



The Use of A Compound Based on Phyllosilicates and Cinnamon Essential Oil for Chicken Broiler's Litter

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■ Keywords

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ABSTRACT

The objective of this study was to evaluate the effectiveness of a litter conditioning compound (LCC) based on 98% phyllosilicates (kaolinite) and 2% cinnamon essential oil to be applied on the litter of broiler houses. Animal performance, prevalence of footpad lesions, and litter quality (water activity, pH, and moisture) were evaluated, in addition to the effect on the incidence of *Salmonella* spp., evaluated by testing shoe-drag swabs, fecal samples, and organs. Twenty-one broiler houses with similar structure and rearing conditions were distributed in a completely randomized design with three treatments and seven replications each, as follows: T0: control, without the use of LCC; T100: application of 100 grams of LCC per m² of litter per week; T200: application of 200 grams of LCC per m² of litter per week. There was no significant effect of treatments on performance variables; however litter pH was lower when 200g/m² was used in comparison with the control treatment, a result not verified for moisture and water activity ($p>0.05$). Our findings demonstrated significant reduction in the percentage of footpad lesions in birds that received the LCC (T0: 63.37%; T100: 41.38% and T200: 27.24%). A reduction in the number of positive flocks for *Salmonella* spp. Was also observed (with overall positivity rates of 17.86%, 12.14%, and 5% for treatments T0, T100, and T200, respectively). It is concluded that the product reduces the incidence of *Salmonella* spp. and also significantly reduces the incidence of footpad lesions, two important factors for the poultry industry.

INTRODUCTION

Antibiotics as growth promoters (AGPs) allowed to improve animal performance and to reduce feed contamination by pathogenic microorganisms (Brenes & Roura, 2010), mainly when associated with feed ingredients of good quality (Gonzales *et al.*, 2012). On the other hand, consumer pressure due to possible threats of bacterial resistance caused by the indiscriminate use of AGPs in poultry lead to several restrictions regarding the use of antibiotics in the European Union since 2006 (Mezalira *et al.*, 2014). Thus, many studies have been carried out to test some alternative additives using natural plants, such as essential oils (EOs), since they may maintain animal performance results (Brenes & Roura, 2010).

When compared to AGPs, Eos are less toxic, leave no residue, and are considered safe for animals and humans (Hashemi & Davoodi, 2011). Their mechanism of action in bacteria is related to the enhancement of membrane permeability, leading to a loss of ions with reduced membrane potential that makes the proton pump inefficient, leading to ATP depletion (Bakkali *et al.*, 2008). Cinnamon oil (*Cinnamomum verum*) is a potent antimicrobial agent and acts against foodborne microorganisms. When used in association with other essential oils, such



as thyme and clove oil, it has a synergistic antimicrobial effect and may reduce microbial resistance (Lu *et al.*, 2011).

Broiler chicken litter is one of the main sources of dissemination and perpetuation of pathogens in poultry houses, as birds spend most of their time on it. Its physical and chemical characteristics are directly related to animal welfare and reflect on productive efficiency and losses, mainly those related to pododermatitis (Jong *et al.*, 2014). Voss-Rech *et al.* (2019) reported that in six consecutive flocks, with nine repetitions, 28.4% of the litter collected in the Southern region of Brazil were positive for *Salmonella* spp. mainly in the first and second flocks, remaining positive in up to four subsequent flocks. Additionally, contaminated flocks show higher chances of cross contamination in the slaughterhouse, especially under high water activity (Aw) conditions due to increased bacterial multiplication (Dunlop *et al.*, 2016A).

Phyllosilicates make up a group of minerals that includes micas, smectites, and kaolinites, among others. Their chemical and physical properties can be modified in laboratories, forming thermostable polymers with good porosity (Shoonheydt *et al.*, 1999) and making them an option for controlling litter moisture (Azevedo *et al.*, 2012). They have catalytic activity, which provides decomposition of organic molecules and adsorption of heavy metals (Guerra *et al.*, 2006). This way, they make for a reactive product with a high capacity for technological applications (Azevedo *et al.*, 2014). Literature lacks information on the potential of phyllosilicates to capture and immobilize substances, their effects on chemical changes in pH, humidity, and drying power of poultry litter, as well as on their antimicrobial properties. In this context, the objective of this study was to evaluate the effectiveness of a so called litter conditioning compound (LCC) based on 98% phyllosilicates (kaolinite) and 2% cinnamon essential oil on broiler's performance, litter quality, incidence of footpad lesions, and reduction of *Salmonella* spp. in broiler flocks with history of positivity for this bacterium.

MATERIALS AND METHODS

The experiment was carried out in poultry houses located in the west of Santa Catarina, Brazil. They belonged to an integrated agroindustry and approximately 260,000 birds were evaluated under similar rearing and infrastructure conditions. Each poultry house had approximately 1,200 m², automatic feeders, and nipple-type drinkers.

All animals were male, with an average population density of 10.5 birds per m². Broiler litter were made of pine shavings (*Pinus elliottii*) that had previously hosted at least five broiler flocks, and with history of positivity for *Salmonella* spp. The broilers' diet was based on corn and soybean meal, formulated according to the needs of the birds, as recommended by Rostagno *et al.* (2017). The interval time prior to the accommodation of the flocks and disinfection were carried out according to the company's technical guidelines. Physical and chemical laboratory analyzes were performed at the Animal Nutrition Laboratory of the Animal Science Department of the State of Santa Catarina University (UDESC) and the *Salmonella* spp. search was performed by a private laboratory accredited by the Brazilian Government.

TREATMENTS

The 21 poultry houses were distributed in a completely randomized design with three treatments of seven replications each, as follows: T0: control, without the use of LCC; followed by two treatments with LCC at the following dosages: T100: application of 100 grams per m² per week; T200: application of 200 grams m² per week. For the variables related to the litter characteristics, a 3 x 5 factorial arrangement (3 LCC levels and 5 litter collections) was used, with seven replicates each.

According to the manufacturer, the litter conditioning compound (LCC) consists of 98% phyllosilicates (kaolinite) and 2% cinnamon essential oil. The product was sprinkled superficially on the litter and manually over the entire length of the poultry house. Applications were performed one day before housing and 07, 14, 21, 28, 35 days after housing, and also one day before slaughter (42 days of age).

VARIABLES

The performance data and percentage of paws unfit for commercialization were provided by the slaughterhouse. Only severe footpad lesions were considered.

Litter samples were collected randomly on days 0 (before housing), 09, 23, 35, and 42 days of life, and all analyzes were performed in triplicate. Full deep litter profile samples of approximately 200 g were generated from randomly obtained subsamples. The areas near and below the feeder and drinking fountain were avoided. The pH reading was performed



according to the methodology described by Silva & Queiroz (2002), which consisted of diluting the sample with distilled water (2:1) with the aid of a pH meter (Testo, model 206). To measure the water activity (A_w), the AquaLab® equipment (Decagon Devices Inc., Pullman, WA, USA) was used, with a measuring range from 0.030 to 1,000 A_w and accuracy of ± 0.003 . For litter moisture percentage determination, the samples were weighed in trays with a known tare weight and taken to a forced air circulation oven (55 °C) until weight stabilization. Then, the percentage of moisture was calculated from the relationship between the initial and final weights of the sample, discounted from 100. Shoe-drag swabs and fresh feces for *Salmonella* spp. search were collected also on days 0 (before housing), 09, 23, 35 (after housing), and 1 day before slaughter (42 days of life), according to the methodology described in Normative Instruction no. 20 of the Ministry of Agriculture, Livestock and Supply (Brasil, 2016). Additionally, 10 chicks were humanely euthanized by cervical dislocation and a pool of organs (liver, heart, and spleen) was collected, as well as yolk and cecum. Moreover, on days 09, 23, 35 of age, and 1 day before slaughter, 05 birds per flock were necropsied and pools of cecal tonsil, gallbladder, and fresh feces were collected for *Salmonella* spp. search. The samples were sent under refrigeration to an accredited laboratory.

STATISTICAL ANALYSIS

All variables were subjected to normality testing (Shapiro–Wilk) and, subsequently, to the analysis of variance. In cases of significant differences between treatments, the LCC dosages effects were assessed by polynomial regression, and litter sampling days effects were compared by Tukey's test ($p < 0.05$). For *Salmonella* spp. results (presence or absence), descriptive statistics were used.

ETHICS COMMITTEE

This work was approved by the Ethics Committee on Animal Use (CEUA) of the State University of Catarina (UDESC) under protocol number 9438130319.

RESULTS AND DISCUSSION

Results of zootechnical performance are presented in Table 01. There was no effect of the treatments on performance variables in the period from 1 to 42 days of life; that is, the addition of LCC did not cause a

negative effect. Similar results were reported in two different studies (Lucca *et al.*, 2012; Bruno *et al.*, 1999), where no statistical differences were found in the performance variables of broilers up to 42 days of age when using conditioners for broiler litter (calcium hydroxide, aluminum sulphate, calcium sulphate, 48% calcium sulphate + 28% expanded phyllosilicate) and agricultural gypsum, respectively.

Table 01 – Effect of litter conditioning compound on animal performance variables.

Treatment	FI, Kg	WG, Kg	FC	Viability, %
T0	5.25	3.11	1.69	96.25
T100	5.32	3.11	1.71	96.37
T200	5.22	3.07	1.70	96.30
<i>p</i> -value	0.895	0.839	0.882	0.25
CV (%)	3.97	5.64	4.01	24.43

FI = feed intake; WG = weight gain; FC = feed conversion; CV = coefficient of variation;

T0: control; T100: 100 g/m² per week; T200: 200 g/m² per week.

On the other hand, McWard & Taylor (2000) and Oliveira *et al.* (2003) found improvement in the feed conversion and weight gain of broilers when comparing untreated litter with litter that received acidified clay and aluminum sulphate, agricultural gypsum, simple superphosphate and hydrated lime, respectively. According to Jong *et al.* (2014), wet litter leads to a reduction in the consumption of water, feed and, consequently, a reduction in weight, and an increase in mortality, feed conversion, and the percentage of footpad lesions, harming animal welfare by a reaction induced by pain, in addition to increasing the condemnation of parts or whole carcasses in slaughterhouses, which causes losses to companies and increased production costs. In the present study, the litter moisture found was always below 23% (Table 02), desirable values that did not cause negative effects on the performance of the birds. The untreated litter (T0) had lower moisture when compared to the litter that received 100 g of LCC ($p < 0.05$).

There was a significant reduction (36.13%) of footpad lesions in broilers reared on litter that received T200 when compared to the control group (Figure 01). This result is important from a productive point of view, since the paws can be better used, increasing profitability and contributing to animal welfare. Litter with high humidity favors the appearance of paw and leg lesions, and dirty feathers, which increases bacterial contamination in the birds, facilities, and litter. The zootechnical performance is also impaired when compared to birds raised on farms that manage to keep litter dry. McWard & Taylor (2000) performed



Table 02 – Effect of treatments and sampling period on the average values of pH, moisture, and water activity of broiler chicken litter under different treatments.

	Treatment (T)		
	pH	Moisture, %	Water Activity
T0	8.49 A	20.27 B	0.869
T100	8.50 A	21.93 A	0.886
T200	8.31 B	20.91 AB	0.871
p	<0.001	0.034	0.080
Sampling day (SD)			
Zero	8.66 A	22.20 A	0.849 B
09	8.61 A	19.46 B	0.814 C
23	8.57 A	20.87 AB	0.858 B
35	8.19 B	21.27 AB	0.929 A
42	8.14 B	21.40 AB	0.927 A
p-value	<0.001	0.023	<0.001
p-value TxSD	0.678	0.385	0.877
CV (%)	2.65	12.63	5.06

A, B Different letters in the same column differ by Tukey's test ($p < 0.05$). CV = coefficient of variation. T0: control; T100: 100 g/m² per week; T200: 200 g/m² per week.

pH = $8,51 + 0,0007X$ ($R^2=0,06$); Moisture = $20,27 + 0,030X - 0,0001X^2$ ($R^2=0,04$).

an economic estimation with the control and group regarding injuries and observed that, in addition to the reduction of healthy paws, there was a worsening with losses due to other injuries, with a smaller margin of 0.089 €/chicken, which is a considerable difference considering slaughter volume (Jong *et al.*, 2014).

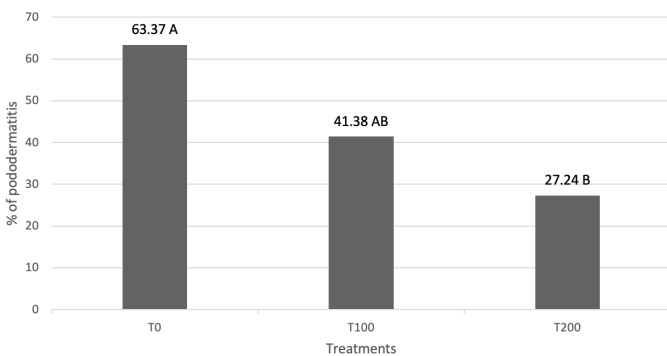


Figure 1 – Percentages of paws unfit for commercialization from flocks that received the different treatments at 42 days of age.

A, B Different letters in the same column differ by Tukey's test ($p < 0.05$). Coefficient of variation = 22,15%. T0: control; T100: 100 g/m² per week; T200: 200 g/m² per week.

Figure 02 shows the results for *Salmonella* spp. in the different samples and periods, according to the treatments tested. A total of 140 samples were analyzed for each treatment, on day zero (swab before housing) and in different periods (01, 09, 23, 35, and 42 days) and locations (shoe-drag swab, yolk, cecum, gallbladder, cecal tonsil, and feces), where 25, 17, and seven positive samples were observed for treatments T0, T100 and T200, respectively, in the different tissues/places analyzed. Flocks became

positives for *Salmonella* spp. mainly after 23 days of age. According to Voss-Rech *et al.* (2019), this is an expected finding, since flocks that reuse litter develop intestinal microbiota quicker, with greater chances of detecting positive flocks for *Salmonella* spp. According to these authors, there is still a high correlation, with recurrences of this pathogen in the following flocks when closer to slaughter.

Figure 02 – *Salmonella* spp. incidence in shoe-drag swabs (S), pool of organs (O), yolk (Y), cecum (C), gallbladder (G), tonsil (T) and feces (F) on days zero (before housing), one, nine, 23, 35 and 42 of age.

Day	0		1					9					23					35					42				
	S	O	Y	C	G	T	S	F	G	T	S	F	G	T	S	F	G	T	S	F	G	T	S	F			
T0									X	X						X	X			X	X	X	X	X			
T0																X	X										
T0																						X	X	X			
T0								X							X	X	X							X			
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T0: control; T100: 100 g/m² per week; T200: 200 g/m² per week.

Infection pressure is low in the poultry house before housing due to proper disinfection management; however, *Salmonella* spp. can remain in the environment (Pedersen *et al.*, 2008) which increases the likelihood of contamination of birds and carcasses in the slaughterhouse (Volkova *et al.*, 2010). Voss-Rech *et al.* (2017) found that *Salmonella* Heidelberg remained in broiler litter even after several procedures such as 14 days of interval between flocks, feather removal and burning, litter fermentation, and lime treatment, indicating the persistence capacity of this pathogen. According to Deblais *et al.* (2018), this is due to the ability of certain bacteria to capture and incorporate DNA from others, by horizontal transfer of plasmids with genes related to resistance. Bacterial resistance to disinfectants may be related to the presence of efflux mechanisms of toxic substances by



bacterial cells, which may use resistance mechanisms similar to those of antibiotics (Stefani *et al.*, 2018). A linear decrease in the number of positive samples for *Salmonella* spp. was observed on a dose-dependent manner (Figure 03), where a higher dosage of LCC led to lower percentages for the bacterium (treatments T0, T100 and T200 and 17.86%, 12.14%, and 5%, respectively). These values show that there was an effective use of LCC, with a better effect for the dosage of 200 g/m²/week. It is important to note that at T0 it was already possible to observe positive flocks from 9 days of age onward, and this increase in positivities was gradually greater as the age of the birds increased. It should be noted that all poultry houses in this study had history of positive flocks for *Salmonella* spp., which shows the effect of the treatments.

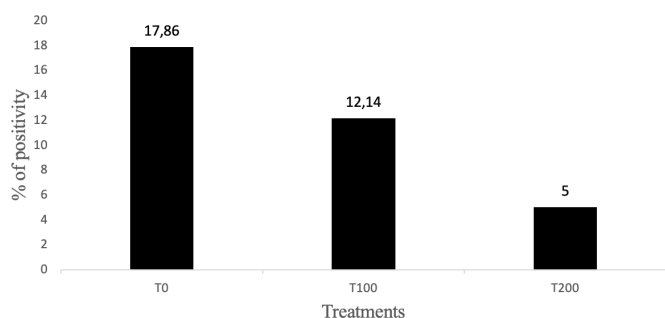


Figure 03 – Overall incidence of *Salmonella* spp. according to the treatment.
T0: control; T100: 100 g/m² per week; T200: 200 g/m² per week.

Our findings show a pH reduction when LCC (T200) was used compared to T0 and T100 (Table 02), which did not differ from each other. The pH of the litter has a direct influence on the levels of ammonia in the air: the lower the pH, the greater the ammonium:ammonia ratio, i.e., more ammonia will be converted into the non-volatile ammonium ion (Ndegwa *et al.*, 2008). No effects of treatments on water activity were observed.

There was a reduction in pH as the age of the birds increased (Table 02), with values significantly lower after 35 days of age ($p < 0.001$). With litter acidification, NH₃ emission is reduced and the environment becomes more hostile to bacterial multiplication. Moreover, the effect of pH on microbial activity interferes with the decomposition of uric acid into NH₃, which is important to the volatilization of NH₃ in the environment.

Means for litter moisture differed statistically between days zero (before housing) and day nine, when a slight drop was observed, probably due to the rise in ambient temperature in the initial days after housing. However, it is noteworthy that the average litter moisture found in all periods of analysis are considered dry according to Collett (2012), who

indicates that the litter is wet when it has moisture above 25%. Water consumption increases with age (Williams *et al.*, 2013), as well as litter humidity, which should be reduced mainly by removing wet and hard litter, proper ventilation, and its continuous turning over (Collett, 2012). When the litter is considered dry, the evaporation rate due to air velocity is reduced, being unable to remove the amount of water needed and, on the other hand, the volume of water increases in the final growth phase of the birds, both by consumption and by excreta production (Dunlop *et al.*, 2015). The temperature is reduced through the use of fans and sprinklers. In this experiment, the water activity showed a behavior different from that of moisture, with a significant increase from 35 days after housing, as described by Dunlop *et al.* (2015). Water activity illustrates the potential for growth of microorganisms in a given location (Dunlop *et al.*, 2016B). This theory was confirmed in the present study, since higher values of Aw were observed (35 and 42 days) along with higher numbers of positive samples for *Salmonella* spp. (Figure 2).

CONCLUSION

The use of litter conditioning compound (LCC) in the tested dosages did not affect the performance of broiler chickens, reduced the incidence of footpad lesions, and the presence of *Salmonella* spp., with the dosage of 200 grams per m² per week showing the best results.

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