

Effects of a slatted floor on bacteria and physical parameters in litter in broiler houses



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ABSTRACT

In this study, a new housing system for broiler was tested. This system consisted of a slatted floor area and a littered area with the aim of improving litter quality. Two experimental broiler houses were provided. In house 1, a slatted floor was installed below the drinker and feedlines. Littered areas flanked the slatted floor. Broiler house 2 reflected conditions in commercial systems, consisting of a full littered area. Litter samples were taken at day 11 and at day 32 of the fattening period. Manure samples were taken at day 32. The total bacteria count (TBC), coliforms, *Escherichia coli* (*E. coli*) and ESBL-producing bacteria were determined. Furthermore, physical parameters (dry matter, water activity, pH) of litter and manure were measured. For statistical analyzes, a generalized linear mixed model (GLIMMIX procedure) was calculated. The floor did not show any significant effect on the bacteria content of the litter. Regarding TBC in litter, the floor showed a tendency for an effect ($F = 5.42, p < 0.1$) with lower contents in house 1. Regarding the manure under the slatted floor, a tendency for a difference between house 1 and house 2 was found for the content of *E. coli* ($F = 5.55, p < 0.1$) with higher contents in house 1. The floor did not show any significant effect on the physical parameters of litter and manure. The results of this experimental study showed no positive effects on the selected litter parameters, but further studies, especially on-farm experiments are necessary to confirm these results.

1. Introduction

Wet litter in poultry houses is an omnipresent problem in commercial production systems. This issue is not new; a study from the year 1923 described wet litter as a major cause of poultry diseases (Dann 1923). Nearly 100 years later, poultry production systems still face problems associated with litter flooring. In accordance with the Council Directive (Council Directive 2007/43/European Council, 2007), broiler chickens in Europe are usually kept in litter flooring systems, where fresh bedding material is provided at the start of the rearing period. Bedding material serves several purposes, like thermal insulation, moisture and excreta absorption as well as aerobic decomposition of excreta. Furthermore, it allows broiler chickens to display natural behaviors, such as scratching, pecking and dust bathing (Collett, 2012, Shepherd and Fairchild, 2010). Thus, bedding material such as wood shavings and sawdust, which are the most commonly used materials (Toghyani et al., 2010), can generally enhance birds' health

and welfare. However, when the rate of water addition (excreta spillage) exceeds the rate of removal (evaporation) (Collett 2012), bedding material can also impair the health and the welfare of the animals. The causes of wet litter are multifactorial. Severe management factors, such as drinker design, indoor climate, litter material and stocking density affect the litter moisture directly (Spindler & Hartung, 2007). Poor litter quality encourages the growth of bacteria and other microorganisms, which can cause subsequent infections. This situation can become more critical when some of the bacteria possess resistance to one or more antibiotic substances (for example, the extended spectrum beta-lactamase (ESBL) producers) and can be transferred via bird to the food chain. A study by Overvest et al. (2011) showed a prevalence of 79.8% for ESBL genes in *Enterobacteriaceae* which were found on retail chicken meat. The detected ESBL genes in chicken meat were identical to ESBL genes which were found in human rectal swab samples (Overvest et al., 2011). Leverstein-van Hall et al. (2011) confirmed these findings; 95% of the analyzed retail meat samples contained ESBL

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Fig. 1. Broiler housing systems: A: House 1, with slatted/littered floor, B: House 2 with commercial conditions (littered floor) (Photography: S. Heitmann).

producers, of which 39% belonged to *E. coli* genotypes. Whether or not the occurrence of bacteria resistant to antibiotics is associated with wet litter is, to the authors' knowledge, hitherto unknown. However, determining approaches to solve the problems of wet litter is equally as difficult and complex as determining the influencing factors thereof. Besides optimizing management (e.g. litter material, air ventilation, drinker design), one approach is to separate the animals from their feces using slatted floors. The idea of keeping broiler chickens on a partially perforated floor (or in fully perforated areas) is not new. The first studies regarding the use of slatted floors were published in the 1950s (Yao, 1959). The aims of these studies were to avoid the usage of litter, reduce the labor for farmers and to determine the appropriate material for these flooring systems (Maus, 1988, Li et al., 2017, de Almeida et al., 2017). Primarily, the focus was laid on the performance and carcass quality, not necessarily on animal welfare. Still, several studies reported that incidences of breast blisters and leg disorders were quite high for broilers raised on perforated (wire) floors (Andrews et al. 1974, Simpson and Nakaue, 1987). How old these aforementioned studies are, as well as the usual husbandry and breeding hybrids used at that time, make a comparison to present-day studies very difficult. In Germany, the usage of slatted floors has gained increased attention over the last decades (Maus 1988, Grashorn et al., 1992, Macke and Van den Weghe, 1997, Najati and Van den Weghe, 2000). Chuppava et al. (2018a) evaluated the effects of different flooring types (litter floor and slatted floor) on antimicrobial resistance in commensal *E. coli* on experimental conditions. They treated turkeys with enrofloxacin. However, there was no clear evidence of any different developments in resistance caused by the flooring systems. Moreover, in their second study (Chuppava et al., 2018b), the flooring system did not have any effect on the development of bacterial resistance to antibacterial agents. The study by Kriewitz (2017) showed no improvement of the litter quality regarding the dry matter: here, one group of broiler chickens were kept in a fully littered cage, and the other group were kept in a partially perforated cage with a littered area. Up until now, there is a lack of comparison data on commercial husbandry with partially slatted areas focusing on the microbial growth in the litter. The present study was part of a project focusing on improving the litter quality by separating birds from their excreta as early in life as possible. The fundamental idea of the project was to design the flooring in such a way as to include a combination of littered areas and a slatted floor. Installing a slatted floor below the drinker and feedline was assumed to control the draining of moisture and water, and consequently, the bedding material should be drier and less loaded with microorganisms. The aim of the present study

was to evaluate the effect of a plastic slatted floor on bacteria and physical parameters in litter, especially *E. coli*, coliforms and ESBL-producing bacteria, as well as the total bacteria count, dry matter, water activity and pH-level.

2. Animals, material and methods

The experiments were performed in accordance with German regulations and approved by the Ethics Committee of North-Rhine-Westphalia, State Department for Nature, Environment and Consumer Protection North Rhine-Westphalia, Germany (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen); file number: 81.02.04.2018.A057. In compliance with the European Directive 2010/63/EC Article 1 5. (f), the present study did not imply any invasive procedure or treatment to the animals. The study was reviewed and approved by the Animal Welfare Officer of the University of Veterinary Medicine Hannover, Foundation, Hannover, Germany.

2.1. Study design

The experiments were conducted on the research farm at the University of Bonn, North-Rhine-Westphalia, Germany. The farm comprises two identical broiler houses (capacity: 500 broiler chickens, stable measurements: 9.6 m x 4.7 m, floor space: 5.3 × 4.7 m) with mechanical ventilation systems (negative pressure). In order to ensure constant climate conditions in both houses, the climate conditions were continuously monitored using a climate computer (PL9400, Stienen Bedrijfslektronica B.V., RT Nederweert, the Netherlands). One broiler house (house 1) was provided with a slatted floor, which was installed below the drinker and feedlines (height: 15 cm) (Fig. 1a). The perforated floor (FIT Farm Innovation Team GmbH, Steinfurt, Germany) was made of plastic, the square openings measured 1.5 cm x 1.5 cm and the struts were 0.5 cm thick. In total, the slatted floor covered 50% of the space. A littered area flanked the slatted floor to the right and to the left. Ramps allowed the birds to access the floor. The second house (house 2) reflected conditions of commercial systems (Fig. 1b), consisting of concrete flooring with wood shavings used as litter material (600 g per m² in both houses). Five hundred broiler chickens (ROSS 308) were kept with a stocking density of about 39 kg/m² in both housing systems. The broiler chickens were fed with a commercial standard fattening diet (chick starter, breeding feed 1 and 2, finisher, Deuka, Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany). They were vaccinated against Newcastle disease (day 13), against Gumboro (infectious bursal disease) (day 18) and against

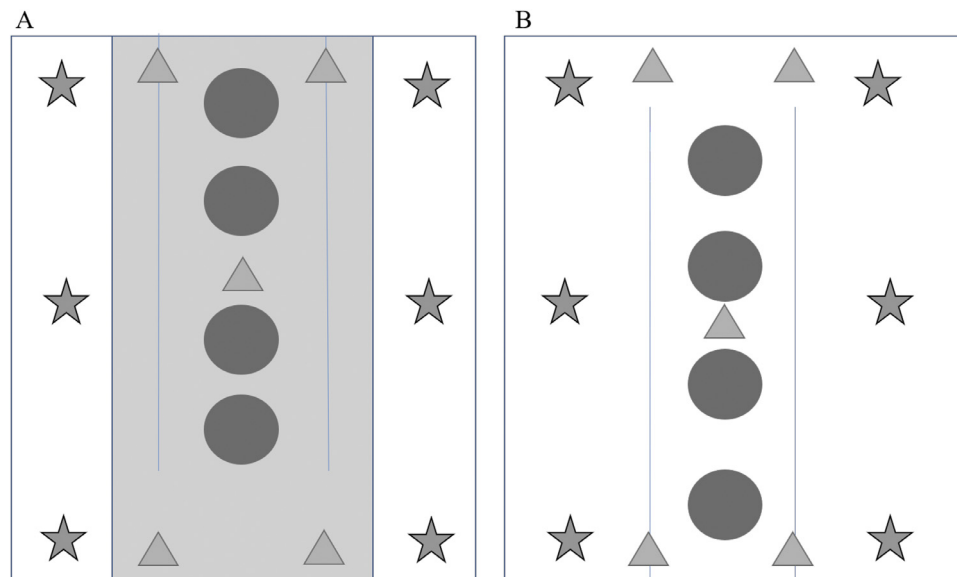


Fig. 2. Schematic diagram of broiler house 1 (A; grey background: slatted area) and broiler house 2 (B). Circles: food boxes, lines: drinkerline, stars: positions for the litter samples, triangles: position for the manure samples (Schematic diagram: S. Heitmann).

infectious bronchitis (day 18). On day 11 and day 32, 50 birds per house were weighted with an animal weight (manual poultry weight, BAT-1, VEIT electronics, Moravany, Czech Republic). After 32 days of life, the target weight of 2016.04 g (broiler house 1) and 1987.37 g (broiler house 2) (Aviagen, 2019) was reached and the broiler chickens were slaughtered. Four fattening periods per broiler house were included in the analysis.

2.2. Litter samples

In each house, litter samples were taken at six positions each at days 11 and 32. These positions were just beside the slatted floor which was laid the entire length of house 1 (Fig. 2). Comparable positions were chosen for house 2. Samples were taken with the aid of a disinfected metal frame (25 × 25 cm) using a disinfected spatula. Inside the frame, the litter was mixed thoroughly with the spatula for 30 s. Subsequently, one litter sample was taken using a disinfected plastic cup (180 cm³) and was then transferred into a sterile bag. These six samples were pooled in one composite sample (one sample bag per house). All collected samples were transported to the laboratory while cooling to about 4°C constantly. In the laboratory, the litter samples were prepared for analyzes.

2.3. Manure samples

Manure samples (a mixture of litter and feces) were taken after each fattening period (day 32) when the perforated floor was removed for cleaning. Here, five positions were sampled in both broiler houses (Fig. 2). Samples were taken and treated the same way as previously described for litter samples.

2.4. Bacteriological analysis of samples

2.4.1. Analysis of total bacteria count

From each pooled litter and manure sample, 25 g were homogenized in 225 mL PBS + 0.01% Tween20, using a stomacher for 120 s at high speed. For the total bacteria count (TBC), a dilution series with 0.9% NaCl solution was created and 100 µL from three dilution steps were spread on blood agar (base) (X914, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) in triplicate. After incubation for 24 h at 37°C, the recovered bacterial colonies were enumerated, and the colony-forming

units (cfu) were calculated per gram litter or manure.

2.4.2. Detection of coliforms, *E. coli* and ESBL-producing bacteria

From each pooled litter and manure sample, 25 g of litter were homogenized in 225 mL Luria-Bertani (LB)-broth (Lennox, X964, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) using a stomacher for 120 s at high speed. Thereafter, a dilution series with 0.9% NaCl solution was produced and 100 µL of each dilution were plated in triplicate on coliform chromogen-agar plates (CL45, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The plates were incubated for 24 h at 37°C and presumed coliforms were counted. In addition, presumed *E. coli* colonies were counted separately. Furthermore, 100µL of each dilution were plated on ESBL screen agar (Brilliance™ ESBL-agar, Oxoid, Wesel, Germany) in triplicate. Plates were incubated for 24 h at 37°C. Subsequently, typical colonies of ESBL-producing bacteria were counted and cfu were calculated per gram litter or manure. Five colonies had suspected *E. coli* colonies (blue on chromogenic coliform agar), and ESBL-producers (blue or turquoise on ESBL screen agar) were streaked on MacConkey agar (CM0115, Oxoid, Wesel, Germany) and plates were incubated for 24 h at 37°C. After incubation, one characteristic *E. coli* colony and suspected ESBL-producers were inoculated on Columbia Agar with 7% sheep blood (PB 5008A, Oxoid, Wesel, Germany) to produce pure cultures. After a further incubation step, the indole reaction was tested with suspected *E. coli* isolates. For this purpose, isolates were incubated in tryptone water (CM0087, Oxoid, Wesel, Germany) for 24 h at 37°C. Then, the Microbact Reagent for Indole (Kovacs, MB0209A, Oxoid, Wesel, Germany) was added. If the incubated tryptone water turned pink, isolates were considered as indole positive and pure cultures of isolates were used to confirm the species by using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonik GmbH, Bremen, Germany) in accordance with the manufacturer's instructions.

2.5. Dry matter, water activity measurements and pH-level

Before measuring, the litter sample was mixed thoroughly again for 30 s. Then, a sample of 2 g was taken with a spoon. The dry matter was measured with a moisture analyzer (Moisture Analyzer, MA 40, Sartorius AG, Göttingen, Germany). Furthermore, a second sample of 2 g was taken and the water activity (a_w -value) was measured using an AQS-31 aquaspector (Nagy Messsysteme GmbH, Gäufelden, Germany).

The pH-measurements were conducted in two fattening periods only (2 + 3). Here, 200 mL distilled water was added to 10 g litter or manure. After mixing, the pH-value was measured (pHEnomenal 111 662-1157, VWR, Radnor, USA). For all parameters, measurements were performed three times. Afterwards, the average value was calculated.

2.6. Data analysis

From the counted bacteria, the weighted average value was calculated and converted into cfu/g litter or manure. Afterwards, the counts were \log_{10} - transformed before further analysis. Statistical analyses were conducted using SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). A generalized linear mixed model (GLIMMIX procedure) was calculated for each parameter separately (body weight, TBC, coliforms and *E. coli* in the litter and in the manure, dry matter values and a_w -values). Flooring system (house 1 with slatted and littered floor versus house 2 with littered floor), age (11 d versus 32 d) and the interaction between both were included as fixed factors. Repeated measurements of age per fattening period were considered as a random effect. Concerning the data of weight, repeated measurements of animals per age per fattening period were taken into account. Tukey Kramer t tests were calculated for pairwise comparison. Residuals were tested for normal distribution, using a normal plot. Correlations were calculated using the CORR procedure, specifying the Pearson correlation coefficient. The threshold to determine significance was set at $p < 0.05$, a tendency was defined when p revealed a value < 0.1 .

3. Results

3.1. Body weight and bacteriology of litter samples

Data are presented as least square means (LSM). Residuals were normally distributed. The body weight amounted to 2016.04 g in broiler house 1 and 1987.37 g in broiler house 2. There was no effect of the flooring system ($F = 1.89$, $p = 0.17$), whereas the age was showing an effect ($F = 1635.57$, $p < 0.01$). Furthermore, no interaction effect was detected ($F = 0.91$, $p = 0.34$). The average content of TBC in the litter from house 1 was $9.7 \log_{10}$ cfu/g litter and $10.21 \log_{10}$ cfu/g litter in house 2, with the flooring system showing a tendency for an effect ($F = 5.42$, $p < 0.1$). No age effect was found ($F = 0.11$, $p = 0.75$). In addition, no interaction effect was detected ($F = 0.37$, $p = 0.57$). The term *coliform bacteria* refers to various bacteria from the family of *Enterobacteriaceae*. These include (among others) *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*. Coliforms are characterized by the following common properties: gram-negative, rod-shaped, facultative anaerobic, non-spore forming, lactose fermentation, acid formation and gas at a temperature of 35-37°C. Regarding the coliform content in the litter, the average value in house 1 was $7.35 \log_{10}$ cfu/g litter, whereas in house 2, the content was $8.01 \log_{10}$ cfu/g litter. Here, no significant effect of the flooring system was detected ($F = 2.46$, $p = 0.17$) (Fig. 3). However, the age of the broiler chickens revealed a significant effect ($F = 17.17$, $p < 0.01$), with lower content at day 32 compared to day 11. An interaction effect was not determined ($F = 0.70$, $p = 0.44$). The average content of *E. coli* was found to be $7.50 \log_{10}$ cfu/g litter in house 1 and $8.29 \log_{10}$ cfu/g litter in house 2. Here, no significant effects of flooring system, age or the interaction between both could be detected (all $F > 0.43$, $p > 0.40$) (Figure 4). All suspicious *E. coli*-isolates ($n = 60$) were confirmed positive by MALDI TOF-MS. The ESBL-producing bacteria were not detected in both houses.

3.2. Bacteriology of manure samples

For the manure samples, the TBC content was found to be the same in house 1 and house 2 ($10.06 \log_{10}$ cfu/g). The coliform content was $8.23 \log_{10}$ cfu/g manure in house 1, and $6.03 \log_{10}$ cfu/g in house 2

($F = 4.18$, $p = 0.13$) (Fig. 5). Regarding the *E. coli* content, the manure contained $9.43 \log_{10}$ cfu/g in house 1, whereas in house 2, the manure contained $4.83 \log_{10}$ cfu/g ($F = 5.55$, $p < 0.1$) (Fig. 6). A tendency of an effect of the flooring system was found. Here again, ESBL-producing bacteria were not found in the manure samples.

3.3. Water activity and physical parameters

3.3.1. Dry matter and water activity in litter

Regarding the litter dry matter, the bird age affected the dry matter values significantly ($F = 13.56$, $p < 0.05$). For house 1, the dry matter revealed an average of 62.85%; in house 2, the dry matter reached a mean value of 61.78%. No interaction effect was determined ($F = 0.04$, $p = 0.85$) and no effect of flooring system was detected ($F = 0.11$, $p = 0.75$). For the a_w -value of litter as well, the age was found to have a significant effect ($F = 24.03$, $p < 0.01$), showing an increase throughout the fattening period. Here again, no interaction effect was determined ($F = 0.21$, $p = 0.66$). In addition, no effect of flooring system was detected ($F = 0.1$, $p = 0.77$). The mean values of water activity of each litter are listed in Table 1.

3.3.2. Dry matter and water activity in manure

The dry matter of manure reached a mean value of 37.21% in house 1. In house 2, the manure had a dry matter value of 49.72% ($F = 5.35$, $p = 0.10$).

The mean values of water activity of manure are shown in Table 2.

3.3.3. pH-level of litter and manure

The pH-level in litter in house 1 and in house 2 increased during the fattening period. Detailed data are shown in Fig. 7. In manure, the pH-level was 8.45 at day 32 in house 1, whereas in house 2, the manure had a pH-level of 5.16

3.4. Correlation

The correlation coefficients between measured parameters are listed in Table 3 (for a_w -value) and Table 4 (for pH-value).

4. Discussion

The aim of the present study was to test a new housing system, consisting of a combination of slatted and litter flooring in broiler houses, with regards to potential differences in bacterial growth in litter, especially *E. coli*, as well as physical parameters including dry matter, water activity and pH-level.

4.1. Body weight, bacteriology of litter and manure samples

The weight differences were not significant with 28.67 g more in broiler house 1. This was not consistent with other studies (Chuppava et al., 2018c, de Almeida et al., 2017) in which significantly higher weights were achieved in housings with partially or fully perforated floors. It was assumed that the amount of data was too small and more repetitions of fattening periods have to be performed. Very diverse species and amounts of bacteria exist in poultry litter. Alexander et al. (1968) found that most bacteria found in poultry litter are *Streptococcus* spp., *Enterobacteriaceae* (which include *E. coli*), *Bacillus* spp. and *Staphylococcus* spp. Many of these bacteria are non-pathogenic and belong to the physiological microflora of the intestinal tract of animals (Alexander et al., 1968). Nevertheless, there are also a lot of pathogenic bacteria or facultative pathogens, incorporated by the animals via dust, excreta or water (Siegmann and Neumann 2012). Reducing the content of pathogenic bacteria in the litter might therefore have a positive effect on animal health and should be strived for in poultry husbandry. Separating the animals from their feces by using slatted flooring systems is one potential approach to reduce the

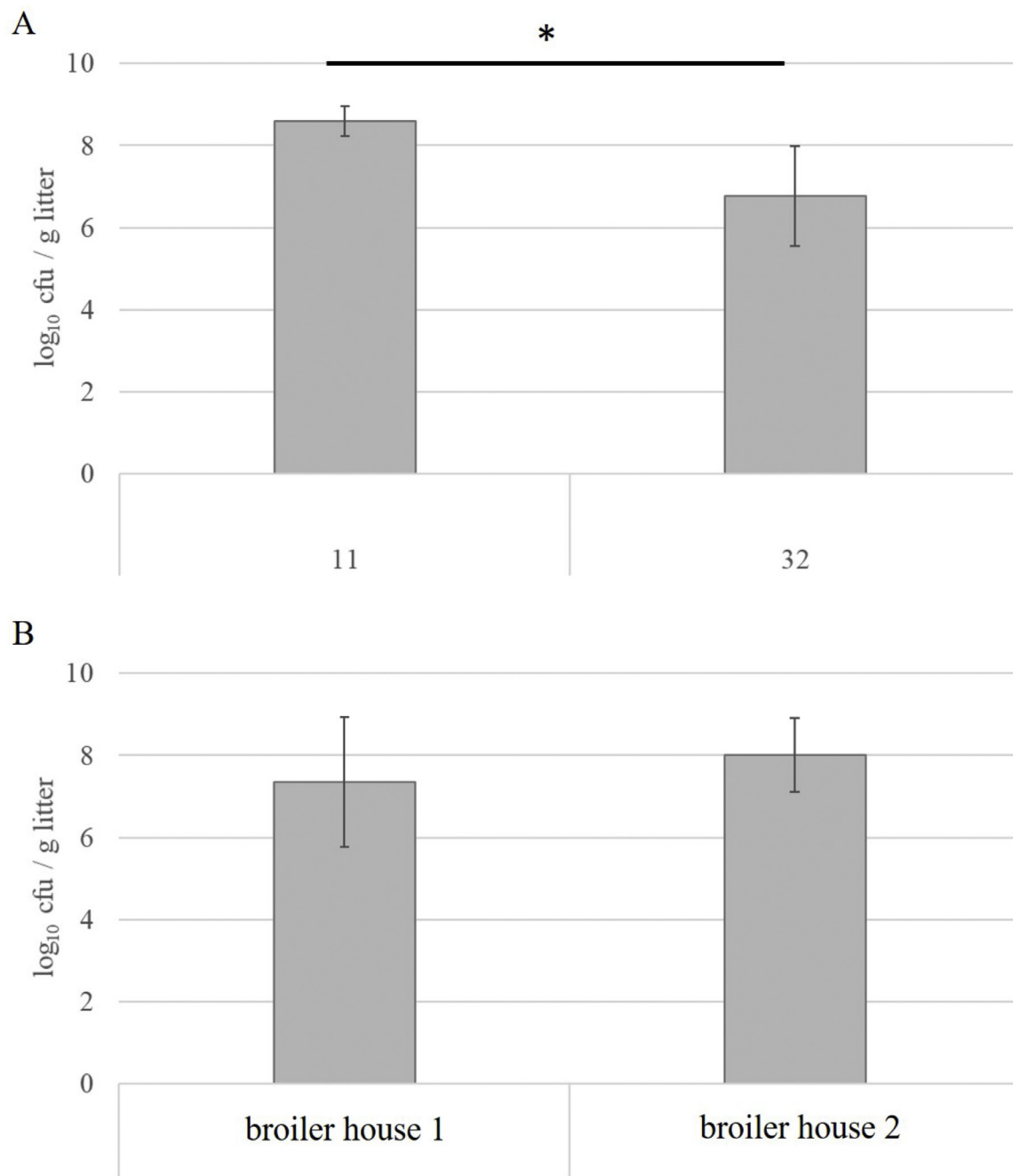


Fig. 3. Average coliform content (LSM \pm standard deviation [SD]) in litter. A show the age effect, which is significant between day 11 and 32, whereas B shows no significant effect of flooring system regarding the content of coliforms in litter (broiler house 1: slatted/littered floor, broiler house 2: littered floor). * $p < 0.01$

infection risk of the animals. However, in the present study, installing a slatted floor did not affect the growth of bacteria in general. Regarding the litter samples from broiler house 1 and broiler house 2, the samples did not differ in bacterial content, neither in the TBC, nor in the content of coliforms nor in the content of *E. coli*. However, the slatted floor showed a tendency regarding the TBC in litter with a lower content in broiler house 1. To confirm these results, more fattening periods should be surveyed to collect more data. Installing a perforated floor, without increasing the total ground area, implies that fewer square meters of littered area were available for the same number of animals. Thus, there were proportionally more animals in the littered area than in house 2 without a perforated floor. This should be confirmed via behavioral observations in further studies, e.g. the usage of a slatted floor by broilers (especially at the end of the fattening period) or if the ramp

impeded the birds from climbing on the perforated floor. The study of [Norrington et al. \(2016\)](#) showed that broiler chickens preferred platforms to perches. The platforms had an inclination of 20° and a height of 20 cm, and 50- 100% of the structures were used by the birds. But [Norrington et al. \(2016\)](#) also could show that broilers became more inactive as they got older. In addition, [Malchow et al. \(2019\)](#), tested grids at three different heights (10 cm, 20 cm and 50 cm) and observed that fast growing broiler chickens (ROSS 308) used the lowest grids more frequently. The authors discussed this to be due to difficulties in climbing up the ramp with an inclination angle of 35°. In our experiment, the ramp had an inclination angle of 27°, and the perforated floor a height of 15 cm, which lied between the lowest and the middle grid in the mentioned study. Moving up a ramp requires physical effort, that fast-growing broiler chickens like ROSS 308, are often not able to

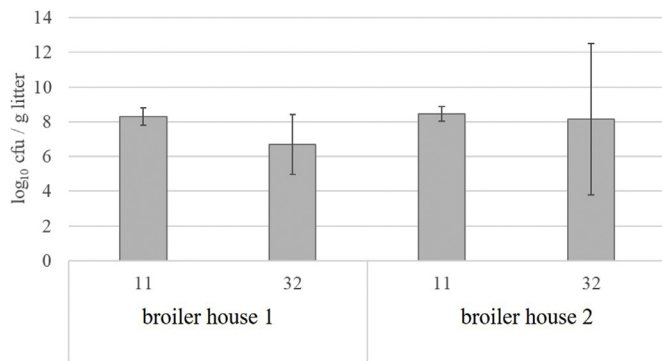


Fig. 4. Average *E. coli* content (LSM ± SD) in litter in house 1 (slatted/littered floor) and in house 2 (littered floor) at day 11 and day 32.



Fig. 5. Average coliform content (LSM ± SD) in manure in house 1 (slatted/littered floor) and in house 2 (littered floor) at day 32, F = 4.12, p = 0.13.

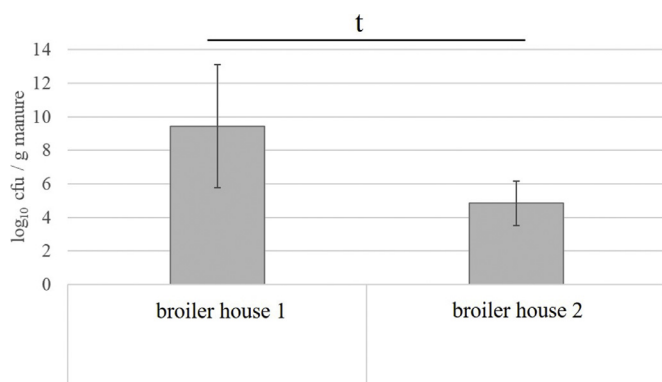


Fig. 6. Average *E. coli* content (LSM ± SD) in manure in house 1 (slatted/littered floor) and in house 2 (littered floor) at day 32, F = 5.55, p < 0.1.

Table 1

Mean values of water activity (*a_w*-value) of litter in house 1 (slatted/littered floor) and in house 2 (littered floor) at day 11 and day 32 with standard deviation (± SD).

	Day 11 Mean	SD	Day 32 Mean	SD
Broiler house 1	0.838	± 0.05	0.973	± 0.97
Broiler house 2	0.836	± 0.06	0.983	± 0.98

perform. So further studies should also focus on the way of locomotion and the ability of broiler chickens to climb up an incline of 27°. However, if proportionally more animals used the littered area in broiler house 1, one would expect the litter quality to be much worse. The microbiological results in this study could not confirm this assumption. In contrast, Kriewitz (2017) found higher proportions of excrements

Table 2

Mean values of water activity (*a_w*-value) of manure in house 1 (slatted/littered floor) and in house 2 (littered floor) at day 32 (F = 3.08, p = 0.18) with standard deviation (± SD).

	Day 32 Mean	SD
Broiler house 1	1	0
Broiler house 2	0.934	± 0.07

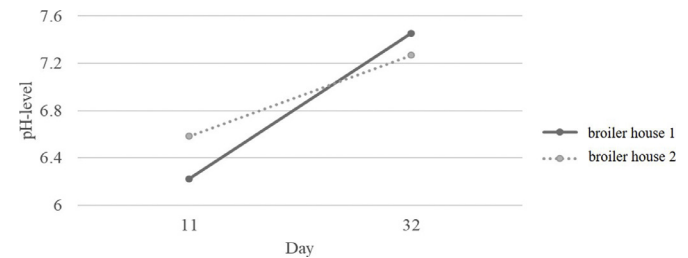


Fig. 7. pH-level of litter (average values) in house 1 (slatted/littered floor) and in house 2 (littered floor).

Table 3

Correlation (Pearson Correlation Coefficient) between the *a_w*-value and the determined bacteria (n = 16). *p < 0.05

	TBC	coliforms	<i>E. coli</i>
<i>a_w</i> -value	0.40	-0.54	-0.18
	p = 0.12	p < 0.03	p = 0.50

Table 4

Correlation (Pearson Correlation Coefficient) between the pH-value and the determined bacteria (n = 8).

	TBC	coliforms	<i>E. coli</i>
pH-value	0.48	0.02	0.36
	p = 0.23	p = 0.95	p = 0.38

and an increase of Salmonella in a study with partially perforated flooring. They reported that animals preferred the littered area and assumed the effects to be due to a higher amount of excrements per m². Regarding the coliforms in litter, it was noted that *E. coli* accounted for the majority of coliforms in the samples. These investigations corresponded to the studies by Terzich et al. (2000). Furthermore, the birds' age affected the content of coliforms significantly. No interaction effect between age and flooring system was found; the effect of age could be observed in both houses. However, the standard deviations were high, so the significant effect should be interpreted with caution. In this regard, more repetitions of fattening periods are recommended in order to produce clearer results. Nevertheless, such an age dependent decrease of coliforms was described before (Hartel et al. 2000, Schefferle 1965). The number of cultivatable coliform bacteria showed a decreasing tendency in both houses. The decrease could be related to the observed increase in pH-value. It is known that the microbiota in litter of broilers changes with increasing pH and that can create a less favorable environment for the growth of bacteria (Madelin and Wathes 1989). Also, Terzich et al., 2000 could show, that the pH-level influences the growth of coliforms. This previous study reported that the coliform content decreased when the pH-level increased from 6 to 8. In the present study, the coliform content decreased within 21 days during the fattening period. There was an increase in the pH-value during this time, which may have been one reason for the decline. In our study, ESBL-producing bacteria were not detected. This is in contrast to other studies which showed a high prevalence of ESBL-producing bacteria. For

instance, Geser et al. (2012) found a prevalence of 63.4% for ESBL producers in chicken housing systems. Friese et al. (2013) confirmed ESBL-producing *E. coli* in all investigated eight broiler farms. One possibility as to why no ESBL-producing bacteria were measured in this study is the fact that the broiler houses had been empty for more than two years prior to these experiments. This might have helped to break possible chains of infection. Another possibility is that the content of ESBL-producing bacteria was below the detection limit (33 cfu/g litter or manure). Furthermore, most probably, there was insufficient time available to develop new resistance. Moreover, there was no medical treatment necessary within the four fattening periods. With regard to the manure under the slatted floor, it was expected that the perforated floor would help the manure to dry off, and in consequence, that fewer microorganisms would grow. Sufficient drying, as Macke and Van den Weghe (1997) reported for the trampoline floor, could not be achieved. The manure under the slatted floor remained moist to wet and thus provided ideal growth conditions for the bacteria. The TBC in house 1 was as high as in house 2, and the coliform and *E. coli* content were even higher than in house 2. As the mixture under the slatted floor mainly consisted of excreta, food and less litter and the comparative sample from house 2 mainly consisted of litter, excreta and hardly any food, this might be the major cause of the results. Thus, this makes such a comparison between both types of samples questionable.

4.2. Dry matter, water activity and pH-level of litter and manure

One of many definitions of wet litter is that litter is deemed to be wet when the moisture content is greater than 25% (Collett 2007). The litter moisture reached 37.15% in house 1, and in house 2, it reached 38.22%. Therefore, in both houses, the litter can be described as clearly wet. Thus, the installation of a slatted floor probably did not lead to an improvement in the litter quality. This is in accordance with a study by Chuppava et al. (2018c). The dry matter in their group with the partly-slatted floor flooring system was lower than in the control group (full littered flooring). The explanation could be the same, as already mentioned above, and should be evaluated in more detail prospectively: by installing the slatted floor, a smaller area of littered floor was available for the same number of animals. At the end of the fattening period, in house 1, there might be more animals in the littered area than in house 2, resulting in an increase in litter moisture. In house 1, the manure had a very low dry matter value at the end of the fattening period, but the dry matter was not higher in house 2. That the manure moisture (62.79%) was so high in house 1 might be due to a poor air movement or air exchange under the perforated floor. However, moisture content is not the only possible way of measuring water in litter. The water activity is closely related to the microbial, chemical and physical characteristics of natural products (Dunlop et al., 2016). Water activity originates from the food safety sector, and there are a few important "critical a_w -values" that describe minimum, optimum and maximum values where bacterial growth was detected. One of the critical values was 0.6 for the growth of any microorganisms and 0.86 for the lowest a_w -value where growth of pathogenic bacteria was observed (Barbosa-Cánovas 2007). These increase risks to flock health, workers' health and food safety. Studies by Opara et al. (1992), Himathongkham et al. (1999) and Eriksson de Rezende et al. (2001) showed that the occurrence of bacteria like *Salmonella* spp. depends on the water activity. Opara et al. (1992) reported that broiler litter surfaces with water activity greater than 0.90 were associated with increased *Salmonella* recovery rates. Owing to pathogens, which have a high relevance in poultry production, like *Campylobacter jejuni* (a_w -minimum for growth: 0.98), *E. coli* (a_w -minimum for growth: 0.95) and *Salmonella* ssp. (a_w -minimum for growth: 0.92-0.95), the a_w -value in litter should be kept to a minimum. In the present study, the water activity was increased in both houses during the fattening period, finally reaching a value of approximately 0.9. This can be described as a critical point for bacterial growth (Dunlop et al., 2016), especially for

pathogens like *Salmonella*, *E. coli* and *Campylobacter*. Furthermore, the water activity reached the highest value in manure in house 1. Even though no differences between house 1 and house 2 were found, flocks in this study had no health problems and no medical treatment was necessary in both groups. Moreover, as already mentioned, the content of coliforms and *E. coli* in litter decreased in both houses during the fattening period, although their a_w -minimum for growth was reached. A negative correlation with the a_w -value was found for coliforms and *E. coli*. One reason for the decrease in coliforms and *E. coli* could be the increasing pH-level during the fattening period. Laboratory studies by Martin et al. (1982) tested which factors influence the survival and growth of coliforms in drinking water distribution systems. The pH of the water influenced the survival of bacteria; 50 percent of the organisms survived for 22 hours at pH 7.3 and 7.9, whereas 50 percent of the organisms survived for only one hour at pH 9.0 (Martin et al. 1982). Also, in litter, the pH value influences the growth of bacteria, especially the growth of coliforms and *E. coli* (Terzich et al. 2000), which was mentioned above. In addition, Schefferle (1965) found that alkaline conditions in litter were associated with fewer abundant gram-negative bacteria. In the present study, the pH-level increased faster and higher in house 1 than in house 2. This situation could also explain the decreasing content of coliforms and *E. coli*, which was also higher in house 1 than in house 2. Here, a positive correlation was found between the increasing pH-level and the growth of TBC, coliforms and *E. coli*. However, the correlation coefficients were quite low, so a higher database would be necessary to confirm these results.

The presented study did not find any effects of a slatted floor on bacteria, especially *E. coli*, coliforms, ESBL-producing bacteria and total bacteria count and physical parameters in litter, such as dry matter, water activity and pH-level. We found numerically differences. However, these could not be confirmed statistically, especially as standard deviations in the dataset were high. Therefore, more replicates should be conducted to strengthen the results.

4.3. Conclusion

The aim of this study was to evaluate the effects of a slatted flooring system with a littered area (50%/50%) on bacteria growth and physical parameters. In this experimental study, no significant effects caused by the slatted floor on bacteria growth, dry matter, water activity or the pH-level of the litter were shown. For a final evaluation of a slatted-litter floor combination, more data are necessary. Also, in further studies, the possible effects of the new husbandry system on animal welfare (usage of slatted floor by animals, effects on footpad health, hocks and breast) should also be included in the investigations.

5. Ethical Statement

The experiments were performed in accordance with German regulations and approved by the Ethics Committee of North-Rhine-Westphalia, State Department for Nature, Environment and Consumer Protection North Rhine-Westphalia, Germany (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen); file number: 81.02.04.2018.A057. In compliance with the European Directive 2010/63/EC Article 1 5. (f), the present study did not imply any invasive procedure or treatment to the animals. The study was reviewed and approved by the Animal Welfare Officer of the University of Veterinary Medicine Hannover, Foundation, Hannover, Germany.

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Declaration of competing interest

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

- Alexander, D. C., Carrière, J. A., & McKay, K. A. (1968). Bacteriological studies of poultry litter fed to livestock. *Canadian Vet. J.* 9(6), 127–131.
- Andrews, L. D., Seay, R. L., Harris, G. C., & Nelson Jr., G. S. (1974). Flooring materials for caged broilers and their effect upon performance. *Poultry Sci.* 53(3), 1141–1146. <https://doi.org/10.3382/ps.0531141>.
- Aviagen. (2019). ROSS 308/ ROSS 308 FF Broiler: Performance and Objectives. <http://eu.aviagen.com/tech-center/download/1339/Ross308-308FF-BroilerPO2019-EN.pdf> (accessed 06.01.2020).
- Barbosa-Cánovas, G. V., Fontana Jr, A. J., Schmidt, S. J., & Labuza, T. P. (2007). *Water activity in foods: Fundamentals and Applications* Editors(1 ed). Carlton, Australia: Blackwell Publishing, IFT Press 33pp.
- Chuppava, B., Keller, B., Meißner, J., Kietzmann, M., & Visscher, C. (2018a). Effects of different types of flooring design on the development of antimicrobial resistance in commensal *Escherichia coli* in fattening turkeys. *Vet. Microbiol.* 217, 18–24. <https://doi.org/10.1016/j.vetmic.2018.02.018>.
- Chuppava, B., Keller, B., Abd El-Wahab, A., Meißner, J., Kietzmann, M., & Visscher, C. (2018b). Resistance of *Escherichia coli* in Turkeys after Therapeutic or Environmental Exposition with Enrofloxacin Depending on Flooring. *Int. J. Environ. Res. Public Health*, 15(9), <https://doi.org/10.3390/ijerph15091993>.
- Chuppava, B., Visscher, C., & Kamphues, J. (2018c). Effect of Different Flooring Designs on the Performance and Foot Pad Health in Broilers and Turkeys. *Animals*, 8(5), 70. <https://doi.org/10.3390/ani8050070>.
- Collett, S. R. (2007). Strategies to manage wet litter. *Aust. Poult. Sci. Symp 2007. Aust. Poult. Sci. Symp* (pp. 134–144).
- Collett, S. R. (2012). Nutrition and wet litter problems in poultry. *Anim. Feed Sci. Tech.* 173(1–2), 65–75. <https://doi.org/10.1016/j.anifeeds.2011.12.013>.
- Council Directive 2007/43/European Council, 2007. Laying down minimum rules for the protection of chickens kept for meat production. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:182:0019:0028:EN:PDF> (accessed 06.01.2020).
- Dann, A. B. (1923). Wet Litter in the Poultry House. *Poultry Sci.* 3(1), 15–19. <https://doi.org/10.3382/ps.0030015>.
- de Almeida, E. A., Arantes de Souza, L. F., Sant’Anna, A. C., Bahiense, R. N., Macari, M., & Furlan, R. L. (2017). Poultry rearing on perforated plastic floors and the effect on air quality, growth performance, and carcass injuries—Experiment 1: Thermal Comfort. *Poultry Sci.* 96(9), 3155–3162. <https://doi.org/10.3382/ps.pex131>.
- Directive 2010/63/EU of the European Parliament and of the Council, 2010. On the protection of animals used for scientific purposes. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF> (accessed 06.01.2020).
- Dunlop, M. W., Moss, A. F., Groves, P. J., Wilkinson, S. J., Stuetz, R. M., & Selle, P. H. (2016). The multidimensional causal factors of ‘wet litter’ in chicken-meat production. *Sci. Total Environ.* 562, 766–776. <https://doi.org/10.1016/j.scitotenv.2016.03.147>.
- Eriksson de Rezende, C., Mallinson, E., Tablante, N., Morales, R., Park, A., Carr, L., & Joseph, S. (2001). Effect of dry litter and airflow in reducing *Salmonella* and *Escherichia coli* populations in the broiler production environment. *J. Appl. Poultry Res.* 10(3), 245–251. <https://doi.org/10.1093/japr/10.3.245>.
- Friese, A., Schulz, J., Laube, H., von Salviati, C., Hartung, J., & Roesler, U. (2013). Faecal occurrence and emissions of livestock-associated methicillin-resistant *Staphylococcus aureus* (laMRSA) and ESBL/AmpC-producing *E. coli* from animal farms in Germany. *Berl. Munch. Tierarztl. J.* 126(3–4), 175–180.
- Geser, N., Stephan, R., & Hächler, H. (2012). Occurrence and characteristics of extended-spectrum β -lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet. Res.* 8, 21.
- Grashorn, M., Bessie, W., & Neumann-Fuhrmann, D. (1992). Which ground under their feet. *Deutsche Geflügelwirtschaft und Schweineproduktion*, 48, 1395–1401.
- Hartel, P. G., Segars, W. I., Sumner, J. D., Collins, J. V., Phillips, A. T., & Whittle, E. (2000). Survival of fecal coliforms in fresh and stacked broiler litter. *J. Appl. Poultry Res.* 9(4), 505–512. <https://doi.org/10.1093/japr/9.4.505>.
- Himathongkham, S., Nuanualsuwan, S., & Riemann, H. (1999). Survival of *Salmonella enteritidis* and *Salmonella typhimurium* in chicken manure at different levels of water activity. *FEMS Microbiol. Lett.* 172(2), 159–163. <https://doi.org/10.1111/j.1574-6968.1999.tb13464.x>.
- Kriewitz, J.-P. J. (2017). *Die Ausbreitung von Salmonella Enteritidis in Gruppen von Masthühnern nach experimenteller Infektion unter dem Einfluss einer unterschiedlichen Fütterung und Haltung* Doctoral thesis. University of Veterinary Medicine Hannover, Foundation.
- Leverstein-van Hall, M. A., Dierikx, C. M., Stuart, J. C., Voets, G. M., van den Munckhof, M. P., van Essen-Zandbergen, A., Plattee, T., Fluit, A. C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M. J. M., & Mevius, D. J. (2011). Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17, 873–880. <https://doi.org/10.1111/j.1469-0691.2011.03497.x>.
- Li, H., Wen, X., Alphin, R., Zhu, Z., & Zhou, Z. (2017). Effects of two different broiler flooring systems on production performances, welfare, and environment under commercial production conditions. *Poultry Sci.* 96(5), 1108–1119. <https://doi.org/10.3382/ps.pew440>.
- Macke, H., & Van den Weghe, H. (1997). Kotbelüftung in Trampoline-Ställen. *Landtechnik—Agricultural Engineering*, 52(2), 94–95. <https://doi.org/10.15150/lt.1997.2565>.
- Madelin, T. M., & Wathes, C. M. (1989). Air hygiene in a broiler house: Comparison of deep litter with raised netting floors. *British Poultry Science*, 30(1), 23–37. <https://doi.org/10.1080/00071668908417122>.
- Malchow, J., Puppe, B., Berk, J., & Schrader, L. (2019). Effects of Elevated Grids on Growing Male Chickens Differing in Growth Performance. *Front. Vet. Sci.* 6(203), <https://doi.org/10.3389/fvets.2019.00203>.
- Martin, R. S., Gates, W. H., Tobin, R. S., Grantham, D., Sumarah, R., Wolfe, P., & Forestall, P. (1982). Factors affecting coliform bacteria growth in distribution systems. *J. Am. Water Works ASS.* 74(1), <https://doi.org/10.1002/j.1551-8833.1982.tb04841.x>.
- Maus, F. (1988). Prüfung unterschiedlicher Laufboeden in der Broilermast. / Test of different floors for broiler chickens. German. *Deutsche Geflügelwirtschaft und Schweineproduktion*, 22, 627–633.
- Najati, S., & Van den Weghe, H. (2000). Die Kot/Einstreubelüftung in der Hähnchenmast—eine Gesamtbewertung. *Landtechnik—Agricultural Engineering*, 55(5), 366–367. <https://doi.org/10.15150/lt.2000.1953>.
- Norring, M., Kaukonen, E., & Valros, A. (2016). The use of perches and platforms by broiler chickens. *Appl. Anim. Behav. Sci.* 184, 91–96. <https://doi.org/10.1016/j.applanim.2016.07.012>.
- Opara, O. O., Carr, L. E., Russek-Cohen, E., Tate, C. R., Mallinson, E. T., Miller, R. G., Stewart, L. E., Johnston, R. W., & Joseph, S. W. (1992). Correlation of Water Activity and Other Environmental Conditions with Repeated Detection of *Salmonella* Contamination on Poultry Farms. *Avian Dis.* 36(3), 664–671. <https://doi.org/10.2307/1591762>.
- Overdeest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens, Z., & Kluytmans, J. (2011). Extended-Spectrum β -Lactamase Genes of *Escherichia coli* in Chicken Meat and Humans, the Netherlands. *Emerg. Infect. Dis.* 17(7), 1216–1222. <https://doi.org/10.3201/eid1707.110209>.
- Scheffler, H. E. (1965). The Microbiology of Built Up Poultry Litter. *J. Appl. Bacteriol.* 28(3), 403–411. <https://doi.org/10.1111/j.1365-2672.1965.tb02170.x>.
- Shepherd, E. M., & Fairchild, B. D. (2010). Footpad dermatitis in poultry. *Poultry Sci.* 89, 2043–2051. <https://doi.org/10.3382/ps.2010-00770>.
- Siegmund and Neumann. (2012). *Kompendium der Geflügelkrankheiten* (7 ed.). Hannover, Germany: Schlütersche vet.kolleg 234pp.
- Simpson, G., & Nakaua, H. (1987). Performance and carcass quality of broilers reared on wire flooring, plastic inserts, wood slats, or plastic-coated expanded metal flooring each with or without padded roosts. *Poultry Sci.* 66(10), 1624–1628. <https://doi.org/10.3382/ps.0661624>.
- Spindler, & Hartung (2007). Abschlussbericht zum Projekt “Untersuchungen zur Besatzdichte bei Masthühnern entsprechend der RL 43”, gefördert durch das Niedersächsische Ministerium für Ernährung, Landwirtschaft, Verbraucherschutz und Landesentwicklung (Final Report Analyses of the stocking density of chickens kept for meat (broiler) according to Council Directive 2007/43/EC supported by: Ministry of Food, Agriculture and Consumer Protection and Regional Development in Lower Saxony)..
- Terzich, M., Pope, M. J., Cherry, T. E., & Hollinger, J. (2000). Survey of pathogens in poultry litter in the United States. *J. of Appl. Poultry Res.* 9(3), 287–291. <https://doi.org/10.1093/japr/9.3.287>.
- Toghyani, M., Gheisari, A., Modaresi, M., Tabedian, S. A., & Toghyani, M. (2010). Effect of different litter material on performance and behavior of broiler chickens. *Appl. Anim. Behav. Sci.* 122(1), 48–52. <https://doi.org/10.1016/j.applanim.2009.11.008>.
- Yao, T. S. (1959). The influence of slatted floor and litter floor on the genetic variations in chickens. *Poultry Sci.* 38(6), 1472–1473. <https://doi.org/10.3382/ps.0381472>.