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Reduction of ammonia emissions by applying probiotics on litter in a commercial breeding poultry house

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Abstract. Agricultural sectors account for a part of total ammonia emissions, including poultry. This is especially true in breeding poultry houses, where birds live on litter for several months. The purpose of the research was to reduce ammonia emission and to improve birds living environment. The study was performed in two breeding poultry houses: the test house (ProLG) and the control house (ConLG). The study starts when young breeding birds (Ross 308) are housed at 19 weeks of age until the birds are eradicated at 60 weeks of age. At the test house the probiotic mixture in a powder form was manually spread 10 g per m^2 before the birds were placed, and it was spread manually once a week on litter and over manure pits throughout the lifetime 5 g per m^2 of probiotic mixture. The amount of ammonia in ProLG and ConLG houses was measured in the fifth week after the start of the study and afterwards every four weeks at the same 6 points each time and the condition of the litter was assessed on a 5-point scale. As the age of the poultry increases, the number of measurements also increases. Electricity consumption was calculated every month for the test housing and for the control housing. The trial is still ongoing, initial results showed that amount of ammonia has decreased compared to the control house, indicating that the probiotics can be used efficiently to reduce ammonia in the manure of the birds and improving the microclimate in poultry houses, but subsequent results did not give the expected results - the efficacy of probiotics had not been yet approved.

Key words: ammonia, *Lactobacillus farcimins*, *Lactobacillus rhamnosus*, manure, poultry.

INTRODUCTION

Reducing ammonia (NH3) emission into the environment has been an important topic in the world and in the Baltic States already for a long time. According to the data of the Ministry of Agriculture of Republic of Latvia, NH3 emission are mainly generated by agricultural production as a result, and these activities accounted for 85.8% of the total ammonia emission in Latvia in 2016 (MARL, 2018). One of the agricultural sectors is poultry farming and demand for poultry meat is growing worldwide and in Latvia as well. Poultry farming is a rapidly growing agricultural sector: according to the data of the Ministry of Agriculture, in 2013 there were more than 4.5 million poultry in Latvia, in 2017 more than 4.7 million poultry and in 2019 already more than 5.6 million poultry (LDC, 2020).

An equally important problem in poultry farming is the maintenance of proper litter condition to provide birds with a high-quality, safe, welfare-friendly living environment. Evaporation of ammonia not only worsens the health of birds (eg respiratory burns, eye irritations, pododermatitis, etc.), reduces feed intake and productivity, but also reduces the value of poultry litter as a fertilizer due to nitrogen loss (Nuernberg et al., 2016; Pezzuolo et al., 2019). Scientists analyse and study the possibilities of bird manure management, their processing technologies, possible positive, as well as harmful effects on the environment, carbon, nitrogen and phosphorus in manure without losing their economic value. At the same time, opportunities are being sought to reduce and concentrate manure without harming bird health (Wei et al., 2017; Drozdz et al., 2020). There are studies describing attempts a treatment of manure by enriching litter with biological materials such as probiotics and / or enzyme mixtures to promote NH_3 degradation. It has been found that the probiotics can reduce the NH_3 content of the litter, alter the litter microbiota, making it more favourable for birds, and reduce the formation and accumulation of toxic compounds (Dornelas et al., 2017; Ibrahim et al., 2018; Naseem & King, 2018).

Chen et al. (2018) described a study showing the ability of *Lactobacillus rhamnosus* to utilize ammonia *in vitro*. In the obtained results, the team shows and explains how *L. rhamnosus* can reduce the level of ammonia ions in media containing ammonium chloride, and concludes that *L. rhamnosus* is able to fix ammonia nitrogen and promote ammonia nitrogen transformation.

A study has been described in which the enrichment of litter with probiotics by spreading it in powder form on the litter before placing broilers, on days 15 and 35 of the production cycle, has resulted in at least 36% reduction in ammonia emissions at the end of the cycle compared to a control house where probiotic powder was not scattered on the litter. There are also positive trends in the reduction of mortality and the increase of live weight of birds in the housing where bedding was treated with probiotics (Pezzuolo et al., 2019). Also Mahardhika et al. (2019) made research findings that adding a mixture of probiotics (*Lactobacilus sp*. 10^9 CFU mL^{-1} and Bacillus sp. 109 CFU mL^{-1}) to drinking water during bird life can reduce ammonia excretion from litter by 36.22% comparing the readings on the 35th day of the cycle, but better results were shown by spraying the probiotic mixture in the form of an aerosol on the bedding, where the ammonia release was reduced by 57.72%, as well as the combination of these methods reduced the ammonia release by 68.89% compared to the control group.

Therefore, the aim of our study was to evaluate the effect of a mixture of litter and probiotics (starting at 10 g per m^2, hereinafter 5 g per m^2 per week) on litter condition, ammonia emissions and electricity consumption in a commercial breeding poultry house.

MATERIALS AND METHODS

Technical details

The research was conducted from April 2020 to March 2021. In this article the data from the first to the thirty-fourth week of the study has been analysed. The study was performed in two identical breeding poultry houses in the same farm: the test house and the control house. Both houses are 1,350 m^2 large, the arrangement of the buildings in relation to the east-west. Buildings without windows, only artificial light 12 hours a day, reverse-flow ventilation system. In both cases the litter material are dedusted softwood shavings distributed before the start of the production cycle, 90 m^3 of shavings in each house. The feeding system is based on circular feeders for fodder and drinking lines with nipples. The diet was arranged based on the standard needs of the breeders.

The ammonia level in each house was measured with a portable single-gas detector 'Micro IV'(manufacturer 'GfG Europe Ltd'), ammonia detection from 1 ppm up to 200 ppm, before the use every time the device was calibrated in fresh air outdoors.

Probiotics composition

The mixture of probiotic is in a powder form, consisting of lactic acid bacteria - *Lactobacillus farcimins* CNCM-I-3699 – 7.8.10^6 GU / g and *Lactobacillus rhamnosus* CNCM-I-3698 – 7.8.10^6 GU / g.

Procedure

The study starts when young breeding birds (Ross 308) are housed at 19 weeks of age until the birds are eradicated at about 60 weeks of age. There were 9116 birds housed in the experimental house (experimental group, ProLG) and 9192 birds in the control house (control group, ConLG) at the beginning of the research.

At the experimental house the probiotic mixture in a powder form was manually spread 10 g per m^2 before the birds were placed, and it was being spread manually once a week on the litter and over the manure pits on the slates throughout the lifetime of the birds, each time 5 g per m^2 of probiotic mixture. Throughout the study per m^2 were used 210 g of probiotic mixture.

The amount of ammonia in ProLG and ConLG houses was measured in the fifth week after the start of the study and afterwards every four weeks at the same 6 points each time 40 cm above the surface, shown in Fig. 1. Ammonia level measurements and condition of litter was carried out every time in the same time: from 09.00 AM to 10.00 AM, 5 minutes in each point. As the age of the poultry increases, the number of measurements also increases - from 17 weeks to 25 weeks of study is measured every three weeks, from 26 weeks to 31 weeks of study measurements are done every two weeks, after 32 weeks of study ammonia is measured once a week.

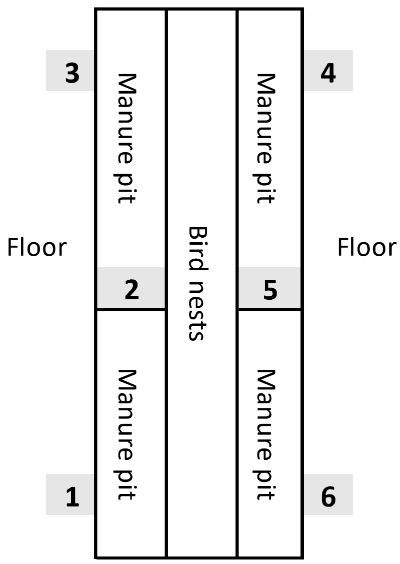

Figure 1. The plan of the breeding poultry houses (ProLG and ConLG). Ammonia measurement and litter quality evaluation points are marked with numbers 1 to 6.

Litter quality was also evaluated on the same days as the ammonia measurements. The condition of the litter was assessed visual on a 5-point scale. The 5-point scale of the litter is shown in Table 1.

Table 1. 5-point scale of the litter

Points	Explanation
5 points	Litter dry, loose, no compaction
4 points	Litter dry and loose at least 90% of the usable area for birds, small compacts
3 points	Litter dry and loose at least 70% of the usable area of the birds, slightly damp litter below the watering lines
2 points	Litter dry and loose at least 50% of the usable area of birds, wet litter under watering lines, a crust is formed
1 point	Litter dry and loose is less than 50% of the usable area of birds, wet litter under all watering lines

Electricity consumption is calculated every fourth weeks beginning with fifth week for the test house and for the control house.

The volume and composition of the litter will be analyzed after the trial for each house.

Data statistics processing

The ammonia readings obtained for each measurement for each house separately (ProLG and ConLG) were counted together and divided by the number of measurements and the average value was obtained. The same approach was used to calculate the litter rating averages. To detect statistical significance was used t-test, as well as standard deviation was determined.

RESULTS AND DISCUSSION

In the first weeks of the study, the house microclimate stabilized, and the hens adapted to the new housing and feeding conditions, so data for the first study 4 weeks were not included in this article. From week 5 to week 13 of the study, ammonia concentrations at the ProLG group house were lower than at the control house. During this period, ammonia levels in the ProLG group remained on safe for health and productivity level below 10 ppm, but exceeded it in the ConLG group. Significantly higher ($p < 0.05$) average ammonia level in the air of ConLG house was found in week 9, when it reached even 13.7 ± 5.8 ppm (Fig. 2).

Calculating the percentage of ammonia contact, we found that the spreading of probiotics to litter in study week 5 provided 50% less ammonia in the air and in week 9 it was 51% less. Thus, the addition of probiotics for up to 9 weeks of the study (i.e., 63 days) in the poultry breeding house provided healthy ammonia concentrations in the air, which is a very good and important indicator in poultry farming. Other researchers have achieved similar ammonia reductions in the poultry house air. Using the same technology for spreading probiotics on litter on day 49 (i.e., study week 7), a 36% reduction in ammonia emissions was achieved, thus providing better housing conditions throughout the growing season of broilers (Pezzuolo et al., 2019).

As our study continued, from week 13 of the study, ammonia concentrations in both houses were similar and fluctuated around 10 ppm, which is a very good indicator of bird welfare, see Fig. 2. This low level of ammonia remained until study week 17. So, by day 119, our chosen dose of probiotics ensures safe levels of ammonia in the air for birds.

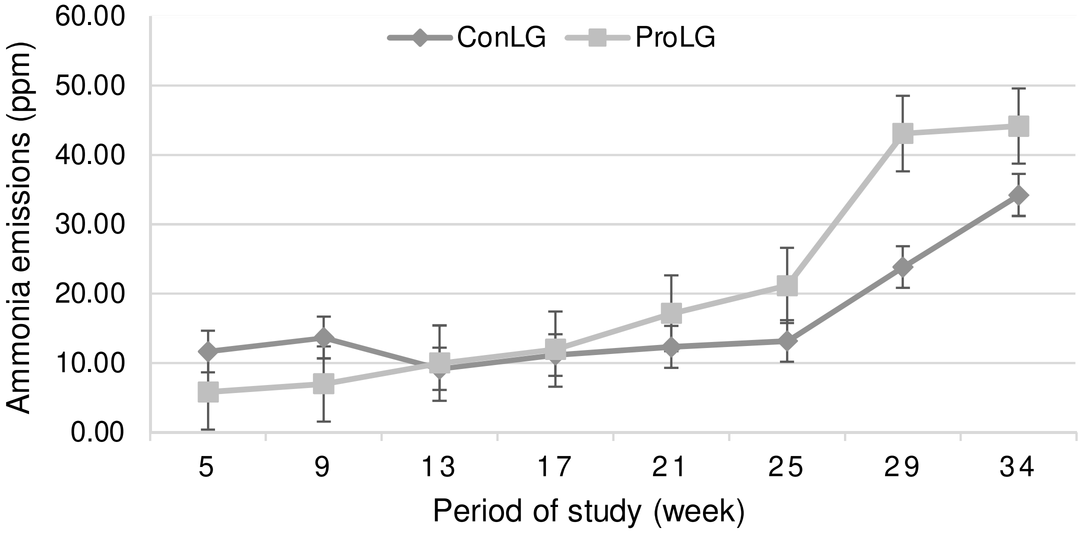

Figure 2. Average levels of ammonia concentrations for the control house (ConLG) and for the test house (ProLG).

The situation began to change from the 21st week. We found that the concentration of ammonia in the air in the ConLG group house was lower than in the house where a probiotic mixture was added to the bedding (ConLG 12.3 ± 3.9 ppm and ProLG 17.2 ± 5.7 ppm, respectively). At study week 29, ammonia concentration in both study houses exceeded 25 ppm, and in the air of ProLG house it was significantly ($p < 0.05$) higher than in ConLG. Such ammonia concentrations can already have an impact on bird health and productivity (Naseem & King, 2018). Although volatile, but still relatively high (above 25 ppm) ammonia concentrations in the ConLG group house remained until the end of the study. In turn, the ammonia concentration in the house of the ProLG group continued to increase, reaching the highest one observed during the study level of 44.2 ± 9.3 ppm at the end of the study, see Fig. 2.

Although the spreading of probiotics on litter at the beginning of the study showed a positive effect on air ammonia concentrations, at the end of the study (weeks 21 to 34 of the study) no reduction in ammonia emissions was achieved, as it had been achieved in the 35-day study of Mahardhika et al. (2019) and Pezzuolo et al. (2019) 49-day study. One of the possible reasons for the increase in ammonia levels after study week 21 (after 147 days) could be the relatively long lifespan of breeding hens, during which the housing microclimate is affected by different weather and temperature changes, making it difficult for housing staff to maintain a constant microclimate.

Simultaneously with the ammonia readings, the litter quality was also assessed using a 5-point scale. As shown in Fig. 3, litter quality was initially assessed with 5 points. This assessment was maintained up to and including study week 21. From week 25 of the study, the litter quality in the ProLG house deteriorated compared to the control house. At this study stage, we also observed an increase in ammonia readings. In poultry houses, ammonia emissions from litter are caused by a chemical reaction between water and uric acid, as well as by the uricase enzyme secreted by Gram (-) bacteria. Moisture, heat and protein content in fecal masses are known to be a favourable environment for various (including pathogenic) microorganisms (Mahardhika et al., 2019).

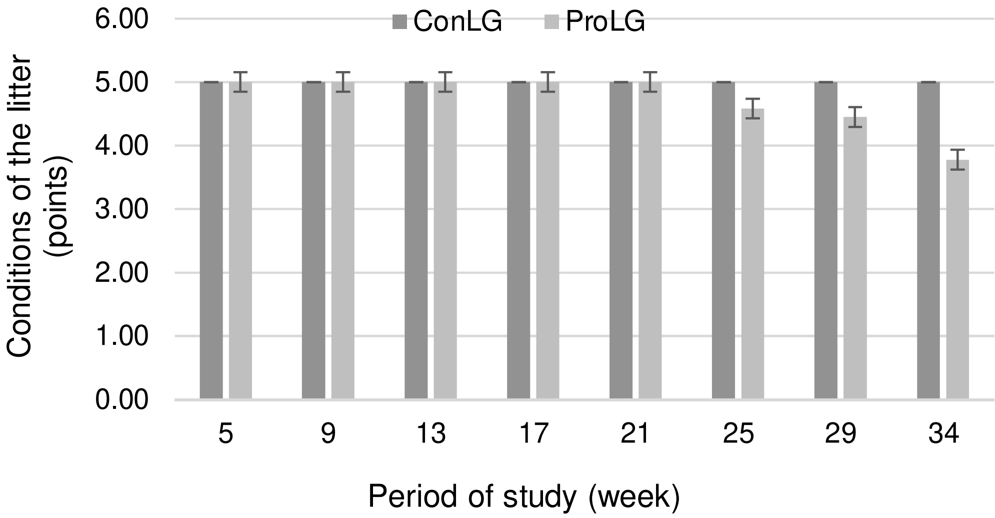

Figure 3. Average levels of litter condition for the control house (ConLG) and for the test house (ProLG).

In poultry farming, electricity consumption is one of the indicators that should be reduced. The ability to improve energy efficiency, thus reducing production costs, is an important economic indicator. Optimizing the operation of ventilation system is one of the ways to do that. In addition, the operation of ventilation equipment affects the air quality. Therefore, in our study, we focused on electricity consumption for the control house (ConLG) and for the test house (ProLG). As shown in Table 2, the amount of electricity consumed at the test site is slightly lower than at the control site. This may indicate that the test site was less ventilated, but this did not prevent ProLG from maintaining relatively good air quality until week 13 of the study. At the same time, this could be one of the reasons for the relatively high ammonia readings and for the relatively poorer litter quality from study week 25 as well. However, this difference in energy consumption is so small that the explanation for the differences is ambiguous.

Table 2. Electricity consumption for the control house (ConLG) and for the test house (ProLG)

Housing	Week 5 (kWh)	Week 9 (kWh)	Week 13 (kWh)	Week 17 (kWh)	Week 21 (kWh)	Week 25 (kWh)	Week 29 (kWh)	Week 34 (kWh)
Control housing	1,792	2,430	3,807	5,728	6,129	6,173	5,566	4,516
Test housing	2,212	2,067	3,814	5,850	6,111	5,614	5,162	4,448

In general, we can say that in the initial phase of the study, up to week 13 (day 91), the spreading of ammonia probiotics on the litter provides birds with a significantly lower ($p < 0.05$) and most importantly safe amount of ammonia vapor in the air. Ammonia levels remained low (below 10 ppm) for the next 4 weeks. It should be noted that this was not achieved by the increased use of the ventilation system, as the electricity consumption in the ProLG group house is even lower than in the control house.

In the second study phase, the efficiency of probiotics decreased from the study week 21, and ammonia emissions were significantly ($p < 0.05$) higher in ProLG than in ConLG at study week 25. It could be due to the poor litter condition of the ProLG home, where ammonia can be increased due to various chemical processes, contributed to the release of ammonia into the air. Research is still in progress to determine whether and how probiotics change the structure of bedding or break down bedding material by changing its properties. However, it should be noted that although the litter condition at the ConLG group house was assessed as slightly better, the amount of ammonia in the air of this house also exceeded 25 ppm. Such a high rate of ammonia, which is one of the predisposing factors for respiratory disease and can reduce growth and productivity rates, has been found at the end of the study in both probiotic and control houses (Naseem & King, 2018).

CONCLUSIONS

Lactobacillus farcimins CNCM-I-3699 - $7.8.10^6$ GU / g and *Lactobacillus rhamnosus* CNCM-I-3698 - $7.8.10^6$ GU / g mixture 10g / m^2 before the birds were placed and 5 g / m^2 once a week spreading on litter up to study week 13 in the ProLG house provides significantly lower ($p < 0.05$) ammonia concentrations in the house, with 51% lower levels in the ninth study week. The amount of ammonia has remained below 10 ppm for the next 4 weeks, which is an important result of improving the microclimate of the breeding poultry house.

Starting from study week 21, the probiotic *Lactobacillus farcimins* CNCM-I-3699 - $7.8.10^6$ GU / g and *Lactobacillus rhamnosus* CNCM-I-3698 - $7.8.10^6$ GU / g mixture 5 g / week the effect of applying on litter rapidly reduced and ammonia concentration was significantly ($p < 0.05$) greater in the test house at study week 25 than in the control house. At the same time, we found a deterioration in the quality of litter.

This work is going to be continued with the analysis of the amount and composition of litter, which will be carried out after the completion of the full research.

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