PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 42:e06990, 2022
DOI: 10.1590/1678-5150-PVB-6990

Original Article
Livestock Diseases

Ammonia gas for bacterial control in poultry litter[1]

Richard F. Muniz[2], Willian R. Oliveira[3], Rhaquel S. Pereira[3], Cristiani V. Pasqualotto[2], Luciana R. Santos[2], Laura B. Rodrigues[2], Bruno S, Mendonça[2], Luciane Daroit[4] and Fernando Pilotto[2]*

ABSTRACT.- Muniz R.F., Oliveira W.R., Pereira R.S., Pasqualotto C.V., Santos L.R., Rodrigues L.B., Mendonça B.S., Daroit L. & Pilotto F. 2022. **Ammonia gas for bacterial control in poultry litter.** *Pesquisa Veterinária Brasileira 42:e06990, 2022*. Centro de Diagnóstico e Pesquisa em Sanidade *Animal*, Universidade de Passo Fundo, Av. Brasil Leste 285, São José, Passo Fundo, RS 99052-900, Brazil. E-mail: fernandopilotto@upf.br

The current techniques used in the disinfection of reused poultry litter, such as lime addition, windrowing and plastic cover on the surface, do not guarantee the elimination of pathogenic microorganisms, causing damage to the environment and animal health. Gram negative bacteria, i.e., *Salmonella* and *Escherichia coli*, can be transmitted from one batch to another through reused litter, causing health damage to broilers and humans that consume food contaminated by these agents. Our study assessed the effectiveness of the methods plastic cover on the surface (PCS) and plastic cover on the surface with ammonia gas injection (PCSAI) in the control of Gram negative bacteria. The results obtained, both in laboratory conditions (Experiment 1) and in the field (Experiment 2), demonstrate that the method PCSAI with 0.22% ammonia gas had a significant reduction (P<0.05) of Gram negative bacteria in the period of 48 hours This new methodology for disinfecting poultry litter will allow its reuse in a practical and safe way, improving the preservation of the environment, of the health of broilers and consumers of poultry products.

INDEX TERMS: Ammonia gas, biosecurity, broilers, poultry litter.

RESUMO.- [**Gás amônia para controle bacteriano em cama aviária.**] O reaproveitamento de camas aviárias na criação de frangos de corte é uma prática muito utilizada no Brasil. Essa prática reduz custos de produção e contribui na conservação do meio ambiente. As técnicas atuais utilizadas na desinfecção de camas aviárias reaproveitadas, como adição de cal, enleiramento e lona na superfície, não garantem a eliminação de microrganismos patogênicos porque não geram quantidade suficiente de amônia. O gás amônia, em concentrações elevadas, tem efeito biocida. Bactérias Gram negativas, como as *Salmonelas* e *Escherichia coli*, podem ser transmitidas de um lote para outro através do reaproveitamento da cama, ocasionando prejuízos para a saúde das aves e dos humanos que consomem alimentos contaminados por estes agentes. Este trabalho avaliou a eficácia do método lona na superfície com injeção de gás amônia no controle de microrganismos Gram negativos. Os resultados obtidos demostraram que esse método controlou os microrganismos Gram negativos num período de 48 horas em camas de frangos de corte reaproveitadas. Assim, essa nova metodologia de desinfecção de camas de aviário permitirá sua reutilização de forma prática e segura, melhorando a saúde das aves e dos consumidores dos produtos avícolas.

TERMOS DE INDEXAÇÃO: Gás amônia, biossegurança, frangos de corte, cama aviária.

INTRODUCTION

The reuse of poultry litter in Brazil is a technique deployed in broiler production that aims to reduce production costs and contribute to the environmental preservation. However, the current methods used to disinfect poultry litter do not guarantee the elimination of pathogens that are harmful to the health of broilers and humans (Santos et al. 2012, Lopes et al. 2015, Vaz et al. 2017).

[1] Received on October 12, 2021.
Accepted for publication on November 21, 2021.
[2] Graduate Program in Bioexperimentation, Universidade de Passo Fundo (UPF), Av. Brasil Leste 285, São José, Passo Fundo, RS 99052-900, Brazil. *Corresponding author: fernandopilotto@upf.br
[3] WR Industry, Ed. Nana Business Style, Av. 136, 761, 11º andar, St. Sul, Goiânia, GO 74093-250, Brazil.
[4] Universidade de Passo Fundo (UPF), Av. Brasil Leste 285, São José, Passo Fundo, RS 99052-900, Brazil.

Among the treatment methods of reused poultry litters, the plastic cover on the surface method has shown the best results in the reduction of Gram negative microorganisms (Silva et al. 2007). After broilers are sent to the slaughterhouse, this method consists of placing an 180 micron plastic cover over the poultry litter along the entire length and wrapping its ends in the litter itself in order to avoid the dissipation of gases produced by microbial fermentation. The assessment of the main physical-chemical parameters in poultry litter subjected to plastic cover on the surface showed changes in ammonia production by microbial fermentative processes caused by this method (Gehring et al. 2020).

Ammonia in poultry litter is generated from uric acid degradation by microorganisms present in excreta. Ammonia production depends mainly on the enzyme uricase, which has its action influenced by pH, temperature, oxygen and water presence in the litter (Kim & Patterson 2003). The biocidal action of the compound is still unclear, but its low molecular weight enables passing through cell membranes, raising the cytoplasmic pH and consequently causing the death of bacteria (Kim & Patterson 2003, Santana 2016).

Gram negative bacteria present in poultry litter, such as *Salmonella* and *Escherichia coli*, can cause losses in broiler performance and risks to consumer health. The main zootechnical losses in broilers result from increased mortality, lower feed conversion and increased discard of carcasses in the slaughterhouse (Muniz 2014). Regarding human health, these bacteria can cause food poisoning, especially by *Salmonella*. Furthermore, they contribute to an increase in bacterial resistance, as they cause disease in birds, which requires greater use of antimicrobials for treatment. Thus, this practice generates an increase in resistant microorganisms in the environment and in poultry products, causing, consequently, greater bacterial resistance in humans due to the consumption of foods contaminated by these microorganisms. (Mendonça 2016).

The aim of our study was to assess the effectiveness of the methods plastic cover on the surface and plastic cover on the surface with ammonia gas injection during a 48-hour period in the control of Gram negative bacteria in the poultry litter.

MATERIALS AND METHODS

The research was carried out in two stages (Experiment 1 and Experiment 2) in broiler farms located in the northern region of the state of Rio Grande do Sul, southern Brazil. This study was approved by the Animal Ethics Commission of the "Universidade de Passo Fundo" (UPF, registry no. 038/2017). In Experiment 1, the methods plastic cover on the surface (PCS) and plastic cover on the surface with ammonia injection (PCSAI) were compared by counting Gram negative bacteria in the poultry litter for a period of 48 hours. Meanwhile, Experiment 2 evaluated the reduction of Gram negative bacteria by assessing the method PCSAI in five broiler farms. Microbiological analyzes of Gram negative bacteria were carried out in the microbiology laboratory of the "Faculdade de Agronomia e Medicina Veterinária (FAMV-UPF).

In Experiment 1, after bird removal from the aviary, the equipment was washed and 30 squares of $1m^2$ each were delimited in the poultry litter. Treatments tested were: Treatment 1 (T1): plastic cover on the surface without ammonia application; Treatment 2 (T2): plastic cover on the surface with ammonia gas injection. The treatments were replicated five times and the counting of the microorganisms was performed during the periods of 0, 24 and 48 hours. The poultry litter used in the experiment had been reused for six consecutive batches. In both treatments, peripheral furrows were made in the delimited squares and a litter sample was collected from each experimental unit (sample time zero). Later in T1, the plastic cover was placed on the litter and wrapped on the sides of the open furrow for sealing. Meanwhile in T2, a perforated hose was placed on the litter to inject ammonia and then the plastic cover was applied following the same procedure as in T1. In T2, an injector of ammonia gas was developed to inject ammonia gas (Aveclean®[5]) in a controlled manner for 10 minutes during every hour. With activation of the opening valve, the gas was injected through hoses under the plastic cover of each experimental unit in T2. Approximately 130g (0.11%) of ammonia gas was injected into each experimental unit during the 24-hour period and 260g of ammonia gas (0.22%) in experimental units injected for 48 hours. We considered litter height of 20cm and density of $600kg/m^3$ to calculate the dosage of ammonia gas. Litter sub-samples were collected at three points within each experimental unit for Gram negative bacteria assessment. The subsamples were mixed into a single sample in sterile packaging and then sent to the laboratory in ice styrofoam boxes for analysis. Gram negative bacteria were counted using the most likely number (MLN) technique.

Experiment 2 was carried out in five broiler farms with size of 150m length and 16m width. All farms tested had six lots in litter. After bird removal, the effect of the method PCSAI was evaluated on the reduction of Gram negative bacteria. In these broiler farms, a hose with perforations spaced at 1m was placed on the litter every 3m along the aviary. Then, an 180 micron plastic cover was placed on the litter and hoses and the sides of the cover were wrapped in the litter itself to prevent the escape of ammonia gas. The hoses were coupled with the Aveclean® ammonia applicator, which applied the ammonia gas for 10 minutes per hour over a period of 48 hours (Fig.1). To calculate the volume of ammonia gas per broiler farm, we considered a litter height of 10cm and density of $600kg/m^3$. The volume of ammonia gas applied was 320kg per aviary, being equivalent to 0.22% with ammonia concentration of 99.5%. Three samples were collected per broiler farm (beginning, middle and end of the building), and each sample was composed of 10 sub-samples collected in the respective evaluated regions. The samples were collected before the application of the ammonia gas (time 0), 24 and 48 hours after the beginning of the treatment. The samples

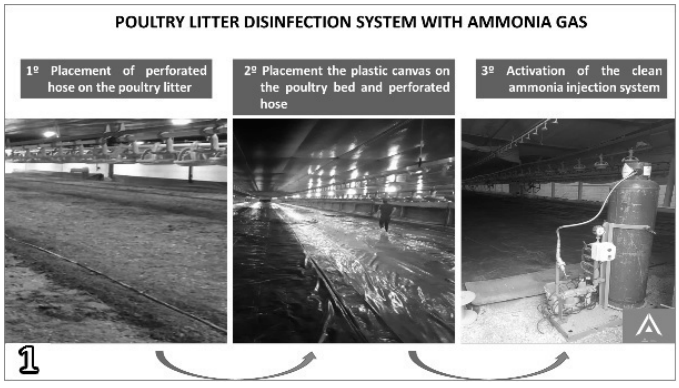

Fig.1. Aveclean system used for ammonia gas application.

[5] Available at <http://estrategiaagro.com.br/portfolio/ver/20/aveclean> Accessed on Feb. 19, 2019.

were placed in sterile bags and sent to the laboratory refrigerated in styrofoam boxes with ice.

For bacteria quantification, 10g of litter sub-samples were homogenized in phosphate buffered saline (PBS) pH 7.4 solution by means of an orbital shaker (150rpm/10 min) and subjected to ten-fold dilutions up to 10^{-5}. Aliquots of 0.1mL of each dilution were plated on MacConkey agar (Acumedia, USA) for total enterobacteria counting. The plates were incubated at 37°C for 48 h (Lu et al. 2003). The equipment for ammonia gas injection was developed by the engineers of the WR Industry, which is in accordance with the technical safety standards recommended by the "Ministério do Trabalho" (Ministry of Labor). The installation of the equipment, acquisition of ammonia in cylinders, transport and application in the broiler farms was carried out by the same company that has authorization from the "Ministério do Trabalho" to perform these activities.

Statistical analysis of the data was performed through analysis of variance and subsequently the means compared by the Dunn test (post-hoc Kruskal-Wallis). The Shapiro-Wilk's test was used to test the data normality.

RESULTS

The results obtained from Experiment 1 (Table 1) demonstrate that the method PCSAI with 0.22% ammonia gas had a significant reduction (p<0.05) of Gram negative bacteria in the period of 48 hours. Meanwhile, there was no reduction in the counting of Gram negative bacteria with the method PCS during the same period. Conversely, there was a significant increase in the count of Gram negative bacteria (p<0.05). We also observed that there was no significant difference in the count of Gram negative bacteria between the application periods of 24 and 48 h in T2. However, there was a numerical bacterial reduction of 100% in the period of 48 h, whereas approximately 60% in the 24 h period.

Experiment 2 confirmed the results found in Experiment 1 in field conditions. We observed that the method PCSAI with 0.22% ammonia gas injection significantly reduced (p<0.05) Gram negative bacteria count in the five broiler farms (Table 2). In addition, the reduction in litter contamination was numerically greater in the 48 hours compared to the 24 hours after application, although no statistical difference was found in four out of the five tested farms.

DISCUSSION

The treatment methods of reused poultry litter (lime addition, windrowing and PCS) exhibit varying degrees of effectiveness, mainly because they do not generate enough ammonia (NH_3) concentration in the litter to eliminate pathogenic microorganisms (Gehring et al. 2020). Ammonia production depends on the action of the enzyme uricase on the uric acid present in the bird excreta. Uricase maximum activity is observed with litter alkaline pH around 8 to 9 (Itaya 1967, Santana 2016). The lime addition method is efficient in raising the litter pH to 8.5-9, which increases ammonia production. However, as the produced ammonia quickly volatilizes, minimum concentrations are not reached to guarantee litter disinfection (Santana 2016). According to Gehring et al. (2020), the lime addition method (600g/m^2) does not raise the litter pH above 10, a pH level that is necessary for litter disinfection. The windrowing method has its microbicidal action mainly based on the temperature increase in the windrow (50 to 60°C). This treatment method does not guarantee litter disinfection because the temperatures in the outer regions of the windrow are not bactericidal and because the enzyme uricase has its activity reduced with temperatures above 35°C, reducing ammonia production (Egute et al. 2010). When the windrow is covered with plastic, the bactericidal efficacy increases because it prevents the ammonia from volatilizing.

Table 1. Count of Gram negative bacteria (log10 cfu/g) in poultry litter treated with plastic cover on the surface and plastic cover on the surface with injection of ammonia gas for a period of 24 and 48 hours

Time	Plastic cover on the surface without ammonia injection (T1) ($\bar{x} \pm s$)	Plastic cover on the surface with ammonia injection (T2) ($\bar{x} \pm s$)	P value
0 hours	3.62 ± 0.33 aA	4.2 ± 00.43 aB	0.047
24 hours	4.83 ± 1.23 abA	1.69 ± 1.92 abB	0.047
48 hours	5.44 ± 0.89 bA	0.00 ± 0.00 bB	0.005
P value	0.016	0.009	----------

a,b,A,B = The averages followed by the same lower case letters in the columns and the same upper case letters in the lines do not differ (P>0.05) by the Dunn test (Kruskal-Wallis post-hoc).

Table 2. Count of Gram negative bacteria (log10 cfu/g) from litter from different broiler farms in the periods of 0, 24 and 48 hours after ammonia injection

Time	Broiler Farms					*P* value
	A	B	C	D	E	
	($\bar{x} \pm s$)	($\bar{x} \pm s$)	($\bar{x} \pm s$)	($\bar{x} \pm s$)	($\bar{x} \pm s$)	
0 hours	4.93 ± 1.24 aA	3.80 ± 0.51 aA	3.84 ± 0.53 aA	2.84 ± 0.40 aB	4.86 ± 0.69 aA	0.040
24 hours	2.96 ± 2.83 abA	0.00 ± 0.00 bAB	0.00 ± 0.00 bAB	2.85 ± 0.11 aA	1.46 ± 2.53 abAB	0.049
48 hours	0.92 ± 1.60 bA	0.00 ± 0.00 bA	0.00 ± 0.00 bA	0.00 ± 0.00 bA	0.96 ± 1.66 bA	0.519
P value	0.049	0.022	0.022	0.049	0.049	--------

a,b,A,B = The averages followed by the same lower case letters in the columns and the same upper case letters in the lines do not differ (P>0.05) by the Dunn test (Kruskal-Wallis post-hoc).

Conversely, the ammonia concentrates at the windrow top due to its volatility, with reduced disinfection near the base of the external part of the windrow. The method PCS exhibits the best result in the disinfection of poultry litters because it can retain ammonia gas under the plastic cover, increasing its concentration in the poultry litter. Furthermore, the efficiency of this method depends on litter sealing by the plastic cover, the humidity and presence of oxygen (Vaz et al. 2017, Gehring et al. 2020). The uricase enzyme has its activity reduced under the absence of water and oxygen. Therefore, reduction in oxygen concentration begins when the plastic cover is placed on the litter, which limits the production of ammonia by bacterial fermentation (Kim & Patterson 2003, Santana 2016). Voss-Rech et al. (2017) reported that the PCS was not efficient in eliminating *Salmonella* Heidelberg in poultry litter. Likely, this outcome was observed because the production of ammonia does not exceed 600ppm in litter under the method PCS (Gehring et al. 2020). The bactericidal concentration for *Salmonella* needs to be above 1,468ppm of ammonia (Koziel et al. 2017).

In our study, we observed an increase in concentration of Gram negative bacteria during the first 48 hours after applying the method PCS. At least 10 days of fermentation is recommended in order to achieve a higher concentration of ammonia in the litter in order to improve its bactericidal efficacy (Silva 2011). As evaluated in the initial fermentation period (48 hours), we speculate that increased bacterial count occurred because the litter is moistened on its surface before being covered with the plastic, with greater water availability for bacterial growth. Gehring et al. (2020) observed that the longer the period with the plastic cover on the litter, the greater the ammonia gas concentration under the plastic cover.

The excellent effectiveness of the method PCSAI is justified because the ammonia concentration is raised to over 2,200ppm in 48 hours, which guarantees litter disinfection. The mechanism of action of intracellular NH_3 is not yet clearly understood. In animal cells, the compound can reach and cross the bacterial cell membrane due to its neutral charge and low molecular weight (17g/mol) (Warren 1962). Inside the cell, NH_3 acts by increasing the cellular pH, possibly as a result of its direct influx, binding to hydrogen ions and by displacing the cellular potassium concentration outside the cell, which destabilizes homeostasis (Luther 2015).

The reduction in Gram negative bacteria count obtained in this study by the action of the ammonia gas is in agreement with the studies already available in the literature. Himathongkham & Riemann (1999) studied the bactericidal effect of ammonia gas against *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, experimentally inoculated in poultry excreta. The authors reported growth inhibition after two days for *E. coli* and *L. monocytogenes* and after six days for *Salmonella* Typhimurium, with a reduction of eight logarithmic units for *Salmonella* Typhimurium and *E. coli* and four units for *L. monocytogenes.*

Ammonia production in the poultry litter by bacterial fermentation increases according to the number of batches carried out on the same litter, from 3,488mg/broiler housed in the first batch to 94,781mg/broiler in the sixth batch on the same litter (Santana 2016). Therefore, further studies will be carried out to verify the behavior of ammonia when injected into the poultry litter. With the presence of litter moisture, ammonia becomes ammonium (NH_4^+), which is not volatile (Liu et al. 2006). Another further study could determine the amount of ammonia produced by the microbial fermentation of the litter, since most of the ammonia-producing microorganisms will be eliminated after its treatment.

The present study demonstrated that the treatment with PCSAI for a period of 48 hours eliminates Gram negative bacteria from the poultry litter. These results are very promising because they optimize resource allocation and logistics of broiler farms due to the reduction of the sanitary void from 15 to six days. This litter treatment enables the production of up to 1.2 more batches in one year, increases the number of flocks in the same poultry litter, reducing contamination of the environment, improvements in the zootechnical performance of animals with increased feed conversion; reduction in the mortality and discard of carcasses in the slaughterhouse with cost optimization and profitability for the agroindustry. In addition, it reduces environmental contamination and the use of antimicrobials, which will promote less microbial resistance and healthier food for consumers.

CONCLUSION

The treatment plastic cover on the surface with ammonia injection (PCSAI) for a period of 48 hours eliminates Gram negative bacteria from the poultry litter.

Acknowledgments.- The "Universidade de Passo Fundo", Aveclean and JBS for the financial support in carrying out this research.

Conflic of interest statement.- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Egute N.D.S., Abrão A. & Carvalho F. 2010. Estudo do processo da geração de amônia a partir de resíduos avícolas visando a produção de hidrogênio. Revta Bras. Pesq. Desenvolv. 12(1):1-6.

Gehring V.S., Santos E.D., Mendonça B.S., Santos L.R., Rodrigues L.B., Dickel E.L., Daroit L. & Pilotto F. 2020. *Alphitobius diaperinus* control and physicochemical study of poultry litters treated with quicklime and shallow fermentation. Poult. Sci. 99(4):2120-2124. <https://dx.doi.org/10.1016/j.psj.2019.11.039> <PMid:32241497>

Himathongkham S. & Riemann H. 1999. Destruction of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. FEMS Microbiol. Lett. 171(2):179-182. <https://dx.doi.org/10.1111/j.1574-6968.1999.tb13430.x> <PMid:10077842>

Itaya K., Yamamoto T. & Fukumoto J. 1967. Studies on yeast uricase. Agricult. Biol. Chem. 31(11):1256-1264. <https://dx.doi.org/10.1271/bbb1961.31.1256>

Kim W.K. & Patterson P.H. 2003. Effect of minerals on activity of microbial uricase to reduce ammonia volatilization in poultry manure. Poult. Sci. 82(2):223-231. <https://dx.doi.org/10.1093/ps/82.2.223> <PMid:12619798>

Koziel J.A., Frana T.S., Ahn H., Glanville T.D., Nguyen L.T. & Van Leeuwen J. 2017. Efficacy of NH3 as a secondary barrier treatment for inactivation of *Salmonella* Typhimurium and methicillin-resistant *Staphylococcus aureus* in digestate of animal carcasses: proof-of-concept. PLoS One 12(5):e0176825. <https://dx.doi.org/10.1371/journal.pone.0176825> <PMid:28475586>

Liu Z., Wang L. & Beasley D.B. 2006. A review of emission models of ammonia released from broiler houses. ASAE Annual Meeting, American Society of Agricultural and Biological Engineers, p.1. <https://dx.doi.org/10.13031/2013.21568>

Lopes M., Leite F.L., Valente B.S., Heres T., Dai Prá M.A., Xavier E.G., Roll V.F.B. 2015. An assessment of the effectiveness of four in-house treatments to reduce the bacterial levels in poultry litter. Poult. Sci. 94(9):2094-2098. <https://dx.doi.org/10.3382/ps/pev195> <PMid:26217027>

Lu J., Sanchez S., Hofacre C., Maurer J.J., Harmon B.G. & Lee M.D. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Appl. Environ. Microbiol. 69(2):901-908. <https://dx.doi.org/10.1128/aem.69.2.901-908.2003> <PMid:12571010>

Luther A.K. 2015. Ammonia toxicity in bacteria and its implications for treatment of and resource recovery from highly nitrogenous organic wastes. Master's Thesis, Graduate Program in Environmental Science, The State University of New Jersey, New Jersey, NJ. <https://dx.doi.org/10.7282/T3668G53>

Mendonça E.P. 2016. Características de virulência, resistência e diversidade genética de sorovares de *Salmonella* com impacto em saúde pública, isolados de frangos de corte no Brasil. Doctoral Dissertation, Universidade Federal de Uberlândia, Uberlândia, MG. <https://dx.doi.org/10.14393/ufu.te.2016.75>

Muniz E., Mesa D., Cuaspa R., Souza A.M. & Santin E. 2014. Presence of *Salmonella* spp. in reused broiler litter. Revta Colombiana Ciênc. Pec. 27(1):12-27.

Santana I.K.S.S. 2016. Emissões de gases de efeito estufa e amônia oriundas da criação de frangos de corte em múltiplos reusos de cama. Doctoral Dissertation, Universidade de São Paulo, Piracicaba, SP. <https://dx.doi.org/10.11606/T.64.2016.tde-20062016-170313>

Santos M.J.B., Samay A.M.A.T. Silva D.A.T., Rabello C.B., Torres T.R., Santos P.A. & Camelo L.C.L. 2012. Manejo e tratamento de cama durante a criação de aves. Revta Eletrôn. Nutritime 9(3):1801-1815.

Silva V.S. 2011. Estratégias para reutilização de cama de aviário. Embrapa Suínos e Aves. Available at <https://www.alice.cnptia.embrapa.br/bitstream/doc/916974/1/estrategisaparareutilizacao0001.pdf> Accessed on Feb. 19, 2019.

Silva V.S., Voss D., Coldebella A., Bosetti N. & Avila V.S. 2007. Efeito de tratamentos sobre a carga bacteriana de cama de aviário reutilizada em frangos de corte. Embrapa Suínos e Aves, Concordia, SC.

Vaz C.S.L., Voss-Rech D., Avila V.S., Coldebella A. & Silva V.S. 2017. Interventions to reduce the bacterial load in recycled broiler litter. Poult. Sci. 96(8):2587-2594. <https://dx.doi.org/10.3382/ps/pex063> <PMid:28371809>

Voss-Rech D., Trevisol I.M., Brentano L., Silva V.S., Rebelatto R., Jaenisch F.R.F. Okino C.H., Mores M.A.Z., Coldebella A., Botton S.A. & Vaz C.S.L. 2017. Impact of treatments for recycled broiler litter on the viability and infectivity of microorganisms. Vet. Microbiol. 203:308-314. <https://dx.doi.org/10.1016/j.vetmic.2017.03.020> <PMid:28619162>

Warren K.S. 1962. Ammonia toxicity and pH. Nature 195:47-49. <https://dx.doi.org/10.1038/195047a0> <PMid:14005047>