Effect of pH-lowering litter amendment on animal-based welfare indicators and litter quality in a European commercial broiler husbandry

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ABSTRACT Several studies have shown that litter moisture is a major reason for foot pad lesions (FPD) and promotes microbial growth of nitrifying bacteria. The aim of the current study was to determine the possible effects of a sodium bisulfate complex (SBS) as a litter additive on FPD, hock burn (HB), and litter parameters. Two application rates of SBS were examined in 2 experiments on a commercial farm. Two groups of about 30,000 broiler chicks each were introduced on spelt granulate spread at 700 g/m^2 and kept for 36 d. In the first experiment (TRT1), 250 g/m^2 SBS was spread on top of litter 20 h before chick placement; in the second experiment (TRT2), SBS was reduced to 150 g/m^2. Each experiment consisted of 1 treatment group (SBS) and a control group without treatment (CON). Both experiments were repeated once. Litter parameters (pH, percentage of dry matter), foot pad, hock condition and body weight of randomly sampled birds (n = 60 per group) were recorded weekly. Mortality rate was higher in SBS groups compared to CON groups (TRT1 2.79 vs. CON 2.03%, TRT2 2.88 vs. CON 2.27%). SBS had no effect on body weight averaged over the whole production period ($P > 0.05$). Incidence of FPD was significantly reduced in both groups treated with SBS compared to CON ($P < 0.05$), with group TRT1 showing the best results. Incidence of HB was not affected by SBS ($P > 0.05$) but by dry matter content ($P < 0.05$). At the beginning, SBS reduced litter pH to 1.7 and 2.0 in TRT1 and TRT2, respectively, compared to 6.5 and 6.7 in CON. Litter pH in TRT groups increased over time and approached pH of control groups by day 15. Results of the current study indicate that SBS treatment may be beneficial regarding foot pad health in broilers. However, further studies are needed to investigate alternative SBS application rates, and to verify the results.

Key words: foot pad dermatitis, litter treatment, broiler, welfare, litter pH

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INTRODUCTION

Associated with an intensified discussion about health and welfare in broiler husbandry, animal-based welfare assessment is increasing. To evaluate husbandry conditions, several animal-based indicators need to be monitored and evaluated on farms and in slaughterhouses (EFSA, 2012; TierSchG, 2017). According to the German Animal Protection Act, every stockman or owner of stock is obliged to assess animal-based indicators (TierSchG, 2017). In broilers and turkeys, foot pad and hock health were shown to be important indicators as they are directly correlated to husbandry conditions and on-farm management (Allain et al., 2009; De Jong et al., 2016). Thus, on-farm assessment of foot pads and hocks enables the stockman to identify and counteract adverse husbandry conditions at a very early stage (Welfare Quality, 2009; Bergmann et al., 2016). However, appropriate and effective measures have still not been suitably established and integrated within flock management and thus must be further improved (Allain et al., 2009; Welfare Quality, 2009; Shepherd and Fairchild, 2010; EFSA, 2012). Of central importance in flock management, is the condition and quality of the litter, since it affects the health of foot pads (Mayne, 2005; Shepherd and Fairchild, 2010; Abd El-Wahab et al., 2011; Taira et al., 2014) and hocks (Haslam et al., 2007) and the ammonia content in the shed (Elliott and Collins, 1982; Miles et al., 2011a). Therefore, several studies have been performed in order to evaluate the effects of different bedding materials on broiler performance and conditional changes of the bedding during production (Grimes et al., 2006; Berk, 2007; Xu et al., 2015) as well as different litter treatments and additives to enhance and maintain litter quality. The litter treatments investigated were calcium oxide (Ruiz et al., 2008), acidified clay, aluminum sulfate, and sodium bisulfate (**SBS**) (McWard and Taylor, 2000). One method of improving litter and air quality, in particular ammonia (NH_3), is to reduce litter pH. Acidifying litter pH leads inter alia to reduced bacterial and

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enzyme activity and thus formation of NH_3 (Li et al., 2013). This practice, and litter treatment in general, is widespread and well-established in the US broiler industry (Jones-Hamilton, 2017) but not established and officially proven in Europe yet. The utilized products (e.g., Poultry Litter Treatment; PLT) mainly consist of SBS (Tasistro et al., 2007; Jones-Hamilton, 2017). In contrast to the European Union, most of the chicks in the US are placed on re-used litter (Nagaraj et al., 2007; Wheeler et al., 2008; Bolan et al., 2010). US poultry housing systems are often without concrete flooring and litter is used for up to 2 yr or even longer without being replaced; however, the top portion may be changed before introducing day-old chicks (Rhodes et al., 2011). The recommended application rates of PLT range between 370 and 490 g/m^2 for litter material newer than 1 yr and between 490 and 730 g/m^2 for litter material older than 1 yr, whereby no further treatment such as tilling is recommended (Jones-Hamilton, 2017). Several studies investigated the effect of SBS and the lowered pH value in poultry litter on foot pad health (McWard and Taylor, 2000; Nagaraj et al., 2007), hock health, and NH_3 content (Terzich et al., 1998b; Tasistro et al., 2007). The studies differed regarding application rates of SBS between 220 g/m^2 (Nagaraj et al., 2007), 244 g/m^2 (Pope and Cherry, 2000; Blake and Hess, 2001; Tasistro et al., 2007; Johnson and Murphy, 2008; Wheeler et al., 2008; Li et al., 2013), 440 g/m^2 (Nagaraj et al., 2007), and 582 g/m^2 (McWard and Taylor, 2000) and between spreading SBS on top of re-used litter (Moore et al., 1996; McWard and Taylor, 2000; Li et al., 2013) or on top of new litter material (Tasistro et al., 2007). In contrast to the husbandry conditions in the US, a cleaning and disinfection procedure must be performed in Germany before housing day-old chicks for a new production cycle (TierSchNutztV, 2017). The bedding material needs to be new, dry, and clean and has to cover the concrete floor. Furthermore, litter material needs to be friable in order to address and promote characteristic behavior such as scratching, foraging and dust bathing (TierSchNutztV, 2017; Welfare Quality, 2009; Dunlop and Stuetz, 2016). Those litter conditions are completely different to re-used litter material as described above. The prevalence and severity of foot pad dermatitis (**FPD**) is mostly influenced by the moisture content of the feces-litter material (Abd-El Wahab et al., 2011). However, water activity should also be taken into account (Dunlop et al., 2016). Another factor that influences the occurrence of FPD is the presence of skin irritating substances such as NH_3 and uric acid (Youssef et al., 2011). NH_3 content in turn depends on the pH and dry matter (**DM**) content of the litter material (Pope and Cherry, 2000; Dawkins et al., 2004; Miles et al., 2011a; Xu et al., 2015; Bergmann et al., 2016). The level of NH_3 volatilization also differs within a shed as litter moisture varies between different functional areas within the shed (Tasistro et al., 2004b; Tasistro et al., 2007).

Currently, the use of SBS as a litter treatment in animal husbandry systems is not explicitly permitted in the European Union and so far no European studies were published. However, SBS is a legal food additive (EU, 231/2012) and furthermore a feed additive (EU, 2015/1414) for pets and other animals not destined for consumption (EU, 2012; EU, 2015). According to the European Food Safety Authority, a dosage of 4,000 mg/kg complete feed is stated as safe for fattening chicken (EFSA, 2014). The objective of the current study was to assess the effects of SBS supplementation to broiler litter on bird health, welfare, and performance as well as the environmental conditions in the shed. No studies are known about SBS litter treatment on straw pellet or straw granule which are common litter materials in Europe and Germany in particular and might affect SBS litter treatment. The lowest application rate which has been published from a field trial was 244 g/m^2. If a further reduction of treatment rate shows notable positive effects on broiler performance it would also support a litter treatment from financial point of view. Finally, an influence on litter parameters and thus on FPD and hock burns (**HB**) due to different feeding strategies (Shepherd and Fairchild, 2010) could be expected under US (maize based feed rations) and European (wheat based feed rations) production conditions. For that reasons, a systematic investigation about SBS treatment under European broiler production conditions is required.

MATERIALS AND METHODS

Experimental Design

The study was conducted on a commercial broiler farm in North-West Germany (October 2016 to March 2017). The farm consisted of 2 broiler grow-out houses which were used for 2 trials. As the maximum stocking density was restricted to 39 kg/m^2 (TierSchNutztV, 2017), each house provided space for 30,000 birds (1,600 m^2). For the first trial, one house housed the control group whereas the litter in the second house was supplemented with a SBS complex and thus, the treatment group. For the second trial, the setting remained the same whereby the level of supplemented SBS in the treatment group differed from trial one. Both trials were repeated once. Besides the differences regarding litter, both grow-out houses were identical concerning technical equipment (e.g., amount and type of feeders and drinkers, ventilation system) and applied management, yet the sheds for the control and treatment group alternated in each trial and repetition.

Animals and Management

A total of 240,000 Ross 308, day-old chicks (Aviagen Group, Huntsville, USA) were used in this study. For each group and cycle within the trial, 30,000 day-old chicks obtained from the same parent stock were housed simultaneously and then raised for 36 d until slaughter. A commercial diet and water were provided ad libitum. The energy levels of the diets were between 12.6 MJ/kg ME (1st week of development) and 13.2 MJ/kg ME (5th week of development), the protein levels ranged between 22.0% and 19.5% CP until slaughter. All other management aspects were in accordance with the national requirements for broiler husbandry (TierSchNutztV, 2017).

Litter and Litter Treatment

A mixture of spelt pellets and spelt granulate (30:70) was used as bedding material in all groups. The bedding material was dispersed once before housing the birds, whereby 700 g bedding material per m^2 was dispersed using a spreader. The bedding material of the treatment groups was supplemented with a 250 and 150 g SBS complex (GRILLO-ACT, Grillo-Werke AG, Duisburg, Germany) per m^2 in trial 1 (treatment 1: TRT1; SBS_{250}) and trial 2 (treatment 2: TRT2; SBS_{150}), respectively. The SBS complex was manually dispersed on top of the bedding material using a lawn seed spreader. Supplementation occurred 24 h before housing the chicks.

Data Collection

The mortality rate (%) was continuously monitored on a daily basis by the flock manager. From the first day of life until slaughtering, the foot pads of 60 randomly selected birds per group were assessed at 7-d intervals. Samples of the birds were collected from different areas within a shed. Additionally, foot pads from 90 birds per group were scored in the slaughterhouse post mortem (p.m.). Both feet of an individual were given scores, the worse foot pad and the highest score between the 2 hock/feet of an individual was taken for analysis. The plantar area of both foot pads of individual birds was graded according to the 5-point scale from Welfare Quality (2009). The score ranges from 0 (no external signs of FPD, swelling or necrosis) to 4 (visible epithelial lesions, massive hyperkeratosis, and necrotic areas covering more than half of the plantar area). The depth of a lesion was not recorded. HB lesions (Score 0—no lesions to Score 4—severe lesions, in accordance with Welfare Quality (2009)) and weight development (FlexScale electronic scale with a precision of ± 1 g; Big Dutchman, Vechta, Germany) were recorded for the same sampled birds.

Litter Samples

Litter samples were collected at 7-d intervals from the day of housing until slaughter. Samples were taken from 3 different areas within each broiler house: around water lines (**DL**), around feeder lines (**FL**) and around the free area (**FA**) (modified from Tasistro et al., 2004a, b). Within the area "water lines" and "feeder lines", 20 subsamples were combined and defined as 1 sample representing each area. For the "free area", 12 subsamples were taken and combined accordingly. Litter samples were taken from the full depth of the litter with a standardized punch. The determination of litter pH was performed using duplicate suspensions of 5.0 g of poultry litter. 140.0 g deionized water was added to the respective sample and the pH was measured by Microprocessor pH 320 with a SenTix electrode (WTW Weilheim, Germany) calibrated using a buffer for pH 4.00, 7.00, and 9.21. DM content of the litter was determined 3 times according to weight loss after drying the pooled litter samples in a forced-draft oven for 24 h at 105°C (Darr method, DIN 52,183). The triple determination of ammonium was done by water vapor distillation and titration, using a semiautomatic distillation device (Vapodest 20 s, Gerhard Comp., Koenigswinter, Germany). NH_3 was released by MgO and calcinated by water vapor in boric acid. Titration with an HCl solution (c = 0.1 mol/l) was applied in order to determine NH_3 and finally NH_4-N was calculated according to VDLUFA (2014) as follows:

$$\text{N in litter sample}(\%) = (\text{HCl}\,(\text{ml})\,{}^*1.40067\,{}^*100)\,/\text{sample weight}\,(\text{mg})$$

Statistical Analysis

Mortality was analyzed descriptively. Results of live weight were logarithmized over the whole period to obtain variance homogeneity. Data were tested with variance analysis (SPSS Vs.24) to calculate differences between groups within treatments by time point and over the whole production period on significance level with $\alpha = 0.05$. Further differences related to live weight were calculated based on the following model:

$$y_{i,j,k} = \mu + \text{time} + \text{group} + \text{barn} + \text{group}\,{}^*\text{barn} + \text{group}\,{}^*\text{time} + \text{barn}\,{}^*\text{time} + e_{ijk}, \text{with time (day of life; } 8, 15, 22, 29), \text{group } (SBS_{250}, SBS_{150}, SBS_0\,(= \text{CON})) \text{ and barn } (1, 2).$$

The results of the foot pad and HB grading at different time points were presented as frequency distribution (percentage per category). Differences were tested with Mann–Whitney U-test ($\alpha = 0.05$). Stepwise forward multiple regression analysis (SPSS Vs.26) was used to analyze the effect of different litter variables on foot pad and hock rating at farm level. The following regression model was constructed:

$$y1, 2 = ß0 + ß1\,{}^*x1 + ß2\,{}^*x2 + ß3\,{}^*x3 + ß4\,{}^*x4 + ß5\,{}^*x5.$$

Table 1. Broiler performance of SBS flocks and control flocks in treatment 1 (TRT1) and 2 (TRT2) at different times (d) (weight: n = 60 birds/date and each group; 2 replications per group).

Treatment	Weight, g (Mean/SD)				P^*	Mortality, %	
	Day 0	Day 8	Day 22	Day 29		Day 1 to 7	Day 1 to 36
TRT1-SBS_{250}	46.3±3.9	206.4±21.5	1077.7±106.8^b	1647.6±215.5	0.687	1.07	2.79
TRT1-CON_{250}	47.1±4.3	206.3±22.2	1047.5±110.7^a	1616.0±179.7		0.66	2.03
TRT2-SBS_{150}	42.0±2.8^a	188.9±27.0	972.2±119.7^a	1551.9±187.5	0.890	1.40	2.88
TRT2-CON_{150}	43.8±2.8^b	183.1±25.1	1004.1±107.5^b	1532.8±169.2		0.89	2.27

Live weight: ^{a,b} column means followed by different superscript letters are significantly different within a TRT ($\alpha = 0.05$).
*Row means from days 0 to 29 significantly different within a TRT $P < 0.05$.

Table 2. Occurrence of foot pad lesions (%) and hock burn (%) in treatment 1 (TRT1) and 2 (TRT2) depending on bird's age (score 1/2 and 3/4 were pooled for paper presentation).

Foot pad lesion (%) Bird age (d)	n/group*	TRT1-SBS_{250}			TRT1-CON_{250}			$P^†$	TRT2-SBS_{150}			TRT2-CON_{150}			$P^†$
		Score 0	1/2	3/4	0	1/2	3/4		0	1/2	3/4	0	1/2	3/4	
8	120	85.0	15.0	0.0	78.3	21.7	0.0	0.122	82.5	17.5	0.0	85.0	15.0	0.0	0.122
22	120	89.2	10.0	0.8	83.3	16.7	0.0	0.055	80.8	17.5	1.7	78.4	20.8	0.8	0.132
p.m.	180	79.4	20.0	0.6	60.6	37.8	1.6	0.011	68.3	30.6	1.1	58.9	39.4	1.7	0.000
Hock burn (%)															
8	120	99.2	0.8	0.0	94.2	5.8	0.0	0.034	96.7	3.3	0.0	98.3	1.7	0.0	0.664
22	120	49.2	50.8	0.0	45.0	53.3	1.7	0.221	74.2	25.8	0.0	73.3	26.7	0.0	0.484
p.m.	180	32.8	66.7	0.5	30.6	68.9	0.5	0.200	28.3	71.7	0.0	22.2	77.2	0.6	0.115

*A total of 60 (on-farm) or 90 (post mortem; 36 d) pairs of foot pads and hocks were scored per group and repetition, both feet and hocks were evaluated, presented data is based on the worse foot or hock.
†Significance level $\alpha = 0.05$; Mann-Whitney U–Test.

y1 = FPD, y2 = HB with depended variables: x1 = time (day of life; 8, 15, 22, 29, 36), x2 = pH (mean across all 3 sample categories Drinker Line (DL), Feeder Line (FL), Free Area (FA)), x3 = DM (mean across all 3 sample categories DL, FL, FA), x4 = NH_4-N (mean across all three sample categories DL, FL, FA), x5 = group (SBS_{250}, SBS_{150}, SBS_0).

To calculate the effects on ammonium, an analysis of variance was calculated with the effects time (day of life; 1, 8, 15, 22, 29, 36), group (SBS_{250}, SBS_{150}, SBS_0), area (DL, FL, FA) and cycle (cycle 1+2 (= TRT1) and 3+4 (= TRT2)). Cycles 1–2 and 3–4 were analyzed separately because of different treatments. The following model was constructed:

$$y_{ijk} = \mu + \text{cycle} + \text{group} + \text{area} + \text{time} + \text{cycle}{}^*\text{group} + \text{area}{}^*\text{time} + \text{group}{}^*\text{time} + \text{group}{}^*\text{area} + e_{ijk}.$$

RESULTS

Growth Rate and Mortality

Results of live bird weight showed differences during the production period in TRT1 and TRT2 (Table 1). No statistical differences between groups within TRT1 and TRT2 were calculated over the period from the 1st to 29th day (TRT1 $P = 0.687$, TRT2 $P = 0.890$). Mortality rates showed an approximate 0.5% higher cumulative 7-d rate in all SBS groups, leading to a higher overall mortality rate in SBS groups compared to control groups (Table 1).

Foot Pad and Hock Health

The results of the foot pad and hock grading from day 8 to 22 and p.m. are presented in Table 2. Foot pad health was positively influenced by both SBS treatments. The higher dosage of SBS resulted in the best foot pad health. At the end of fattening, the percentage of foot pads without lesions was 18.8 percentage points higher in SBS_{250} compared to CON_{250} ($P = 0.011$). With lower SBS treatment (TRT 2), the difference was about 8 percentage points in favor of SBS_{150} flocks compared to CON_{150} ($P = 0.000$). The severity of FPD was relatively low in all groups as only a few birds were rated category 3 or 4. The prevalence of HB was not influenced by SBS application, no differences between groups were measured ($P > 0.05$; Table 2). HB were graded after the 1st week in all groups and hock health decreased to about 67% of birds having HB in TRT 1, 72% (SBS_{150}) and to 79% (CON_{150}) HB in TRT2 in both groups p.m.

Different predictors with a possible impact on foot pad and hock health were calculated with stepwise multiple regression analysis. From all predictors which were tested at significance level of $P < 0.05$, "Group"

Table 3. Litter parameter pH and dry matter content (%) depending on sampling date and sampling area; "Drinker Line" (DL), "Feeder Line" (FL) and "Free Area" (FA) (SBS = Sodium bisulfate; CON = control group).

Parameter	Area	Group	Day of life					
Treatment 1			1	8	15	22	29	36
pH	DL	SBS_{250}	1.67 ± 0.12	3.03 ± 0.21	5.78 ± 0.04	6.27 ± 0.12	6.63 ± 0.27	6.77 ± 0.61
		CON	6.47 ± 0.09	6.10 ± 0.06	6.09 ± 0.28	6.47 ± 0.02	6.83 ± 0.60	8.36 ± 0.07
	FL	SBS_{250}	1.67 ± 0.12	2.35 ± 0.12	4.86 ± 0.05	5.98 ± 0.10	6.76 ± 0.06	7.47 ± 0.09
		CON	6.47 ± 0.09	6.13 ± 0.10	5.87 ± 0.02	6.16 ± 0.02	6.79 ± 0.16	6.43 ± 0.34
	FA	SBS_{250}	1.67 ± 0.12	2.58 ± 0.10	4.93 ± 0.42	6.54 ± 0.16	7.76 ± 0.52	8.41 ± 0.28
		CON	6.47 ± 0.09	6.10 ± 0.00	6.08 ± 0.26	6.87 ± 0.17	8.35 ± 0.30	8.65 ± 0.02
Dry matter (%)	DL	SBS_{250}	90.75 ± 0.05	72.75 ± 3.95	59.90 ± 0.40	61.85 ± 1.15	63.15 ± 0.15	58.95 ± 1.85
		CON	91.55 ± 0.25	76.55 ± 0.85	64.05 ± 2.45	61.10 ± 1.70	61.35 ± 0.35	62.30 ± 0.30
	FL	SBS_{250}	90.75 ± 0.05	83.15 ± 0.25	83.75 ± 0.55	75.20 ± 1.80	78.05 ± 0.45	74.45 ± 1.95
		CON	91.55 ± 0.25	85.80 ± 0.20	80.10 ± 2.60	75.40 ± 0.50	73.70 ± 0.20	67.15 ± 0.25
	FA	SBS_{250}	90.75 ± 0.05	77.65 ± 3.15	77.05 ± 0.95	72.85 ± 0.55	70.25 ± 1.35	60.55 ± 2.55
		CON	91.55 ± 0.25	80.15 ± 2.55	76.85 ± 3.85	74.30 ± 0.20	65.65 ± 0.75	65.50 ± 1.40
Treatment 2								
pH	DL	SBS_{150}	1.96 ± 0.05	4.26 ± 0.20	6.18 ± 0.13	7.42 ± 1.01	7.79 ± 0.86	6.42 ± 1.34
		CON	6.71 ± 0.36	6.07 ± 0.04	6.74 ± 0.36	8.23 ± 0.36	6.22 ± 0.73	5.93 ± 0.91
	FL	SBS_{150}	1.96 ± 0.05	4.58 ± 0.85	6.03 ± 0.25	6.24 ± 0.33	6.80 ± 0.03	6.76 ± 0.38
		CON	6.71 ± 0.36	6.12 ± 0.08	6.10 ± 0.09	6.87 ± 0.13	6.72 ± 0.14	6.83 ± 0.80
	FA	SBS_{150}	1.96 ± 0.05	3.37 ± 0.95	6.15 ± 0.27	8.24 ± 0.47	8.57 ± 0.27	8.92 ± 0.03
		CON	6.71 ± 0.36	6.13 ± 0.04	6.22 ± 0.02	7.92 ± 0.29	8.85 ± 0.04	8.71 ± 0.24
Dry matter (%)	DL	SBS_{150}	90.70 ± 0.20	67.45 ± 0.35	57.95 ± 1.95	63.25 ± 0.35	66.40 ± 3.20	58.75 ± 6.55
		CON	91.95 ± 0,05	73.65 ± 6.95	60.55 ± 2.15	62.30 ± 3.20	56.25 ± 4.05	56.60 ± 5.30
	FL	SBS_{150}	90.70 ± 0.20	81.45 ± 1.05	77.25 ± 1.45	60.00 ± 6.80	75.80 ± 6.20	77.60 ± 2.00
		CON	91.95 ± 0.05	85.50 ± 0.40	80.10 ± 0.60	68.45 ± 5.15	78.15 ± 0.75	78.35 ± 0.25
	FA	SBS_{150}	90.70 ± 0.20	75.80 ± 7.30	71.35 ± 5.15	67.80 ± 0.70	70.80 ± 0.90	67.65 ± 0.15
		CON	91.95 ± 0.05	83.25 ± 1.65	77.45 ± 1.05	73.15 ± 2.95	67.40 ± 0.70	62.70 ± 2.30

($P = 0.046$) influenced foot pad health with $R^2 = 0.379$ (SE = 0.149). The less SBS applied the higher the foot pad lesions were scored (=worst score number).

The significant impact of predictors "time" ($P = 0.000$) and "DM content" ($P = 0.037$) were pointed out regarding HB. With an increased age and decreased DM content skin irritation and dermatitis on hocks were increasing.

Litter Parameters pH Value and DM

Both SBS application rates reduced litter pH from 6.47 and 6.71 in control groups to 1.67 and 1.96 in TRT1 and TRT2, respectively, at first sampling date. Litter pH was below 4 in both groups treated with SBS until day 8 and under 6 until the 15th day in the SBS group TRT1. However, the change in litter pH depended on the sampling areas (Table 3). Data regarding (DM) showed less DM content at the first sampling date in both SBS treatment groups. Especially in the area around the drinker lines, DM content decreased to 59.9% at day 15 in TRT1 and to 57.95% in TRT2, about 4 to 5 points lower compared to the control groups (Table 3). Values below 65% DM were measured from day 15 to day 36 in SBS group TRT1 and TRT2 around drinker lines, with the exception of day 29 in TRT2 (DM = 66.40%).

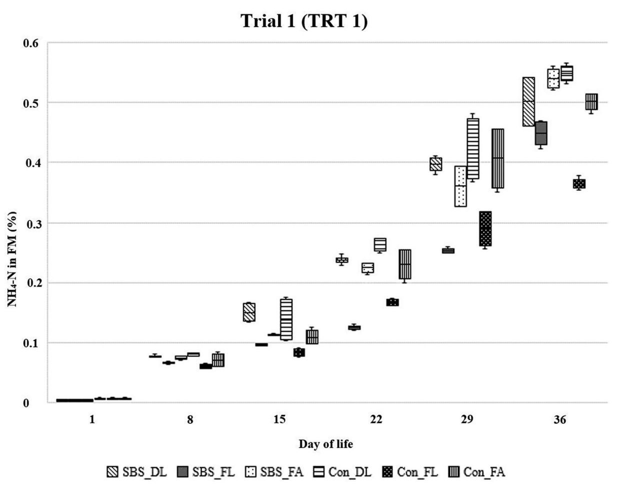

Figure 1. Ammonium-N (%) in fresh mass (FM) within TRT1 (SBS_{250}) at different areas (DL = Drinker Line, FL = Feeder Line, FA = Free Area).

Litter Parameter Ammonium

NH_4-N concentration at the start of production was lower in Trial 1 than in trial 2 (Figures 1 and 2). Within the trials, the NH_4-N content in fresh mass at day 1 of production was between 0.003 (SBS_{250}) and 0.005% (CON) in Trial 1 and 0.017 (SBS_{150}) and 0.022% (CON) in Trial 2, respectively. At day 15 of production, NH_4-N content differed between the sampling areas in all

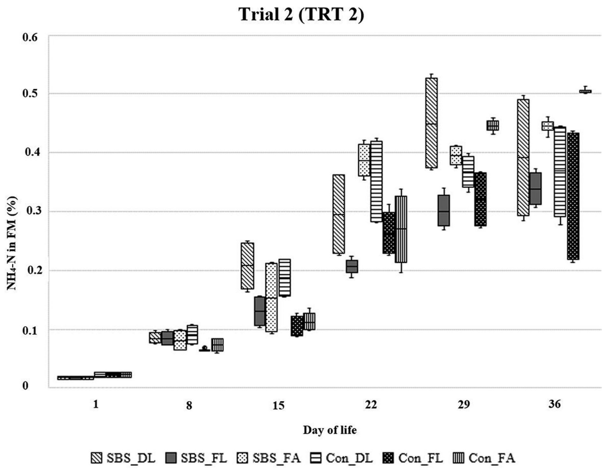

Figure 2. Ammonium-N (%) in fresh mass within TRT2 (SBS_{150}) at different floor points (DL = Drinker Line, FL = Feeder Line, FA = Free Area).

groups, whereby the lowest concentration was measured around the FL. The maximum values of NH_4-N in fresh mass were measured on day 36. Litter samples from TRT1 at the end of fattening resulted in 0.501% NH_4-N (DL), 0.448% NH_4-N (FL), and 0.541% NH_4-N (FA) in the SBS_{250} group and 0.549% (DL), 0.365% (FL), and 0.500% (FA) in the CON group (Figure 1). Compared to that, the level of NH_4-N in TRT2 was lower at day 36 with NH_4-N in the litter at 0.391% (DL), 0.339% (FL), and 0.440% (FA) within SBS_{150} and 0.365% (DL), 0.325% (FL) and 0.505% (FA) within the CON group (Figure 2). Focusing on the amount around water lines (DL), the calculated mean within SBS_{150} was highest at day 29 (0.451% NH_4-N).

The NH_4-N values increased significantly over time ($P = 0.000$). The results of these analyses showed no significant mean effect of the treatment (TRT1 $P = 0.443$ and TRT2 $P = 0.602$). There were significant differences between the means of the sampled areas (TRT1 $P = 0.009$, TRT2 $P = 0.049$).

DISCUSSION

Growth Rate and Mortality

The performance traits of the broilers in all groups are comparable with commercial production figures (Damme, 2017) indicating that the broiler houses were well operated and that the underlying conditions of the study represent practical conditions. Nevertheless, the sample size of 60 birds and 60 feet (resp.180 feet p.m.) per time point and group needs to be considered when interpreting the data. The mortality rate was below the national average of 3.54% (Damme, 2017) in all groups. However, when focusing on 1st week mortality, the mortality rate in the SBS groups was higher compared to the control groups. Other studies with SBS application found no difference between groups (McWard and Taylor, 2000; Nagaraj et al., 2007; Tasistro et al., 2007), but those studies were conducted under well-defined and experimental husbandry conditions, or significantly lower mortality rates in treated groups (Li et al., 2013). Terzich et al. (1998a) also described a positive effect of SBS on mortality rates as less birds suffered from ascites following SBS treatment. Furthermore, Terzich et al. (1998a) postulates that those observations may be consequent to the reduced NH_3 levels in the SBS groups. In the present study, birds were placed on fresh and dry bedding material so NH_3 emission rates were initially not an issue (Tasistro et al., 2007). Another indicator, which has to be addressed when evaluating SBS application regarding health and safety, is the performance of the birds reflected by their live weight. With focus on the overall production period the current experiment showed no significant difference on body weight within treatments, which is in accordance with other studies (Nagaraj et al., 2007; Tasistro et al., 2007; Li et al., 2013). Contrarily, some authors observed increased body weights in SBS treated groups compared to control groups (Terzich et al., 1998b; McWard and Taylor, 2000).

Foot Pad and Hock Health

Foot pad health was better within the SBS treated groups at all samplings. However, very few feet were assessed to score 3 and 4, indicating severe lesions, which may reflect the good management at farm-level, especially in winter when FPD prevalence is expected to be higher than in the summer season (Haslam et al., 2007). Deficient ventilation and an increase of condensation water lead to a higher litter moisture which has proven to be the main factor influencing foot pad health as chicken feet are in permanent contact with litter material during the course of a fattening period (Mayne et al., 2007; Abd El-Wahab et al., 2011). A positive effect of straw granulate compared to chopped straw and wood shaving on foot pad health was investigated by Berk (2007) and could be a reason for less than 2% of scored feet classified as severe lesions in both groups of both trials. Youssef et al. (2010) described that the physical form of litter material, e.g., sharp edges, may promote the occurrence of skin irritations. That could explain the record of first alterations like rhagades or redness in all groups after the first week. At the same time litter moisture increased, especially around waterers, up to 15 to 20%. Litter moisture increased over time and foot pad health became worse in all groups and Mayne et al. (2007) and Youssef et al. (2011) assume that water softens and opens the collagen matrix of metatarsal pads' skin. That would trigger the immune system and also bacteria proliferation would be stimulated (Eichner et al., 2007) and thus promote dermatitis. However, from the first day after applying SBS the DM content in the litter was even below the DM content in the CON groups. That may be explained by the hygroscopic characteristic of SBS (Nagaraj et al., 2007; Li et al., 2013) but would also indicate an effect of a low pH-value by SBS application on FPD. In the present study, the factor "Group" influenced foot pad health ($P = 0.046$) and the less SBS was applied the higher the foot pad lesions were scored (=worst score number). The low initial pH value of less than pH 2 compared to pH 6 in CON groups might have reduced microbial activity and Tasistro et al., (2007) suspected a lasting acidifying effect even though pH increased above pH 6 after day 15 (TRT1) or day 8 (TRT2) in the present study. Furthermore, the low pH-value caused by SBS reduces water activity (aw) in litter and consequently reduces the amount of water the birds' feet get in contact with (Dunlop et al., 2016). The positive effect from SBS supports the results from the experimental study of Li et al. (2013) who even re-applicated 183 g/m^2 SBS during the husbandry period. When litter was treated twice with 220 g/m^2 SBS, Nagaraj et al. (2007) evaluated a trend of improved foot pad health but litter moisture was below 20% during production period.

Within the current experiment litter moisture and time (=age) in combination increased HB prevalence significantly, which supports results from Hepworth et al. (2010). The results in Table 2 show no difference in HB between the SBS and CON groups. Considering the data of DM in litter the increase of litter moisture is similar between groups with ongoing production period. The early occurrence of hock lesions confirms investigations from Bergmann et al. (2016) who monitored hock lesions on Ross broilers in a field trial after 5 d post hatch. Feed composition is described as an impact factor regarding litter quality, and thus as a cause for foot pad lesions and hock dermatitis in consequence of polyuria due to high protein levels and electrolytes like sodium and potassium, for example (Haslam et al., 2007; Shepherd and Fairchild, 2010). This might had an effect on those animal-based indicators in this research but feeding rations were identical in both groups.

Litter Parameters pH Value and DM

DM content decreased with continuous moisture input from excreta, drinker lines and condensation water (Table 3). The differences between several areas in the shed were greater than the differences of DM content between groups. Also Dunlop et al. (2016) observed caked litter underneath waterlines and mostly dry litter in free areas, which clarifies the differences of the litter quality between areas in the shed. The critical DM content of 65% and less, proven by Abd-El Wahab et al. (2011) as a reason and intensifier for FPD, was measured around DL as late as day 15 in both trials and groups. Based on the higher moisture level in litter of SBS groups from the beginning of the production period the hygroscopic characteristic of SBS may become evident. As soon as water is absorbed into SBS the chemical compound dissolves into Na^+, H^+, and SO_4^- (Johnson and Murphy 2008). The released H^+ reduce litter pH and react with NH_3 to form the irreversible component NH_4^+, which would not release nitrogen (N) when litter pH increases again (Johnson and Murphy, 2008).

Litter Parameter Ammonium

Based on recorded NH_4-N, no evidence for lowering NH_3 volatilization was discovered. NH_4-N is significantly influenced by time, as the NH_4-N content in the litter increased over the course of a fattening period. Tasistro et al. (2004b) described the lowest decomposition rate around FL with 17% water content in the litter, followed by FA with a 30% water content and DL with a 55% water content in the litter, which is supported by the present data in Figures 1 and 2 and may explain differences between sample points. However, SBS application did not increase NH_4-N in the litter as recorded by Tasistro et al. (2007) and derived from Tasistro et al. (2004b) who found a strong positive correlation (r = 0.81) between pH and NH_3-N. Decomposition by bacteria may be higher and increases NH_3 volatilization (Tasistro et al. 2004b). As a consequence of low pH in the litter, and depending on the litter water content (Miles et al. 2011b), volatilization of NH_3 should be suppressed as shown by Tasistro et al. (2007). In the same study, the pH value was lower around feeders and in free areas compared to the area around water lines, which is contrary to the current findings. Possibly, sample collection and differences in litter material as well as the amount of material influenced the results, as they spread a 5-cm deep layer of litter material compared to only 700 g/m^2, which were dispersed before chick placement in this study. Moore et al. (1996) could not prove an effect of $NaHSO_4$ on NH_4 in litter after 42 d under experimental conditions (CON 3.27 g vs. SBS 3.91 g $NH_{4/kg}$ litter). Although they top-dressed SBS on re-used litter, the amount of 20 g SBS/kg litter might have been too low to initiate a change. Blake and Hess (2001) published that bacterial decomposition of uric acid increases above litter pH of 7 and uricase activity is highest at pH of 9. Compared to the results in the present study litter pH was below 7 until 22nd day in TRT1 and day 15 in TRT2 in both groups and did not reach pH 9 within both treatments. That could explain similar results of NH_4-N between groups at least until the latter time points. Moreover, McWard and Taylor (2000) assumed that litter pH does not directly correspond with measured NH_3, because the sulfate load from SBS would interfere with the metabolic process of the bacteria in litter and hence reduce the ability to transform NH_3 (McWard and Taylor, 2000). The authors showed that the effect of reducing litter pH below pH 7 by SBS lasted about 5 to 7 d after SBS application, compared to the NH_3 reduction beneath 20 ppm until 30 d after SBS application. Finally, a direct measurement is recommended to prove a relationship between litter quality and NH_3 (Tasistro et al., 2007; Li et al., 2013).

Overall, SBS application to litter did not affect animal health and welfare detrimentally. Seven-day mortality was about 0.5% higher compared to control groups. The reasons are unclear and need to be examined in further studies. Due to the reduction of microbial activity in consequence of a lower pH value and finally a lower aw value, investigations into free water content and water activity (aw) could be useful to describe the relationship between SBS application and foot pad health. Due to less effects on bird's welfare from the lower SBS application rate the economic aspect was neglected and requires consideration in further studies. As foot pad health was better in SBS groups, the results of the study may indicate that the use of SBS as a litter additive is beneficial to birds' health and welfare. Therefore, SBS application may be a useful measure in stock management to prevent or at least reduce FPD, especially severe lesions, and help to increase health and welfare in chicken stock.

ETHICS STATEMENT

The study was carried out in accordance with the German legislations, the German "Animal Protection Act" (TierSchG, 2017) and the "Statutory order for housing and environmental conditions of farm animals" (TierSchNutztV, 2017) as well as the "Guide for the Care and Use of Agricultural Animals in research and Teaching" (Ag Guide, 2010).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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