Efficacy of fluralaner (Exzolt) for the treatment of natural *Allopsoroptoides galli* infestations in laying hens

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ABSTRACT Mite infestations in laying hens can cause losses to producers due to stress, reduced egg production and even death of birds. A new species of mite, *Allopsoroptoides galli* (***A. galli***), Analgoidea: Psoroptoididae, was recently identified in commercial laying farms in Brazil, causing damage due to its highly aggressive infestation that results in a sharp drop in egg production and culling. The present study evaluated the acaricidal action of a formulation containing fluralaner (Exzolt) against *A. galli*. Thirty-four laying hens naturally infested with *A. galli* were equally divided into a fluralaner-treated group and an untreated control group. The fluralaner-treated group received Exzolt in drinking water at a dose of 0.05 mL/kg body weight (equivalent to 0.5 mg fluralaner/kg body weight), twice, 7 d apart. Both groups were followed for 70 d evaluating the level of infestation by counting mites in skin scrapings and assessment of skin lesions. The average mite count of the treated group decreased significantly, dropping from 61.6 to 3.8 mites (D+7 to D+70). The efficacy progressively increased on subsequent days, reaching 98.8% on d +56 post-treatment and 96.9% on d +70. Recovery of skin lesions was observed after administration of Exzolt, showing a marked remission in the degree of lesions (2.5 on d −14 to 0.2 on d +70). The mean number of mites in the untreated control group ranged from 79.3 to 124.1 and the lesion score from 2.6 to 2.9, thus remaining stable throughout the study. The results obtained in the present study demonstrated that Exzolt administered at a dose of 0.05 mL of product/kg body weight (equivalent to 0.5 mg of fluralaner/kg body weight), twice at a 7-d interval, in drinking water was effective in the treatment of the mite *Allopsoroptoides galli* in naturally infested laying hens.

Key words: ectoparasite, mites, stress, acaricide, fluralaner

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INTRODUCTION

The proliferation of ectoparasites has caused significant economic losses in confined poultry production, where these parasites find ideal conditions for infestation. These ectoparasites feed on the animal's blood, lymph, damaged skin, or sebaceous secretions (DeVaney, 1978). The presence of arthropod parasites in a flock of chickens increases the production costs and contributes to spreading of avian diseases due to weakness, stress, anemia, and weight loss in chickens. Signs of an infestation include a decrease in egg production without reduced feed intake, leading to a reduction in the feed conversion ratio (Arends, 1991; Hinkle and Hickle, 2008; Tucci et al., 2020).

Allopsoroptoides galli (***A. galli;*** Analgoidea: Psoroptoididae), a new mite species, has been found with increasing frequency in commercial farms in Brazil (Mironov, 2013). According to the author of the new genus and species (Mironov 2013), this is the first record of feather mites of the Psoroptoididae family parasitizing Galliformes, which aroused the interest of acarologists on the origin of the mite in the wild. The reservoir host of *A. galli* was identified the wild bird *Guira cuckoo* (*Guira*; Cuculiformes: *Cuculidae*), as the reservoir host of *A. galli*. Generally, feather mites have specific hosts (Proctor, 2003; Hernandes et al., 2014).

However, intensively reared chickens provide ideal conditions for *A. galli*. This new parasite is aggressive to chickens, causing severe discomfort with hyperactivity of the birds, which spend considerable time and energy scratching themselves and trying to remove the mites with their beaks. Fifteen days after the initial infestation, the birds present intense itching and severe dermatitis, which are the main clinical manifestations. Currently, little is known about this parasite's biology, ecology, and control (Tucci et al., 2014).

Direct contact is the way of transmission of mites between chickens. After 7 d of birds having initial contact with each other, the first signs of itching occur and intensify with the multiplication of mites. Severe dermatitis are observed after 15 d of the infestations. Regions

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where there is no presence of feathers, for example, under the wings, a higher intensity of dermatitis is identified (Tucci et al., 2020).

The identification of this mite is carried out in the laboratory through morphological characteristics. It is important to be careful when analyzing and collecting mites. Mites do not remain on dead birds. In this way, it is possible to have a false negative result in the diagnosis if the scraping is not performed on live birds (Tucci et al., 2020).

Currently, little is known about this parasite's biology, ecology, and control (Tucci et al., 2014). However, prevention measures must be adopted, such as monitoring the avian to obtain an early diagnosis and mitigate the spread. Understanding the behavior of avian, analyzing the presence of itching, zootechnical dadas, dermatitis, and wetting is necessary. General biosecurity measures are essential to avoid spreading to other houses.

Soares et al. (2016) investigated the economic losses caused by *A. galli*, comparing the performance of artificially infested chickens with an un-infested control group. Although feed intake did not differ between the 2 groups, final body weight, egg production, feed conversion, and egg weight were significantly lower in infested hens; egg production was reduced by 31% in the infested group.

It is believed that *A. galli* had no previous contact with acaricidal substances until their arrival in poultry production houses and that this species is therefore sensitive to the commercially available acaricides. This has been verified by some authors (Soares and Tucci, 2012; Tucci and Soares 2013; Tucci et al., 2013) in tests with botanical acaricides and older chemical products. While these products showed acaricidal action on *A. galli,* they are associated with the inconvenience of being applied via spraying, bathing, and dusting, which causes additional stress to the birds and can result in insufficient efficacy if not applied thoroughly.

A new formulation of an insecticide and acaricide drug containing fluralaner [Exzolt], which is administered in the poultry's drinking water, effectively treated infestations with poultry red mites (*Dermanyssus gallinae*) and northern fowl mites (*Ornithonyssus sylviarum*) in hens (Gassel et al., 2014; Hinkle et al., 2018; Thomas et al., 2018). Fluralaner, an isoxazoline compound for use as a systemic treatment, kills mites by binding to a distinct, previously unrecognized receptor site on γ-aminobutyric acid (**GABA**) and L-glutamate-gated chloride channels, which are widely expressed in the central nervous and peripheral neuromuscular systems in insects and acari (Ozoe et al., 2010; Casida, 2015).

The control of mites, especially *A. galli*, in egg production farms is essential to minimize the discomfort of birds and losses to the producer. Thus, the present study aimed to evaluate the acaricidal efficacy of fluralaner - Exzolt (Gassel et al., 2014; Huyghe et al., 2017), which has successfully been used in the treatment and control of hematophagous mites in commercial laying poultry (Mullens et al., 2017; Thomas et al., 2017; Hinkle et al., 2018) when administered in the drinking water.

MATERIALS AND METHODS

Experimental Design

The study was conducted in Bastos (a city in São Paulo state; S 21°56'15"; W 50°41'15"), using an experimental shed belonging to the Bastos Research and Development Unit/Institute of Biology SP/Brazil. The Ethics Committee previously approved the procedures for Animal Experiments (CETEA/IB) under Protocol No. 165/19.

For the study, a total of 34 white hens, Dekalb breed, 56 wk of age, were purchased from a commercial hen farm in the city of Bastos-SP, where *A. galli* infestations had previously been identified and the animals presented mite-induced skin lesions. The birds were housed in a closed shed, equipped with galvanized wire cages, trough-type feeders, and drinking bowls. The birds received feed and water ad libitum and were subjected to a lighting program with 16 h of daily light.

Thirty-four hens naturally infested with *A. galli* mites were included in this blinded and randomized trial. The animals were distributed homogeneously between the experimental groups by their mean mite count on d −1. Each hen represented an experimental unit. The study consisted of 2 experimental groups of 17 hens each: the **treated group** received the fluralaner formulation (Exzolt) in drinking water at a dose of 0.05 mL/kg body weight, twice, 7 d apart (d 0 and d 7); the **control group** received no treatment.

The efficacy evaluations were carried out by mite counts and quantification of skin lesions. Evaluations were performed on all hens before treatment (d −1) and post-treatment on d 7, 14, 21, 28, 42, 56, and 70, according to the procedure described by Tucci et al. (2019):

Mites were counted by skin scrapings: On each evaluation day, 3 skin areas of at least 1 cm^2 were scraped for each animal − wings, flank, and back. The skin scraping was deposited on a microscope glass slide, mixed with a drop of 0.1-M sodium hydroxide solution and protected with a coverslip. Using a 10X magnification microscope, the field was focused on the top corner of the coverslip and the slide was assessed systemically, counting any adult mites.

Tucci et al. (2019) evaluated the skin lesions as a measure of fluralaner efficacy against *A. galli*, a mean of these values was calculated. The animals' wings, sides, and backs were examined and classified as follows: 0 − no dermatitis; 1 − mild dermatitis; 2 − moderate dermatitis; 3 − median dermatitis; 4 − severe dermatitis; and 5 − very severe dermatitis. For skin lesions efficacy, a mean of these degrees was calculated considering wings, sides, and backs.

Statistical analysis of infestation data for both groups was performed using the Student's t test, considering the probability level of 5%. To evaluate the efficacy against the mite, the following Abbott (1925) was used: Efficacy (%) = 100 × (mc - tm) / mc; (mc: mean of live mites in control animals before treatment and tm: mean of live mites in treated animals after treatment).

RESULTS

The results are shown in Tables 1 and 2.

Skin Scrapings

In the evaluation of d +7, there was a reduction in the number of mites in the treated group and a small increase in the control group, with 61.6 mites in the first group and 91.5 in the second, with statistically significant differences between the 2 groups ($P < 0.05$). On d +14, the treated group showed a great reduction in the number of mites per slide with 41.6 in the treated group, 101.9 in the control ($P < 0.05$) and with a product efficacy of 59.2%. On d +21, the efficacy was 80.7%, with 16.3 mites found in the skin scrapings of the treated group and 84.6 in the control group ($P < 0.05$). On d +28 the efficacy of the product reached a rate of 94.8% with 4.3 mites in the treated group and 82.4 in the control group ($P < 0.05$). On d +44 and +56 after initial treatment, efficacy was 98.3% and 98.8%, respectively, with statistically significant differences between the 2 groups ($P < 0.05$) being 1.8 and 1 .4 mites per slide in the treated group at each evaluation and 103 and 112 in the control group. On the last evaluation day, d +70, the efficacy was 96.9% and 3.8 mites were found on the slides in the treated group and 124.1 in the control group ($P < 0.05$).

Skin Lesions

The action of Exzolt on the skin lesions caused by *A. galli* could be observed from d + 7 (Table 2). The difference in the degree of dermatitis between the treated and control groups was higher from d +14 onward, although the 2 groups showed statistically significant differences ($P < 0.05$) in all assessments from day +7. Table 2 presents the results obtained throughout the experiment, where it can be observed the increase in the action of the product from the first application, with an efficacy of 22.2% after 7 d of the first treatment, 38.5% on d + 14, 48% on d +21 and 61.5% after 28 d. From d +44 onward, there was a marked decrease in the degree of lesions (Figures 4, 5 and 6), with efficacy values above 80%, being 85.2 on d +44 and 85.7% on d + 56. The highest value was observed on d +70 with 93.1% efficacy on lesions.

These results demonstrate that the recovery of lesions caused by the mite parasitism takes a longer time than the acaricidal effect of the product. On d +21, an efficacy of 48% of the product was observed (Table 2), however, in the same period, the number of live mites (Table 1) was already greatly reduced, indicating that although on d +21 the skin of the birds not yet fully recovered, the number of live mites was already quite low.

Table 2. Degree of mite-induced dermatitis produced by *A. galli* on different evaluation days.

	Evaluation day							
	D-14	D7	D14	D21	D28	D44	D56	D70
Exzolt	2.5[a]	2.1[a]	1.6[a]	1.3[a]	1.0[a]	0.4[a]	0.4[a]	0.2[a]
Control	2.6[a]	2.7[b]	2.6[b]	2.5[b]	2.6[b]	2.7[b]	2.7[b]	2.9[b]
Efficacy (%)	-	22.2	38.5	48.0	61.5	85.2	85.7	93.1

[ab]Means followed by the same letter in the same column do not differ from each other at the 5% significance level (Student's *t* test).

The results show that Exzolt has an acaricidal action on *A. galli* resulting in an improvement in clinical signs of parasitism throughout the trial period. Assessing the skin lesion score is essential for following the birds' clinical evolution.

DISCUSSION

Ectoparasite control is an essential part of management in the egg production industry to control the damage caused to birds and the losses caused to producers. Among the ectoparasites, mites belong to the most important group, causing significant losses to the poultry industry. Despite the importance of these arthropods, there are few compounds with an indication for use and registered with regulatory agencies for laying poultry. With only few molecules available, it is challenging to design control programs aimed at a broader and more extended use of active pharmaceutical ingredients and to implement the molecule rotation technique aiming to manage resistance development. Fluralaner is a molecule that was recently introduced in the market (Exzolt) and has since been used with great success in the treatment and control of hematophagous mites in commercial laying farms (Gassel et al., 2014; Huyghe et al., 2017; Mullens et al., 2017; Thomas et al., 2017; Hinkle et al., 2018). The efficacy of Exzolt against infestations with poultry red mites (*D. gallinae)* and Northern fowl mites (*O. sylviarum*) has already been reported (Mullens et al., 2017; Thomas et al., 2017; Hinkle et al., 2018).

In this study, Exzolt showed acaricidal efficacy in the treatment and control of the mite *A. galli* and it effectively reduced mite-induced dermatitis. Similar results to this

Table 1. The mean number of *A. galli* mites found in skin scrapings on different evaluation days.

	Evaluation day							
	D-14	D+7	D+14	D+21	D+28	D+44	D+56	D+70
Exzolt	78.4[a]	61.6[a]	41.6[a]	16.3[a]	4.3[a]	1.8[a]	1.4[a]	3.8[a]
Control	79.3[a]	91.5[b]	101.9[b]	84.6[b]	82.4[b]	103.5[b]	112.1[b]	124.1[b]
Efficacy (%)	-	32.7	59.2	80.7	94.8	98.3	98.8	96.9

[ab]Means followed by the same letter in the same column do not differ from each other at the 5% significance level (Student's *t* test).

study were obtained by Hinkle et al. (2018) in the treatment and control of *O. sylviarum*, where fluralaner showed efficacy above 90% after 3 wk from the initial treatment. A study with strains of *D. gallinae* from Europe and Brazil showed that mites are susceptible to fluralaner but resistant to other molecules tested by the authors (Thomas et al., 2018). During field studies with laying hens, fluralaner effectively controlled *D. gallinae*, showing efficacy above 90% for up to 8 mo after the initial treatment (Thomas et al., 2017) if re-infestation with new mites is strictly prevented by biosecurity measures on the farm.

No adverse effects were seen throughout this study consistent with a study conducted by Prohaczik et al. (2017) which assessed the safety of fluralaner in laying hens by analyzing clinical and laboratory parameters, including egg production and egg quality. The authors have found that the oral administration of fluralaner via drinking water at the recommended treatment dose (0.5 mg/kg body weight, twice at a 7-d interval) was safe for the birds and did not impact egg production or egg quality. A similar study was carried out by Huyghe et al. (2017), which confirmed the safety of fluralaner in breeder chickens, noting that fluralaner did not affect the number of eggs, egg weight, or fertility of birds, nor the hatchability of chicks or chick viability. Furthermore, a field study in Europe showed a significant improvement on animal welfare when treating mite-infested chickens with Exzolt EC - European Commission. (2021).

For an efficient treatment of mite infestations in chickens, in addition to being effective and safe, the compound must be easy to apply. One of fluralaner's significant advantages is the convenient administration via drinking water that is well accepted by producers. It considerably reduces the time needed to treat birds (thousands of birds can be treated in a single period of the day) and eliminates the need for additional equipment or labor. Another essential factor is the immense stress suffered by birds during treatment when using methods such as bathing or spraying, which is eliminated by using the fluralaner product.

CONCLUSIONS

The results obtained in this study showed that Exzolt (fluralaner) at a dose of 0.05 mL of product per kg of body weight (equivalent to 0.5 mg of fluralaner per kg of body weight) administered twice, 7 d apart, in the drinking water was effective in the treatment and control of the mite *Allopsoroptoides galli* and reducing mite-induced skin lesions in naturally infested laying hens.

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DISCLOSURES

The authors declare no conflicting interests

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