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Effect of cypermethrin on the control of lesser mealworm (*Alphitobius diaperinus*) and broiler performance[1]

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ABSTRACT.- Souza C.J.D., Barbosa F.M.O., Puzotti Marujo M.M., Santos E.T., Domingues C.H.F., Oliveira D. & Sgavioli S. 2021. **Effect of cypermethrin on the control of lesser mealworm (*Alphitobius diaperinus*) and broiler performance**. *Pesquisa Veterinária Brasileira 41:e06859, 2021*. Universidade Brasil, Av. Hilário da Silva Passos 950, Descalvado, SP 13690-970, Brazil. E-mail: sarahsgavioli@yahoo.com.br

This study analysed the effectiveness of using different levels of a product based on 6% cypermethrin and 2% citronella on broiler production and control the lesser mealworm (*Alphitobius diaperinus*). A total of 648 one-day-old chicks (Cobb®) which were allocated to one of three treatments (no application of the commercial product - control, and two concentrations of the product 2.00 and 3.33g/m^2) in eight replicates with 27 birds each. The commercial product, which was based on cypermethrin was applied in the chambers with an atomizer. The lesser mealworm population was sampled weekly (1, 7, 14, 21, 28, 35 and 42 days of the experiment) using traps, at three points (front, middle and back) inside the pen. The performance, carcass and cut yields were evaluated. At 21, 28, 35 and 42 days of the experiment, the number of lesser mealworm adults and larvae was lower in the treatment groups involving 2.00 and 3.33g/m^2 of the product than in the control group. On the first and 14th days of the study, the number of lesser mealworms adults was lower in the group treated with 3.33g/m^2 of the product compared to the control group, although the results did not differ statistically from those obtained with 2.00g/m^2 of the product. In conclusion, the application of the commercial product at both concentrations (2.00 and 3.33g/m^2) was effective in combating *Alphitobius diaperinus* adults and larvae, and the performance was not influenced by treatments.

INDEX THERMS: Cypermethrin, lesser mealworm, *Alphitobius diaperinus*, animal production, broiler performance, pest control, insecticide.

RESUMO.- [**Efeito da cipermetrina no controle de cascudinho (*Alphitobius diaperinus*) sobre o desempenho de frangos de corte.**] Este estudo analisou a eficácia do uso de diferentes níveis de um produto à base de cipermetrina a 6% e citronela a 2%, na produção de frangos de corte e no controle do cascudinho (*Alphitobius diaperinus*). Um total de 648 pintos de um dia de idade (Cobb®) foram distribuídos em três câmaras com os tratamentos (sem aplicação do produto comercial - controle, e duas concentrações do produto comercial - 2,00 e 3,33g/m^2) em oito repetições com 27 aves cada. O produto comercial a base de cipermetrina foi aplicado nas câmaras com um atomizador. A população de cascudinho foi amostrada semanalmente (1, 7, 14, 21, 28, 35 e 42 dias do experimento) usando armadilhas, em três pontos (frente, meio e fundo) de cada parcela. Foram avaliados o desempenho, rendimentos de carcaça e cortes. Aos 21, 28, 35 e 42 dias do experimento, o número de adultos e larvas de cascudinho foi menor nos grupos de tratamento envolvendo 2,00 e 3,33g/m^2 do produto quando comparado ao grupo controle. No primeiro e no 14º dia do estudo, o número de cascudinhos adultos foi menor no grupo tratado com 3,33g/m^2 do produto em comparação

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ao grupo controle, embora os resultados não tenham diferido estatisticamente daqueles obtidos com 2,00g/m^2 do produto. A conversão alimentar de 1 a 21 dias de idade foi significativa, com os melhores resultados para essa variável quando aplicado 3,33g do produto por metro quadrado e no tratamento controle. Em conclusão, a aplicação do produto comercial em ambas as concentrações (2,00 e 3,33g/m^2) foi eficaz no combate a adultos e larvas de *Alphitobius diaperinus* e, não influenciou o desempenho.

TERMOS DE INDEXAÇÃO: Cipermetrina, cascudinho, *Alphitobius diaperinus*, controle de pragas, desempenho de frangos de corte, inseticida, produção animal.

INTRODUCTION

The poultry sector faces challenges in terms of ensuring the health of birds and consequently, the health of consumers (Chernaki-Leffer et al. 2011). The infestation of chicken litter by insects such as the lesser mealworm (*Alphitobius diaperinus*) is a worrying factor, since this insect can transmit a number of pathogens to birds (Gazoni et al. 2012). Some of these conditions include necrotic enteritis, caused by *Clostridium perfringens*, which affects mostly young birds (Vittori et al. 2007), and the bacteria *Escherichia coli* and *Salmonella typhimurium*. Insects release these microorganisms via feces, which may cause contamination during the rearing period (Crippen et al. 2012).

The *A. diaperinus*, belongs of the phylum arthropod, insect class, coleopteran order and family tenebrionidae. It is an insect with four wings, shiny back and well-developed jaws. The beetles of the order have a varied feeding habit and habitat, because they feed on all kinds of plant and animal materials (Mendes & Povaluk 2017). Besides the impact on animal health, infestation by the lesser mealworm generates economic losses due to the damage caused to facilities, because during the larval phase, these insects form tunnels in the insulation material and in the thermal protection that may compromise the birds' ambient conditions (Salin et al. 2000).

The use of chemical products is still the most effective way of combating this pest (Mustac et al. 2013, Oliveira et al. 2014, Mendes & Povaluk 2017). One of the most widely used chemical insecticides is cypermethrin, a pyrethroid insecticide (Gazoni et al. 2012, Wolf et al. 2015, Oliveira et al. 2016). Reports have been published on the use of cypermethrin, dichlorvos and triflumuron in Brazil, demonstrating their efficiency in combating the lesser mealworm (Chernaki-Leffer et al. 2011, Oliveira et al. 2016). However, a growing number of publications have described the resistance of pest populations to these compounds in several countries (Lambkin 2005, Hamm et al. 2006, Kaufman et al. 2008, Tomberlin et al. 2008, Hickmann et al. 2018). A significant amount of pyrethroids seem to be suitable for suppressing *A. diaperinus* populations in broiler facilities, such as Ravap (tetrachlorvinphosdichlorvos), cyßuthrin, carbaryl, permethrin, Talstar Professional Insecticide (bifenthrin) and Extinosad (spinosad) by Elanco Corporation (Tomberlin et al. 2008).

The monoterpenes extracted from plants as citronella have an insecticidal action on the central nervous system of insects, which impairs their development (López & Pascual-Villalobos 2010) and have repellent and larvicidal activity (Gurib-Fakim 2006).

The objective of this study was to examine the effect of using different levels of a product based on 6% cypermethrin (pyrethroid) and 2% citronella (monoterpenoid) for control of *A. diaperinus* and performance on broiler production. This research could provide insights to policy makers and private companies that can be used to develop strategies to control the pest population in poultry production.

MATERIALS AND METHODS

This experiment was approved by the Animal Ethics Committee (CEUA, approval no. 017668/17) and conducted in Jaboticabal/SP, Brazil, located between the geographical coordinates 21°14'05" South latitude and 48°17'09" West longitude, and average altitude of 615.01m.

Broiler housing and management. A total of 648 one-day-old male Cobb® broiler chicks were reared in 24 pens (2x1m^2) with 27 birds each until 42 days (d) of age (Sakomura & Rostagno 2016). The birds were housed in three chambers (total area of 36m^2 and 3m in height), these were essential for the isolation of the experimental treatments. Although this may involve risks, we believe the experimental design used in this study is justified, since the environmental conditions inside the chambers were controlled and the experimental replicates were uniformly distributed within the chamber. The chambers were acclimatized with: heating hoods, underfloor heating, refrigeration unit and air exhausts.

The birds were allocated to one of the following three treatments with eight replicates each, in a completely randomized design: control (no application of a commercial product) and two product concentrations (2.00 and 3.33g/m^2), in a total of 24 experimental plots.

The broilers were raised on wood shaving litter using 1.2kg of dry substrate per bird, new and with no chemical treatment. No litter management was performed during the experiment and the lesser mealworm infestation occurred naturally by the coleopterans already present in the experimental facility (Oliveira et al. 2016).

The temperatures and humidities the all chambers were measured using a thermo hygrometer and, the average and absolute maximum temperatures were 30°C and 33°C, respectively, while the average and absolute minimum temperatures were 23°C and 19°C, respectively. The average and absolute maximum relative humidity were 55% and 64% and the average and absolute minimum relative humidity was 46% and 37%, respectively.

To monitor the populations of lesser mealworm adults and larvae before treatment started, eight samples of litter (200g/experimental unit) near the feeder were superficially collected before product application. The samples of the insect population were performed by adapting the methodology of Godinho & Alves (2009). Was performed the statistical analysis of the initial samples between the treatments and repetitions, with no significant difference ($P<0.05$).

The commercial product contained 6% of cypermethrin (Dominus, Jandaia do Sul, Brazil) and 2% of citronella, mineral sulfides as a carrier (containing at least 1% of sulfur) and anhydrous silicate. The chemical formula is -cyano-3-phenoxybenzyl-2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate. The product belongs to the Pyrethroid group. The product was applied according to the tested concentration (2.00 and 3.33g/m^2) in the respective treatment chamber. The application of the product was applied through the atomizer (Dominus, Jandaia do Sul, Brazil), where the product was atomized into the air, like a mist, inside each chamber (Oliveira et al. 2016).

Birds received feed and water ad libitum during the entire experimental period and were raised under thermal comfort, following the recommendations for this particular line described in the Cobb Breeder Management Guide (Cobb-Vantress 2008). The environmental conditions of the three chambers were measured. Temperature and relative humidity were recorded daily using three digital thermo-hygrometers (Instrutemp, ITHT-2250, temperature scale from -50°C to 70°C, precision: ±1°C, São Paulo, Brazil), placed at the birds' height in each chamber.

The chicks were vaccinated in the hatchery (Globo Aves, Itirapina, Brazil) against Marek's disease, infectious bursal disease (IBD) and avian pox. The following vaccination program was completed during the experimental period: IBD (mild strain) on the seventh day using eye drops and Newcastle disease and IBD (hot strain) through the drinking water, using powdered milk (Italac, Corumbaíba, Brazil) as a carrier (2g L^{-1}) on day 14. The adopted light regimen was 23L:1D (light:dark). All vaccines administered were from the MSD Animal Health, São Paulo, Brazil.

Broilers were raised up to 42 days of age and were fed with two diets formulated on corn and soybean meal, adjusted for two phases: starter diet (1-21 days: 12.06MJ/kg metabolisable energy, 21.27% crude protein (CP), 0.88% digestible methionine + cysteine, 0.56% digestible methionine, 1.22% digestible lysine, 0.85% Ca, 0.19% Na, 0.42% P available) and grower diet (22-42 days old: 13.07MJ/kg metabolisable energy, 18.86% CP, 0.77% digestible methionine + cysteine, 0.49% digestible methionine, 1.05% digestible lysine, 0.69% Ca, 0.20% Na, 0.32% P available), following the nutritional requirements established by Rostagno et al*.* (2011).

Performance. The average weight, weight gain, feed intake and feed conversion ratio were evaluated for all phases (1 to 21, 22 to 42 and 1 to 42 days of age). Productive viability was analyzed for the total rearing period (1 to 42 days of age); this variable was calculated as a percentage of surviving animals in relation to the initial number of housed birds (V = 100 − mortality*100/initial number of housed birds). The mortality was recorded daily for the correction of performance parameters as weight (Sakomura & Rostagno 2016) and to calculate the viability. For the performance evaluation, the result of each pen was considered an experimental unit (eight pens or replicates per treatment).

Carcass yield. On the day 42 of grow-out, 48 birds (sixteen per treatment) with average body weights close to the average of the experimental unit were selected and identified with numbered leg bands, were fasted for eight hours and then slaughtered by cervical dislocation, drained and plucked.

Subsequently, the birds were eviscerated and the entire carcass without the feet and head was weighed. The carcass was sectioned into cuts (breast, thigh + drumstick; wing + drummet and back) and the carcass yield was calculated. The pieces were weighed and the individual weight of each broiler at slaughter was taken as a basis to determine the carcass yield. Whereas for the evaluation of carcass and cut yields and larvae in the litter before product application, the analyzed birds/samples were considered experimental units (eight broilers/samples or replicates per treatment).

Population sampling of lesser mealworm adults and larvae. Traps were prepared followed a modified version of the Arends model (Safrit & Axtell 1984). The traps were made of polyvinylchloride (PVC) (Tigre, Rio Claro, Brazil) tubes 3.8cm thick×23cm long with a longitudinal opening of 0.65cm, containing a rolled-up sheet of corrugated cardboard (20×30cm) inside and with only one end closed.

For the population sampling of lesser mealworm adults and larvae, we adopted a completely randomized design with the same treatments described above, but using the sample collection days (1, 7, 14, 21, 28, 35 and 42 days of the experiment) as a split-plot and the trap placement points (front, middle and back) as sub-split plots, both with eight replicates. Traps were considered experimental units (eight traps/treatment/day/point).

Statistical analysis. For the number of lesser mealworm adults and larvae in the Arends traps, the normality of the residuals was also checked using the Shapiro-Wilk test. The number of adults and larvae was analyzed using a generalized linear mixed model (PROC GLIMMIX SAS 9.2) with a negative binomial distribution to control the zero-inflated data. In the models, the trap placement point, treatment, day and their interactions were included as fixed effects and the cage was considered a random effect (Eq. 1). Interactions and main effects that were not significant were dropped sequentially to simplify the models. The final models were confirmed using a forward procedure, by sequentially adding the main effects back into the model again, considering the Akaike information criterion (AICc). A Tukey post-hoc test was used for the comparisons. Significance was considered $P<0.05$. The results are presented as means and standard errors (SEs).

For the number of lesser mealworm larvae and adults in the poultry litter, performance and carcass yield and the normality of the residuals was also checked using the Shapiro-Wilk test and analyzed using a generalized linear mixed model (PROC MIXED SAS 9.2). In the model, the treatment was included as a fixed effect. Among the 15 different covariance structures tested, the model used was chosen based on the lowest value of AICc. A Tukey post-hoc test was used for the comparisons. Significance was considered $P<0.05$. The results are presented as means and SEs.

The model included the fixed effects of treatments (application of a commercial product), Days (1, 7, 14, 21, 28, 35 and 42 days of the experiment), and the Treatment*Day interaction, by means of experimental model.

RESULTS

Before the application of treatments, the average + standard error for number of lesser mealworm adults were: 75.00±19.22, 75.50±14.72 and 76.50±22.19 for chambers 1, 2 and 3, respectively and larvae 80.50±24.79, 77.67±26.93 and 80.33±30.67 for chambers 1, 2 and 3, respectively, was similar ($P>0.05$).

The number of lesser mealworm adults (*F*-value = 3.49 and DF = 479) and larvae (*F*-value = 13.31 and DF = 475) was influenced by the interaction between treatments and days ($P<0.0001$ and $P<0.0001$, respectively) (Table 1). After the 21st day of the study, there was an evident decrease in the number of lesser mealworm adults and larvae following the application of both product concentrations (2.00 and 3.33g/m^2) compared to the control treatment. At 21, 28, 35 and 42 days of the experiment, the number of lesser mealworm adults and larvae was lower in the treatment groups involving 2.00 and 3.33g/m^2 of the product than in the control group. On the first and 14th days of the study, the number of lesser mealworms adults was lower in the group treated with 3.33g/m^2 of the product compared to the control group, being statistically different only for the first day of the experimental period.

The same was true for the number of larvae on the first day. When we evaluated the number of larvae in the control group, a difference was detected between the days of the study, with the highest numbers found at 28, 35 and 42 days and the lowest at 1, 7 and 14 days of age.

Feed conversion was influenced ($P=0.0491$) from 1 to 21 days of age, with the best results for this variable were obtained with treatment control and 3.33g of the product (Table 2) and the treatments did not affect (P>0.05) the yields of the carcass or cuts (Table 3).

DISCUSSION

Promising results were obtained regarding the control of this insect.

The number of larvae in the control treatment, on the other hand, increased over the duration of the study, corroborating the results presented by Silva et al. (2001). Pyrethroid-based insecticides act rapidly on beetles of the species *Alphitobius diaperinus*, yielding positive results in less than four hours following product application (Tomberlin et al. 2008).

In poultry farming, a low number of lesser mealworm adults and larvae is desirable, given the losses caused by *A. diaperinus* to commercial facilities (Salin et al. 2000). Moreover, the lesser mealworm is a known reservoir for many human and poultry pathogens (Axtell & Arends 1990).

Table 1. Effect of treatments and days for the number of lesser mealworm adults and larvae in the population sampling of the traps

	Day	Treatment		
		Control	2.00g/m²	3.33g/m²
Number of adults	1	2.5860±0.7941Aa	1.7290±0.5530ABa	0.2030±0.1080Ba
	7	2.2967±0.7128Aa	1.8384±0.5839Aa	1.3040±0.4328Aa
	14	4.3850±1.3267Aa	0.9883±0.3429ABa	0.7599±0.2772Ba
	21	4.4966±1.3296Aa	0.5944±0.2289Ba	0.5493±0.2156Ba
	28	7.4163±2.1466Aa	0.6346±0.2407Ba	0.3302±0.1492Ba
	35	3.9870±1.1869Aa	0.5548±0.2172Ba	0.2466±0.1225Ba
	42	7.8803±2.2764Aa	0.4423±0.1836Ba	0.2433±0.1214Ba
Number of larvae	1	1.8876±0.5254Ac	0.5581±0.2018ABa	0.1670±0.0921Ba
	7	0.9786±0.3062Ac	0.6569±0.2274Aa	0.4559±0.1745Aa
	14	1.2478±0.3803Ac	0.2239±0.1088Aa	0.2057±0.1044Aa
	21	10.9518±2.6608Ab	0.0388±0.0398Ba	0.0417±0.0428Ba
	28	48.0879±11.3820Aa	0.1089±0.0705Ba	0.0831±0.0619Ba
	35	43.0886±10.2081Aa	0.3544±0.1469Ba	0.0416±0.0427Ba
	42	41.1526±9.7534Aa	0.1197±0.0744Ba	0.0833±0.0620Ba

Means followed by different letters in columns and rows, respectively, differ significantly by Tukey's test at 5% probability; ± Standard error.

Table 2. Effect of treatments on broiler performance from 1 to 21, 22 to 42 and 1 to 42 days of age

Treatment	Mean weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion
		1 to 21 days of age		
Control	906.93±11.74	835.49±11.74	1,242±2.32	1.48±0.02AB
2.00g/m²	890.29±20.48	818.85±20.48	1,245±2.26	1.53±0.04A
3.33g/m²	944.73±15.92	874.27±15.92	1,239±2.80	1.41±0.03B
Probability	0.0718	0.0719	0.1926	0.0491
		22 to 42 days of age		
Control	2,977±46.58	2,139±52.12	3,630±74.57	1.72±0.01
2.00g/m²	3,076±17.15	2,256±15.52	3,769±23.71	1.67±0.01
3.33g/m²	3,038±24.84	2,162±36.92	3,718±46.49	1.70±0.02
Probability	0.1094	0.0957	0.1938	0.0842
	Viability (%)	Weight gain (g)	Feed intake (g)	Feed conversion
		1 to 42 days of age		
Control	95.83±1.63	2,975±46.58	4,865±73.16	1.63±0.00
2.00g/m²	98.61±0.68	3,074±17.15	5,014±23.32	1.63±0.01
3.33g/m²	99.08±0.61	3,037±24.85	4,952±47.37	1.64±0.02
Probability	0.0948	0.1093	0.1513	0.9237

Means followed by different letters in columns differ significantly by Tukey's test at 5% probability; ± Standard error.

Table 3. Effects of treatments on broiler carcass and cuts yields

Treatment	Percentage (%)				
	Carcass	Back	Breast	Drumstick + thigh	Wing
Control	73.73±0.63	16.24±0.63	29.35±0.47	20.45±0.25	7.08±0.09
2.00g/m2	74.07±0.64	16.00±0.57	29.47±0.46	20.71±0.30	7.11±0.08
3.33g/m2	73.25±0.58	16.99±0.47	30.65±0.34	20.43±0.22	7.25±0.07
Probability	0.1894	0.4149	0.0629	0.6866	0.2752

± Standard error.

Despins & Axtell (1995) evaluated the feeding behavior and growth of broilers fed the larvae of lesser mealworm. The average weight of the birds fed the larvae was 173g lower than that of the animals which only received feed. In addition to their lower mean body weight, the birds showed signs of stress after being fed the larvae; e.g., high vocalization, feces with high moisture content and the presence of larval cuticles. After ingesting lesser mealworm adults, birds may suffer damage caused by the elytra, which injure their gastrointestinal tract, leaving it vulnerable to the entry of pathogens (Japp et al. 2010) and thereby compromising nutrient digestion and absorption.

Besides the question raised above, the lesser mealworm exoskeleton composition includes chitin, a polysaccharide (Henry et al. 2015) that is indigestible by monogastric animals (Sánchez-Muros et al. 2014). Chitin is a linear polymer of b-(1-4) N-acetyl-d-glucosamine units, which reduces protein digestibility in broiler chickens (Khempaka et al. 2011). As such, it might have compromised the birds' feed conversion ability. Ballitoc & Sun (2013) observed a downward trend in the feed conversion ratio following the addition of 0 to 10% of *Tenebrio molitor* larvae meal in broiler diets and attributed this result to the proteins bound to chitin and therefore, indigestible by birds. Other authors observed reduced digestibility in broilers fed a diet containing a meal of the insect's larvae (Belluco et al. 2013).

It is known that lesser mealworm population in poultry houses are difficult to control. Studies have shown that the use of insecticides remains the main control strategy for insects used inside poultry houses (Mustac et al. 2013, Oliveira et al. 2016).

CONCLUSION

The application of the commercial product with 6% of cypermethrin and 2% of citronella at both concentrations (2.00 and 3.33g/m^2) was effective in combating *Alphitobius diaperinus* adults and larvae in chambers and did not affect the broilers performance until 42 days of age.

Conflict of interest statement.- The authors declare that they have no conflict of interest.

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