally, at the end of the trial, 20 hens per group were randomly chosen and sacrificed, and classified using the lesion scoring scale developed by Johnson and Reid,<sup>(12)</sup> which provides a numerical classification of macroscopic intestinal lesions caused by coccidia ranging from 0–4 according to the degree of intestinal anatomopathological lesions.

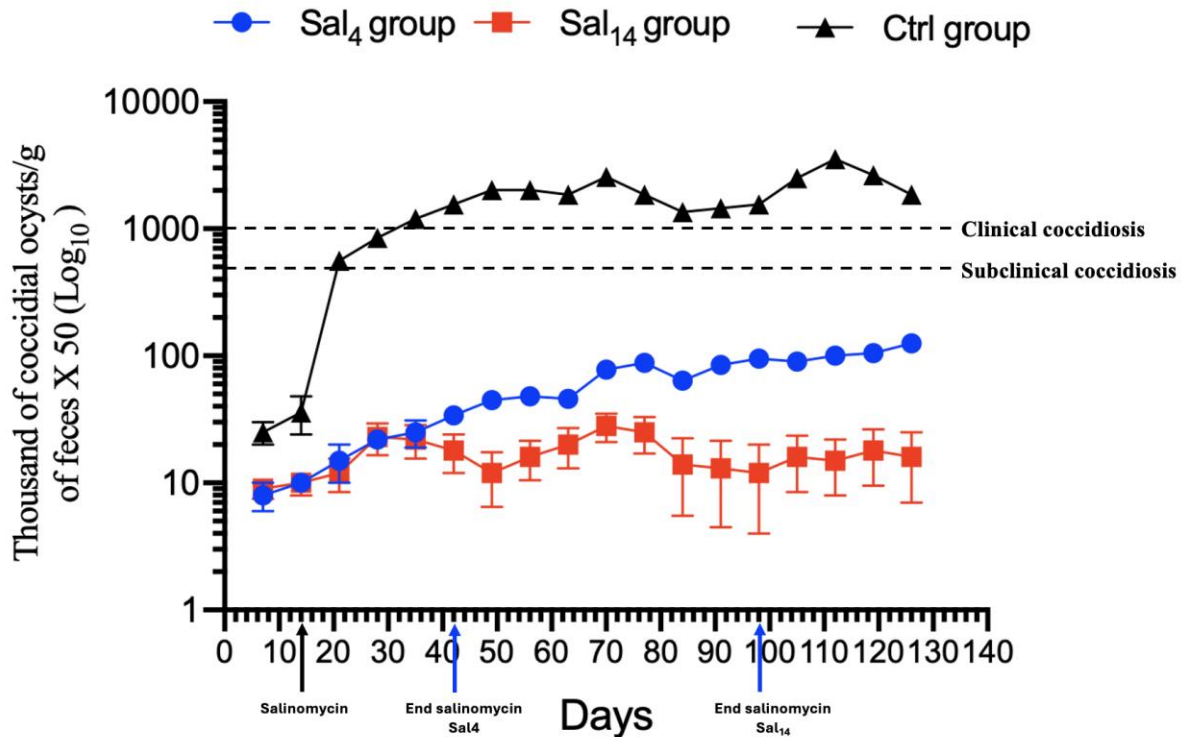
#### *Statistical analysis*

Uniformity is a parameter used to measure body weight variability in a flock. It is linked to good husbandry practices and is sensitive to bacterial or parasitic diseases. This test was calculated using the average weight  $\pm 10\%$  of the mean weight obtained in 100 randomly selected birds. The pullets between both ranges represent the uniformity percentage of the population sample. The pullets were weighed individually in weeks 4, 10, and 14 in the three groups. ANOVA and t-test were performed to compare the groups for statistical analysis.

## Results

### *Oocyst count*

**Figure 1** shows the means and standard deviations of oocyst loads taken from hens' feces. When performing a t-test analysis, statistical differences were found between the three groups ( $P < 0.01$ ); the results reveal that the Sal<sub>14</sub> group had better control of oocyst counts than the Sal<sub>4</sub> group, and obviously, the two medicated groups (Sal<sub>4</sub> and Sal<sub>14</sub>) had lower oocyst counts than the control group.



**Figure 1.** Means  $\pm$ 1 SD of the number of oocysts found per gram of feces (values expressed Log<sub>2</sub> as 1/50 given the McMaster technique) in the groups treated with salinomyacin (Pisacox®) either for 4 weeks (Sal<sub>4</sub>; until day 35) or for 14 weeks (Sal<sub>14</sub>; until day 105).

Low or basal counts: Less than 5 000 oocysts per gram (OPG) of feces is often considered normal for poultry with some acquired immunity or on treatment. Moderate counts: 5 000–50 000 OPG may indicate increased exposure. These levels should be monitored, especially in young birds. High counts: Greater than 50 000 OPG indicate significant infection and generate clinical signs of coccidiosis, mortality, and diarrhea, especially if combined with stress.<sup>(3, 8, 12)</sup>

### *Lesions assessment*

In this test, coccidiosis was often manifested as a subclinical disease. Hence, necropsies and their assessment were performed based on Johnson and Reid's<sup>(12)</sup> recommendations to avoid confusion with other diseases. These results are presented in **Table 1**. It must be noted that coccidiosis lesions were evaluated immediately after randomly slaughtering hens, thus avoiding postmortem changes in the intestine, as they start very quickly and can be confused with coccidiosis. For the diagnosis of coccidiosis, the intestine was divided into three sections: duodenum, middle part (including jejunum and ileum), and caudal third (including caeca and rectum). The severity of the lesions is generally proportional to the number of oocysts ingested by the bird and the course of the disease, which correlates positively with weight loss and the type of droppings.

**Table 1.** Macroscopic lesion scores of the intestines for each of the twenty randomly selected hens

<b>Hen</b>	<b>Ctrl group</b>	<b>Sal<sub>4</sub></b>	<b>Sal<sub>14</sub></b>
1	+2	+1	+1
2	+3	+1	+0
3	+2	+2	+1
4	+3	+1	+0
5	+2	+0	+0
6	+3	+2	+0
7	+3	+1	+0
8	+2	+0	+0
9	+3	+0	+0
10	+3	+2	+1
11	+2	+0	+0

12	+3	+0	+1
13	+2	+1	+1
14	+3	+0	+0
15	+2	+1	+1
16	+3	+0	+1
17	+2	+1	+0
18	+2	+0	+0
19	+3	+2	+1
20	+3	+1	+1

The score was per treatment (Sal<sub>4</sub> and Sal<sub>14</sub>) and in the Ctrl group. +0 = Normal (absence of injuries), +1 = Mild injuries, +2 = Moderate injuries, +3 = Severe injuries, +4 = Very severe injuries, with mortality in the group.

The highest score of macroscopic intestinal lesions for each of the evaluated treatments was found in the Ctrl group (average = 2.55, corresponding to the classification of severe lesions +3). In the Sal<sub>4</sub> group, treatment presented a very low lesion score (average = 0.8; corresponding to the classification of mild lesions +1), while the Sal<sub>14</sub> group presented the lowest score of intestinal lesions (average = 0.35; corresponding to the classification of normal +0).

**Mortality.** Mortality was highest in the control group. Necropsy of daily mortality showed cecal coccidiosis and a mortality of 11 %, mainly due to *E. tenella*, probably in combination with other coccidia. In contrast, the Sal<sub>4</sub> and Sal<sub>14</sub> groups showed much less severe lesions and a low rate of clinical signs, with 2.33 % and 1.9 % mortalities for Sal<sub>4</sub> and Sal<sub>14</sub>, respectively (**Table 2**).

**Table 2.** Hen mortality and viability in the treatments evaluated

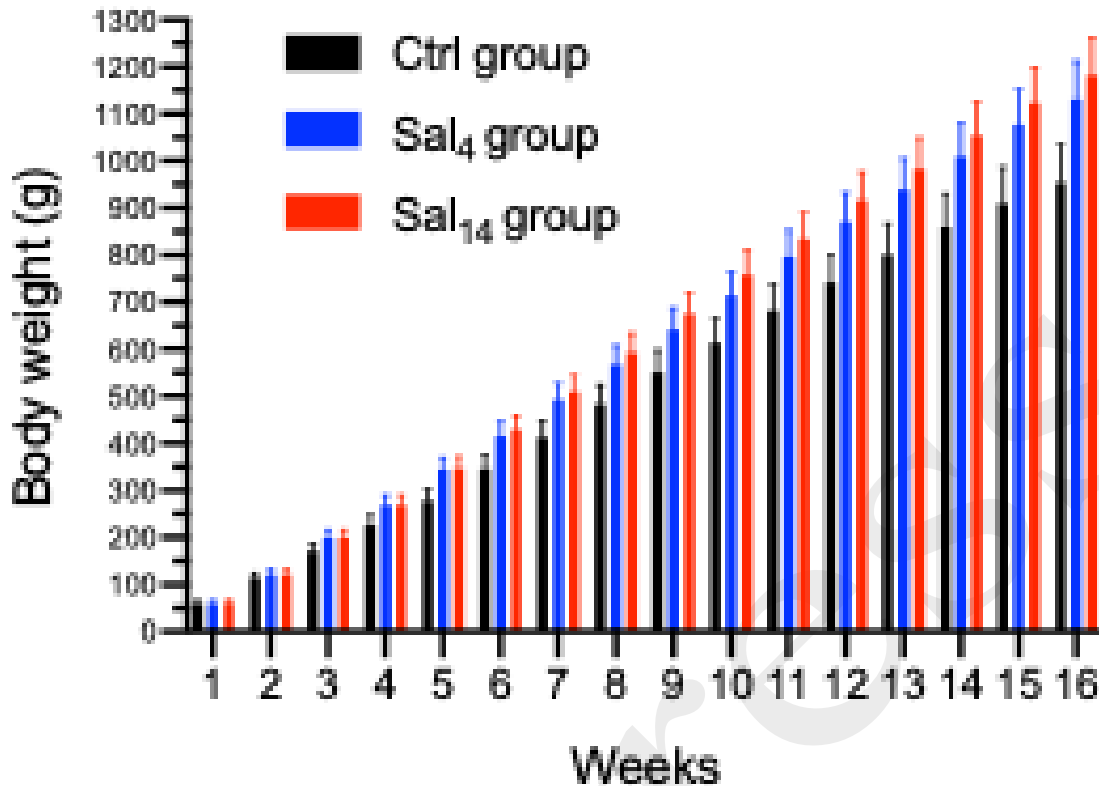
Values	Ctrl group	Sal <sub>4</sub> group	Sal <sub>14</sub> group
Total mortality	110	23	19
Mortality rate (%)	11	2.33	1.9
Mortality due to coccidiosis	98	13	2
Mortality due to coccidiosis (%)	9.8	1.33	0.2

Treatments evaluated were Sal<sub>4</sub> and Sal<sub>14</sub>, as well as mortality in the Ctrl group at the end of the study. n = 1 000 hens.

Total mortality and % mortality correspond to the accumulated mortality in all weeks of treatment, including mortality due to selection, mortality, colibacillosis, coccidiosis, etc. Mortality due to coccidia and % mortality due to coccidia correspond to all those birds in which lesions suggestive of coccidiosis were found at necropsy.

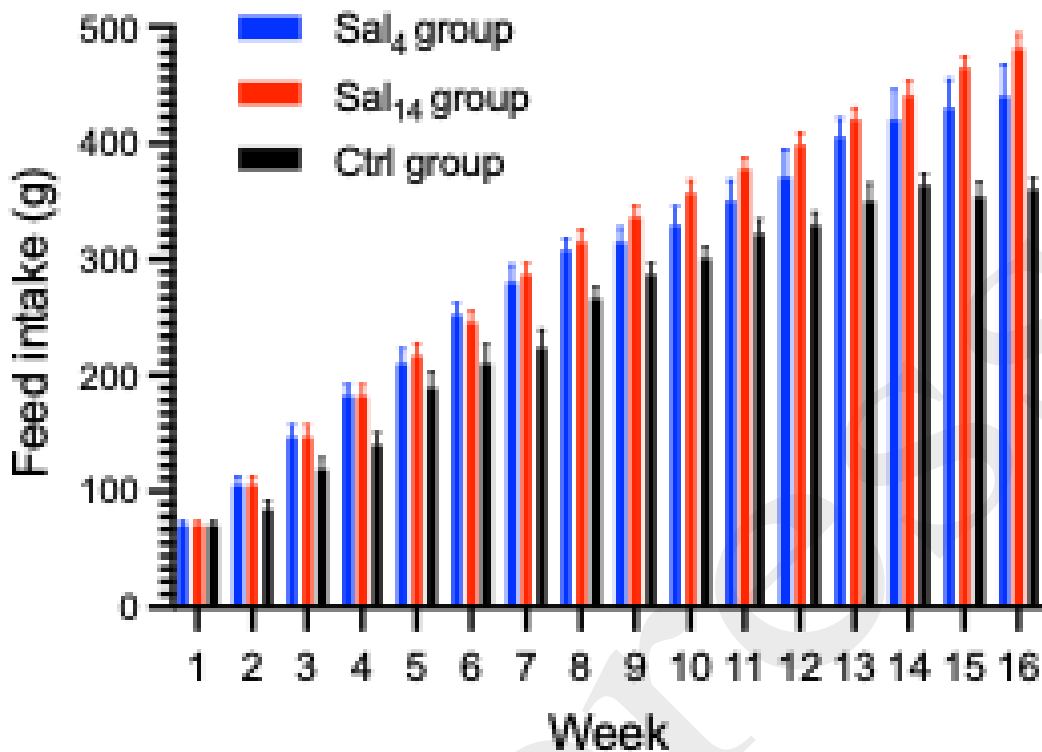
#### *Body weight and daily weight gain*

The results of the weekly body weight assessment are presented in **Figure 2**. Using ANOVA and t-test, a statistical difference ( $P < 0.5$ ) was found from the second week; at the beginning of the study, the average weights had no significant differences. From week four onwards, the average weights between treatments were different, being higher in hens from Sal<sub>4</sub> and Sal<sub>14</sub>, and within the expected parameters of the flock. Sal<sub>14</sub> obtained the best parameter in terms of final weight and daily weight gain compared to Sal<sub>4</sub> treatment ( $P < 0.05$ ) and evidently vs the control group ( $P < 0.01$ ).



**Figure 2.** Average flock weights, where it can be observed that the Ctrl group without salinomycin begins to decrease its average weekly weight, falling below 223 grams, compared to the Sal<sub>4</sub> treatment and especially Sal<sub>14</sub>.

Feed consumption was similar among hens from the three groups in week 1; variations were observed and coincided with the beginning of clinical coccidiosis in the control group. Sal<sub>4</sub> and Sal<sub>14</sub> groups remained healthy within the expected consumption parameters for the flock, while the control group fell below the desired consumption by 11 g. This can be observed in **Figure 3**. Using ANOVA and t-test, a statistical difference was found between the treated and the control groups in feed intake in the second week, but not between the treated groups.



**Figure 3.** Weekly mean feed intake of replacement hens. Feed consumption started to differ from week two onwards in the Ctrl group. In groups Sal<sub>4</sub> and Sal<sub>14</sub> this parameter remained within the desired parameters. Towards the end of the trial, the Sal<sub>14</sub> group had better feed consumption than the Sal<sub>4</sub> group.

Uniformity results show (**Table 3**) that on week 4, control, Sal<sub>4</sub>, and Sal<sub>14</sub> groups were 244, 270, and 270 g, respectively; week 10 (552, 717, and 759 g) and week 14 (833, 1 022, and 1 056 g). In week 4, there was no significant difference between the three treatments, with an average of 85 % uniformity in the three groups. At week 10, the difference in uniformity of the control group compared to Sal<sub>4</sub> and Sal<sub>14</sub> was statistically evident ( $P < 0.05$ ). At week 14, the Sal<sub>4</sub> and Sal<sub>14</sub> groups maintained a similar uniformity

in contrast to the control group, which presented a uniformity of 71 % at 3 weeks of starting laying ( $P < 0.01$ ).

**Table 3.** Uniformity percentages of the evaluated treatments

Uniformity (%)			
Group	Week 4	Week 10	Week 14
Ctrl	84	73	71
Sal <sub>4</sub>	85	82	80
Sal <sub>14</sub>	85	83	82

## Discussion

It has been claimed that recommended doses of salinomycin, as used in this trial, are safe for chickens, but salinomycin can cause toxicity if supplemented excessively.<sup>(13, 14)</sup> As with all anticoccidial drugs, resistance may develop, particularly when used continuously, cycle after cycle. Therefore, integrated coccidiosis control measures, such as rotation of anticoccidial drugs and incorporation of vaccination along with salinomycin, are essential.<sup>(15, 16)</sup> Except for tiamulin, salinomycin is biocompatible with most active ingredients used in poultry diets. It acts early in the coccidia life cycle, causing an ionic imbalance across its membrane, destroying the sporozoite and immature schizonts within the intestinal mucosa cells.<sup>(17)</sup>

The environmental impact of salinomycin use in poultry medicine is negligible for humans. However, care should be taken when disposing of poultry litter.<sup>(18)</sup> Because coccidia that affect birds reproduce in the cells of the intestinal mucosa of the bird, affecting the digestion processes and absorption of nutrients and additives, it results in a decrease in weight gain and skin pigmentation, favoring the predisposition to the

presentation of other diseases. It has been established that approximately 50 000 oocysts per gram of feces or more are linked to significant *Eimeria* spp. —infection, causing clinical outbreaks of coccidiosis, mortality, and diarrhea—. Ten thousand to 50 thousand oocysts per gram of feces may indicate greater exposure, such as in cases of subclinical coccidiosis. A thousand to fewer oocysts per gram of feces suggests a load that requires monitoring to normal levels in poultry.<sup>(9, 15, 18)</sup>

It is well known that oocyst count is the most useful parameter in monitoring coccidiosis in poultry production. It is advisable to keep records of oocyst counts independent of the anticoccidial program utilized. Additionally, it should be noted that the *Eimeria* species, more often found in significant quantities, are the least pathogenic. The emergence of clinical coccidiosis after an anticoccidial treatment is considerably rare, as in the case of the treatment with salinomycin (Sal<sub>4</sub> and Sal<sub>14</sub>). In contrast, the control group presented significantly high oocyst count, and such values are compatible with the gastrointestinal pathology found, i.e., diarrhea and mortality. These differences become evident when the productive parameters of the groups treated with Sal<sub>4</sub> and Sal<sub>14</sub> are compared with those of the hens from the control group.

The score of macroscopic intestinal lesions in the Sal<sub>4</sub> and Sal<sub>14</sub> groups fluctuated between 0–1. No uniform zero score was expected as salinomycin does not entirely suppress infections with *Eimeria* spp. This feature is essential, considering that it allows a low level of coccidial infection, which promotes the development of immunity and is key to maintaining intestinal health in hens during the laying cycle. Pullets in the control group presented lesions with scores as high as 3, i.e., focal whitish lesions, transversely elongated, visible on the serosal surface of the duodenum (*E. acervulina*), as well as a creamy mucous exudate in the middle portion of the small intestine. Lesions of *E. tenella*

were also detected, characterized by marked typhlitis (the inflammatory process of the caeca), a thickened and scarred mucosa with coagulated blood.

Enteritis was observed in different degrees, such as intestinal dilatation, which contained hemorrhagic material. In addition, the intestine was flaccid due to loss of muscle tone. In addition to animal welfare, avian clinical coccidiosis increases production costs, not only due to the use of treatment with anticoccidial drugs but also owing to the treatment of associated diseases.<sup>(19)</sup> The control group presented acute coccidiosis with a morbidity of 100 % and a mortality due to coccidiosis of 9.8 %. Coccidiosis is not only characterized by clinical causing diarrhea and mortality. Additionally, the surviving birds have a deteriorated appearance that has been associated with poor digestion, low weight gain, and delayed laying.<sup>(20, 21)</sup> In contrast to the Sal<sub>4</sub> and Sal<sub>14</sub> treatments, 1.33 % and 0.2 % mortality rates were presented, respectively.

The results of this study showed that the average weights remained similar until week 4 of the test; subsequently, because of coccidiosis, the evaluated groups began to differentiate, noting a lower average weight and lower weight gain in the hens of the control group. This is characteristic of the onset of coccidiosis with ruffled feathers, depression, and decreased feed consumption, and in the case of *E. tenella*, poor skin pigmentation and lower weight gain, low egg production, and a higher number of dirty eggs in laying hens. Morbidity for this parasitosis is usually 100 %, and mortality is variable. Regarding feed intake, the groups treated with the ionophore maintained the predetermined intake for the strain. In contrast, the control group decreased its intake from week two of the test, which coincides with clinical coccidiosis. As mentioned, the consequence is the loss of intestinal integrity, which affects the use of nutrients and increases maintenance costs.

## Conclusions

Clinical coccidiosis readily develops in replacement pullets raised in inadequate litter conditions, emphasizing the need to protect them with anticoccidial drugs, i.e., salinomycin. Also, the number of oocysts per gram of feces influences the productive results of poultry. However, although the use of salinomycin for four weeks (Sal<sub>4</sub>) successfully controls clinical coccidiosis, the degrees of intestinal lesion scores suggest some subclinical coccidiosis. However, a statistically significant decrease in the oocyst count detected in group Sal<sub>14</sub> compared to Sal<sub>4</sub>, especially from week 6 onwards ( $P < 0.05$ ) reveals that treatment for Sal<sub>14</sub> is more effective. Thus, lesions found in Sal<sub>4</sub> group reached a 2+ on the Johnson and Reid's<sup>(12)</sup> scale, while lesions decreased in the Sal<sub>14</sub> group to almost zero.

In this trial, the intestinal portions most affected by coccidia were the duodenum and the caeca. Finally, given that efficiently obtaining replacement hens is a priority for the egg production units and considering that salinomycin is not linked to the problem of bacterial resistance, a 14-week treatment scheme is postulated as viable and harmless.

### **Data availability**

The original datasets used in this research, and if applicable, supporting information files, are deposited and available for download at the SciELO Dataverse repository.

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### **Conflict of interest**

The authors confirm that they do not have any conflicts of interest.

### **Author contributions**

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Methodology: H Sumano, L Gutiérrez

Writing-original draft: L Gutiérrez, H Sumano.

Writing-review and editing: L Gutiérrez, H Sumano.

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