Impact of water sanitation on broiler chicken production and welfare parameters

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Primary Audience: Live Production Managers, Flock Supervisors, Researchers

SUMMARY

The objective of the study was to assess the impact of drinking water sanitation on performance and welfare outcomes in broiler chickens kept on fresh or used litter. We hypothesized that under a more challenging environment (used litter), the impact of water sanitation would differ to that in a less challenging environment. Seventy-two pens housed 1,944 male broilers for 35 D. The treatments included a tap water control, water with fully activated ClO_2, water with partially activated ClO_2, and water with iodine, offered to birds housed on either clean or used pine shavings. The latter resulted from housing 20 birds per pen that had been vaccinated with Coccivac-B. The intended setup was successful as birds on used litter showed worsened feed conversion, body weights, and daily gains early in the production cycle, although these differences were lost over time. Water sanitation did result in altered drinking behavior, with fewer drinking bouts for birds on iodine treatment than for birds on control and fully activated ClO_2 treatments. Iodine-treated birds showed less severe footpad lesions than birds on other treatments. Similarly, birds on fresh litter had smaller lesions than those on used litter. Iodine- and fully activated ClO_2–treated birds experienced less chronic stress than control birds. The impact of water sanitation did not differ when birds were in a challenging environment (used litter) compared with those in a cleaner environment. Water sanitation did provide some benefits for production (body weight and daily gain) and welfare outcomes (footpad dermatitis and chronic stress).

Key words: animal welfare, broiler chicken, Cl, iodine, production, water sanitation

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DESCRIPTION OF PROBLEM

Hygienic drinking water is important for disease prevention, food safety, and reduction of antibiotic use [1]. Drinking water management could affect the exposure of broiler chickens to pathogenic microorganisms, via drinking line contamination and litter conditions, with litter moisture as a factor for oocyst sporulation [2]. For instance, a "biofilm" could develop in drinking waterlines, which could expose birds to pathogens [3]. A biofilm contains surface-associated microorganisms or can be described as an assemblage of microbial cells [4] within waterlines, including potential pathogens [5]. Furthermore, waterlines with a biofilm or other particulates can result in leaky drinkers,

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increasing litter moisture [6]. A method to remove these biofilms is to sanitize waterlines. Disinfectants (e.g., chlorine dioxide [$\mathbf{ClO_2}$]) and (organic) acids are used for this purpose [4]. Iodine could also be used as a disinfectant, although data concerning the long-term use of iodine are limited [7]. Iodine supplementation to broilers under varying stocking densities showed improvement in production parameters under the relatively lower stocking densities (720 and 540 cm^2/bird) but not under the high stocking density of 360 cm^2 [8]. Provision of iodine in diets of laying hens resulted in improved feed conversion and fewer damaged eggs during a 150-D provision period starting at 18 wk of age [9].

Although drinking water sanitation is commonly used in poultry production to clean drinking lines between flocks [5], few studies have investigated the impact of this procedure. Sanitation during production may provide benefits by preventing biofilm buildup during production, especially during the first days of production when water flow rate is low and house temperatures are high [5]. One challenge with conducting small-scale studies in research settings is that they do not provide conditions similar to the larger scale challenge of commercial production. To better evaluate products under research conditions, sanitation models have been developed, resulting in some insult or challenge to the birds [10]. The use of a coccidial challenge is an established research model in part owing to the coccidial infection in the intestine, allowing for increased access to secondary bacteria [2].

Waterline management can influence leg health through exposure to wet litter. Footpad dermatitis (**FPD**) is a common issue in broiler production, for instance, 38% of commercial fast-growing birds with moderate to severe lesions were reported in the Netherlands [11]. Litter moisture plays an important role in FPD and hock burns (dermatitis on hocks) [6, 12, 13]. Moisture levels are affected by a combination of diet, water quality, water intake, and litter management [14].

Unpalatable water related to either the presence of microorganisms or sanitation products could possibly induce a chronic stress response in broiler chickens because of a long-lasting lack of controllability (choice) [15]. The chronic stress experienced can be quantified by studying characteristics of the adrenal glands. Adrenal glands produce stress-associated hormones such as corticosterone, and they respond to chronic stress by showing hyperplasia and hypertrophy [16] because of increased adrenal activity in response to the stressor [17]. Broiler chickens with an impaired gait showed higher relative adrenal gland weights than birds with a normal gait [18]. With the left gland appearing more sensitive to chronic stress than the right one [19], variation in asymmetry can be a measure to quantify chronic stress response.

Water sanitation may provide an appropriate method to avoid performance and welfare-related losses during broiler production. Therefore, the objective of this study was to determine the effectiveness of water sanitation treatments for broiler chickens housed on fresh or used litter using broiler production parameters (weight, daily gain, mortality, feed conversion), water consumption parameters (drinking frequency and water intake), and aspects of welfare (FPD, hock burn, chronic stress) as response variables.

MATERIALS AND METHODS

The Virginia Tech Institutional Animal Care and Use Committee reviewed and granted approval for this experiment (protocol: 17-232). The experiment included a 4*2 factorial setup, with 4 water treatments equally distributed over 2 litter treatments (either fresh or used litter).

Pre-Experiment Generation of Used Litter

The poultry facility contained 72 pens, divided into 9 blocks. To generate used litter, we housed 720 Cobb males for 21 D in 36 pens of dimensions of 1.37 m × 1.37 m (20 birds/pen). The birds were housed on approximately 5-cm fresh pine shavings, with *ad libitum* access to commercial feed and untreated water through nipple drinkers. These pre-experimental birds were vaccinated with CocciVac B52 [20] with ca. 1,250 oocytes per bird by mixing this live coccidiosis vaccine through their feed, offered on feed trays when birds were placed in their pens at the age of 1 D. Lighting and the

Table 1. Water sanitation treatments provided at the pen level (n = 72) to 1,944 broilers from day 0 to day 34 with water quality targets and mean observed values (±SD) from the front of the house (Bin) and the back (last waterline).

Treatment group			Control: tap water	Full-ClO_2: fully activated chlorine dioxide[1]	Part-ClO_2: partially activated chlorine dioxide[2]	Iodine: iodine-based treatment[3]
Water quality target	pH		7.0 ± 0.6	N/A	6 ± 1	N/A
	Total chlorite (ppm)		–	–	5 ± 1	–
	Free ClO_2 (ppm)		–	2 ± 1	N/A	–
Observed values	pH	Bin	7.52 ± 0.23	6.80 ± 0.28	6.86 ± 0.35	2.89 ± 0.19
		Back	7.17 ± 0.29	6.76 ± 0.23	6.89 ± 0.23	2.93 ± 0.17
	Total chlorite (ppm)	Bin			5.59 ± 0.43	
		Back			5.51 ± 0.81	
	Free ClO_2 (ppm)	Bin		2.09 ± 0.86	2.17 ± 1.00	
		Back		0.67 ± 0.43	1.04 ± 0.50	

N/A not applicable, but was assessed.

– indicates not assessed.

[1]One thousand microliters of AquaPrime NeoKlor and 500 µL of AquaPrime Activator were mixed for 5 min to activate ClO_2 and then added to 10 L of tap water after 5 min.

[2]One thousand microliters of AquaPrime NeoKlor and 500 µL of AquaPrime Activator were mixed and directly added to 10 L of tap water.

[3]Consists of 0.42% iodine and 99.58% inert ingredients: 1.3 mL per 1 L of tap water.

ventilation regime were similar to commercial standards, with 24 h of light until day 7 and 18 h of light thereafter and tunnel ventilation to maintain temperatures (35°C on day 1, lowered gradually to 22°C on day 21). At 21 D of age, these birds were euthanized by manual cervical dislocation, and used litter was piled in the center of the facility. The used litter was mixed manually and returned to the same 36 pens the next day to ensure equal distribution of litter.

Experiment

In total, 1,944 male Hubbard × Ross 708 broiler chicks were allotted to 8 experimental treatments using 9 replicate pens of 27 chicks per pen (1.88 m^2), with a stocking density of ca. 30 kg/m^2 at day 35. Treatments were arranged using a 4*2 factorial arrangement of treatments including a tap water control, water with fully activated ClO_2, water with partially activated ClO_2, and water with iodine, offered to birds housed on either clean 5 cm of fresh pine shavings or used pine shavings as noted previously. The treatments were blocked within the house and randomly assigned across each block. Diet consisted of *ad libitum* access to standard corn–soybean meal–based starter (crumbles), grower, and finisher (pellets) feed provided from days 0–14, days 14–28, and days 28–35, respectively. Broiler feed was formulated to meet the requirements of broilers [21]. In the first week, brooder heat lamps were provided; thus, birds received 24 h of lighting from day 1 to 7, which was reduced to 18 h for the remaining experiment.

Water Treatment The control water treatment consisted of tap water provision without addition of any disinfectant. Two different applications of AquaPrime NeoKlor (7.5% sodium chlorite solution [22]) and BioSentry AquaPrime Activator (a blend of mineral and organic acids) in a 2:1 ratio were tested in treatments "Full-ClO_2" and "Part-ClO_2." The third treatment ("Iodine") consisted of provision of iodine (0.42% iodine and 99.58% inert ingredients [22]) in tap water. The water treatments were prepared in 100-L bins and automatically pumped through the waterlines to nipple drinkers (5 per pen). Diluted water was added to the bins daily, providing birds with ad libitum access. Before the start of the trial, water quality of the tap water was analyzed at an external laboratory, and outcomes were within normal ranges for sodium, calcium, magnesium, pH, nitrate, sulfate, conductivity, total dissolved solids, hardness, total, coliform, iron, manganese, chloride, and fluoride.

During the trial, water quality was assessed daily (days 0–34) at the beginning (bin near the entrance of the facility) and the end of the waterline from the drinker line in the back of the facility between 9:00 h and 10:00 h. Measurements included free ClO_2 (Full-ClO_2 and Part-ClO_2 treatments), total chlorite (Part-ClO_2 treatment), and pH (all treatments; Table 1), and assessment was performed similarly as described by the manufacturer [23]. A titration kit [24] was used to assess free ClO_2 in 100 mL of water, which was collected in a 250-mL Erlenmeyer flask. Two drops of phosphate buffer of pH 7.0 was added, and the mixture was gently swirled. Thereafter, one pack of potassium iodide was added, and the mixture was titrated until the light yellow color disappeared. One drop of starch solution was added, and titration was continued until the blue color disappeared. Free ClO_2 (ppm) was calculated using the following formula, with A representing the titration reading:

$$\text{Free } ClO_2 \text{ ppm} = \frac{\frac{A}{800}}{100} * 0.113 * 67452$$

After the free ClO_2 test, the same water sample was used to assess total chlorite using the Hach titration kit. A dissolved oxygen pillow was added, and the mixture was gently swirled before placing it in a dark location for 5 min. Then, we titrated the mixture until clear. Total chlorite (ppm) was calculated using the following formula, with B representing the titration reading:

$$\text{Total chlorite ppm} = \frac{\frac{B}{800}}{100} * 0.113 * 16863$$

pH levels were assessed using a digital pH meter [25] with 0.05 accuracy. The meter was placed in the water sample, and the measurement was read after the number stabilized.

Measurements

Intestinal Lesions Macroscopic intestinal coccidial lesions were scored in 3 random birds per pen at day 20, using a method adapted in the study by Johnson and Reid [26]. The duodenum, jejunum, and ceca were scored separately by 3 trained observers, which were blinded for treatments. The scoring system entailed a 5-point categorical scale: 0 = no gross lesions; 1 = small petechiae on serosa, with ca. 1–10 lesions per intestinal section; 2 = numerous petechiae on serosa, orange mucus may fill the intestine, with more than 10 lesions per section; 3 = intestinal wall ballooned and thickened, with a roughened mucosal surface, intestinal contents filled with pinpoint blood clots and mucus, and could include fewer, but larger lesions; and 4 = the intestinal wall is ballooned for most of its length, contains blood clots and digested blood, has a putrid odor, and is greatly thickened. The lesion scores were averaged within a pen.

Production Parameters Body weight (kg) and feed intake were recorded at the pen level on days 1, 14, 28, and 35 to calculate mortality-corrected feed conversion ratio (**mFCR**) and average daily gain (**ADG**) for days 0–14, days 0–28, and days 0–35. Pen mortality, recorded daily, was used to correct mFCR, which is calculated as follows:

$$\text{mFCR} = \frac{\text{Total feed intake}}{\text{Total pen weight} + \text{total mortality weight}}$$

Body weight was divided by the number of remaining birds per pen to obtain average body weight per bird. Both provided feed and remaining feed were weighed at the pen level to obtain feed intake data. Mortality was checked daily, and body weight, feeder weight, and cause of death were recorded.

Water Intake Daily water intake (L) was measured between 9:00 h and 10:00 h for each water treatment (n = 4) by measuring the bin's remaining water and subtracting it from the water level on the previous day. Thus, water level (height) was measured daily, and the associated volume was recorded. One bin of water provided the water supply for all pens within that water treatment group; thus, there was only a single measurement of water intake for each set of 18 pens within a treatment. The lack of replication (a single recording for 18 pens) for daily water intake resulted in observational data.

Drink Frequency Drinking behavior was recorded from videos on days 6 and 7 (week 1), days 19 and 20 (week 3), and days 33 and 34 (week 5). The parameter was recorded each week in the same sample of 32 pens. The parameter was recorded for 16 pens per sampling day, with cameras [27] mounted at approximately 50 cm of height, directed at the drinker line. The next sampling day, the 16 cameras were moved to the other 16 sampled pens. The sampled pens were located in the middle of the poultry facility. Drinking frequency (number of drinking bouts per bird) was assessed for three 30-min periods, starting at 10:00 h, 16:00 h, and 21:30 h per recording day. A drinking bout was defined as one or more successive drinks by a single bird without stopping for ≥5 s. Thus, if a bird stopped for ≥5 s, it was counted as 2 separate bouts. Three observers recorded the behavior and were blinded for treatments, with all treatments equally divided among observers. Observers showed agreement based on two 30-min observations of one pen (92 and 94% agreement). Average number of drinking bouts per bird per 30-min observation was calculated (total bouts/ remaining number of birds in a pen).

Foot and Leg Health Footpad dermatitis and hock burns were recorded from 3 birds per pen on days 21, 28, and 34. Both footpads and hocks were scored on a 5-point categorical scale, ranging from no lesion (score 0), a small superficial lesion (score 1), a medium or large superficial lesion (score 2), a deep, small or medium lesion (score 3), to a deep and large lesion (score 4) [28]. Birds were chosen randomly within the pen, and the worst (highest) score of 2 feet and hocks were recorded for each [29, 30] because those lesions were considered most impactful for bird welfare. Footpad dermatitis and hock burn scores from 3 birds were averaged per pen.

Chronic Stress Response Variation in adrenal gland weight asymmetry was used as an indicator of chronic stress [16, 19]. Left and right glands are usually asymmetric, but when this asymmetry differs (greater difference between left and right) between treatments, it can indicate differences in chronic stress responses. Adrenal glands from 3 randomly chosen birds per pen were dissected at day 35 and fixed in 10% buffered formalin solution. The adrenal glands were weighed on a precision scale [31] (to the nearest 0.1 mg), and the absolute asymmetry was calculated by subtracting the right adrenal weight from the left adrenal weight.

Litter Moisture Litter moisture was assessed at day 0 for both litter treatments and at day 35, pooling the litter from all pens within a treatment. The collected litter was weighed, dried at 100°C for 48 h, and weighed again to record percentage of moisture per litter sample. Day 0 litter moisture was 6.9% for fresh litter and 14.7% for used litter. Day 35 litter moisture was 27.9% for fresh litter and 27.8% for used litter. Litter moisture content on day 35 was 29.36% for control treatment, 28.7% for Full-ClO_2 treatment, 28.6% for Part-ClO_2 treatment, and 24.7% for iodine treatment.

Statistical Analysis

All data were analyzed using SAS 9.4 [32]. Data taken from 3 birds per pen were averaged at the pen level (intestinal lesions, FPD, hock burns, and adrenal gland weights). Distributions of residuals were assessed based on visual assessment of QQ plots. Normally distributed data were analyzed using mixed models and Tukey–Kramer post hoc tests. Independent variables were "water treatment," "litter treatment," and their interaction. For FPD, hock burns, and water intake, "age" was an additional independent factor expressed in days. "Block" was included as a random factor in the model.

Normally distributed variables were body weights (kg), mFCR, ADG (g), FPD (0–4 score), adrenal gland asymmetry (mg), and daily water intake per bird (mL). Variables without normal distribution were cumulative mortality, hock burn scores (0–4 score), intestinal lesion scores (0–4 score), and drinking bouts (n/bird). The latter were analyzed nonparametrically using a Kruskal–Wallis chi-square test, including a Bonferroni correction for pairwise comparisons. Differences were deemed significant if P-values were <0.05. All variables were reported at the pen level as least square means with SEM or raw means with SE unless stated otherwise. No interactions between litter and water treatments were found for all normally distributed dependent variables; thus, only main effects are reported.

RESULTS

Intestinal Lesions

In the experiment, a mild coccidial challenge was induced for birds kept on used litter, indicated by the difference in lesion scores between

Table 2. Body weight, mortality-corrected feed conversion ratio, average daily gain (least square means ± SEM), and cumulative mortality (median ± SE) of broilers kept on fresh vs. used litter and receiving a water sanitation treatment (tap water [control], fully activated chlorine dioxide, partially activated chlorine dioxide, iodine).

	Litter treatment			Water treatment				
Age (d)	Fresh	Used	SEM	Control	Full-ClO_2	Part-ClO_2	Iodine	SEM
Body weight (kg)[1]								
0	0.044	0.045	0.0002	0.044	0.045	0.045	0.044	<0.000
14	0.491[a]	0.476[b]	0.003	0.480	0.489	0.487	0.479	0.004
28	1.592	1.584	0.016	1.558[B]	1.610[A]	1.607[A,B]	1.577[A,B]	0.020
35	2.349	2.349	0.020	2.328	2.378	2.365	2.324	0.024
Mortality-corrected feed conversion ratio[1]								
0–14	1.100[a]	1.103[b]	0.001	1.103	1.100	1.101	1.100	0.001
0–28	1.440	1.442	0.008	1.452	1.442	1.438	1.433	0.009
0–35	1.541	1.546	0.008	1.554	1.539	1.547	1.534	0.009
Average daily gain (g)[1]								
0–14	31.94[a]	30.83[b]	0.20	31.09	31.75	31.61	31.10	0.28
0–28	55.27	54.96	0.59	54.06[B]	55.89[A]	54.78[A,B]	54.73[A,B]	0.70
0–35	65.85	65.83	0.58	65.25	66.67	66.29	65.14	0.68
Cumulative mortality (median %)[2]								
0–35	0.0 ± 0.10	0.0 ± 0.12	-	0.0 ± 0.11	0.0 ± 0.10	1.0 ± 0.18	0.50 ± 0.18	-

[a,b]Different superscripts denote a pairwise significant difference at $P < 0.05$ within a treatment and row.

[A,B]Different superscripts denote a tendency for a pairwise difference at $P < 0.10$ within a treatment and row.

[1]Analyzed with mixed models for normally distributed data.

[2]Analyzed using a Kruskal–Wallis chi-square test.

litter treatments. Birds kept on used litter tended to have a higher lesion score on day 20 (median score of 0.44, minimum: 0, maximum: 1.67) than birds that were kept on fresh litter (median score of 0.11, minimum: 0, maximum: 1.44; $P = 0.060$). Lesion scores did not differ between birds kept on different water treatments ($P = 0.931$).

Production Parameters

Production parameters (body weight, mFCR, ADG, and mortality) per treatment are shown in Table 2. Mean body weights did not differ between treatments on day 0 ($P \geq 0.266$). Body weights on day 14 differed between litter treatments, with birds on used litter resulting in reduced body weight (15-g difference; $P = 0.0002$). Body weights on day 28 differed between water treatments ($P = 0.050$), with birds on Full-ClO_2 treatment tending to be heavier than birds on tap water treatment (51-g difference; post hoc $P = 0.080$). Body weights on day 35 tended to differ between water treatments ($P = 0.095$).

mFCR between days 0 and 14 differed between litter treatments ($P = 0.049$), with increased feed conversion when broilers were raised on used litter compared with fresh litter (1.103 vs. 1.100, respectively). Water treatment did not affect mFCR from days 0 to 14 ($P > 0.500$). Water and litter treatments did not affect mFCR between days 0 and 28 and days 0 and 35 ($P > 0.210$).

ADG between days 0 and 14 was higher for birds on clean litter than for those on used litter (1.109-g difference; $P < 0.001$; Table 3), but the difference diminished over time ($P = 0.559$, days 0 to 28; $P = 0.973$, days 0 to 35). ADG between days 0 and 14 did not differ among water treatments ($P = 0.219$), but did tend to differ between days 0 and 28 (1.83 g higher in Full-ClO_2 than in control treatment; $P = 0.052$), and tended to differ between days 0 and 35, with slightly higher ADG in the ClO_2 treatments ($P = 0.098$). Cumulative mortality tended to differ among water treatments ($P = 0.098$) but not differ among litter treatments ($P = 0.831$).

Water Intake

Overall daily water intake differed over time ($P < 0.001$) but not among water treatments

Table 3. Mean ± SE adrenal gland weights and gland asymmetry weights (right minus left gland weight) for each water treatment (n = 51–54).

	Adrenal weights (mg)		
Treatment	Left	Right	Gland asymmetry (mg)
Control	80.50 ± 2.96	66.03 ± 2.37	11.65 ± 2.16[b]
Full-ClO_2	71.46 ± 2.45	68.86 ± 3.27	0.98 ± 2.17[a]
Part-ClO_2	80.82 ± 2.91	73.03 ± 3.41	6.49 ± 2.16[a,b]
Iodine	76.17 ± 2.30	71.30 ± 2.36	3.03 ± 2.21[a]

[a,b]Different superscripts denote a pairwise significant difference at $P < 0.05$.

(P = 0.990). Generally, water intake per bird increased over time from 15.5 mL per bird on day 0 to 371 mL per bird on day 33.

Number of drinking bouts per bird differed among water treatments (Figure 1, control: 2.86 ± 0.14 bouts; Full-ClO_2: 2.89 ± 0.14; Part-ClO_2: 2.81 ± 0.14; Iodine: 2.49 ± 0.15; P = 0.009). Pairwise comparisons showed less frequent drinking in the iodine treatment than in the control and Full-ClO_2 treatments (Bonferroni-corrected P = 0.028 and P = 0.022, respectively). Furthermore, a tendency for fewer drinking bouts was observed in the Iodine treatment than in the Part-ClO_2 treatment (Bonferroni-corrected P = 0.055). No differences between litter treatments were found (P = 0.109). Birds showed fewer bouts in the afternoon (median ± SE: 2.46 ± 0.07 bouts per bird) and evening (2.57 ± 0.11) than in the morning (2.96 ± 0.08; Bonferroni-corrected $P < 0.001$ and P = 0.083, respectively). All birds showed fewer bouts in week 5 than in weeks 3 and 1 (Bonferroni-corrected $P < 0.001$ for both comparisons).

Foot and Leg Health

Footpad dermatitis scores (0–4) were generally low but differed among water treatments (P = 0.026), litter treatments ($P < 0.001$), and age ($P < 0.001$). Birds receiving the Iodine treatment had better FPD scores than those receiving Full-ClO_2 treatment (0.334 difference; P = 0.030; Figure 2) and tended to have better scores than those receiving Part-ClO_2 treatment (0.291 difference; P = 0.083) and the control group (0.290 difference; P = 0.076). Birds on fresh litter showed better footpad scores (score: 0.765 ± 0.092) than birds on used litter (score: 1.283 ± 0.084; $P < 0.001$). In all birds, FPD worsened with age, from 0.573 ± 0.133 in week 3, 1.080 ± 0.087 in week 4, to .391 ± 0.083 in week 5, with significant increases from week 3 to 4 ($P < 0.001$) and week 4 to 5 (P = 0.015). No water or litter treatment effects were found for hock burns (water: P = 0.772, litter: P = 0.988). There was an age effect, with worse hock burn scores found in older birds (Bonferroni-corrected $P < 0.001$).

Chronic Stress Response

Absolute adrenal gland asymmetry differed among water treatments ($P < 0.001$; Table 3), with less asymmetry between left and right glands in Iodine-treated birds than those in the control group (P = 0.004) and less asymmetry in Full-ClO_2–treated birds than those in the control group ($P < 0.001$). No litter treatment effect was found (P = 0.187).

DISCUSSION

For this study, the hypothesis was that drinking water sanitation during broiler chicken

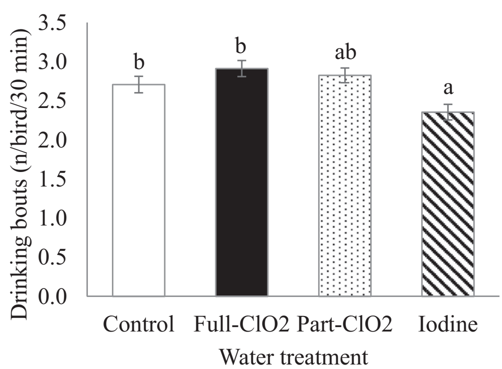

Figure 1. Median number (±SE) of drinking bouts per bird per 30-min observation for each water sanitation treatment. Different superscripts (a, b) indicate a difference between treatments ($P < 0.05$).

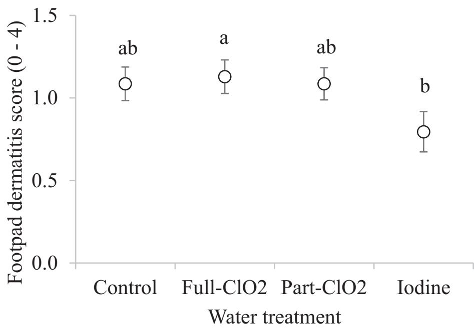

Figure 2. Least square means ± SEM of footpad dermatitis scores (based on worst footpad and average of 3 birds per pen) per water sanitation treatment (n = 216). Different superscripts (a, b) indicate a difference between treatments ($P < 0.05$).

production would reduce the impact of a mild challenge of used litter. In other words, we expected an interactive relationship between water sanitation treatments and litter treatments, with used litter representing a mild coccidial challenge. Although the intended experimental design was confirmed by the higher lesion scores and poorer mFCR and body weight for birds on used litter than for birds on fresh litter, we were not able to show an interaction between water treatment and litter. However, both the water sanitation and litter treatments affected production and welfare outcomes, with differences found in body weights, FPD, and chronic stress responses.

Production Parameters

Generally, few differences were found in production values among water treatments. Throughout the study, body weights in all treatments were higher than breeder standards (e.g., raw mean of 2.32 kg in our study vs. 2.26 kg at day 35 [33]). The lack of differences among water treatments may be due to the overall water quality as laboratory analysis showed good water quality before the start of the trial. Yet birds in the Full-ClO_2 treatment group did show increased body weights on day 28 and ADG (days 0 to 28) compared with those in the control group, although the difference was no longer significant on day 35.

Previous work resulted in production-related benefits when iodine was added to drinking water at a concentration of 2 ppm, yet under moderate stocking densities only [8]. The use of another strain of broilers, the difference in ppm (5 ppm in the present study), and having different stocking densities as additional treatments may have contributed to the different outcomes when comparing with the present study. Iodine has also been reported to result in high livability in broilers (96%) when provided at a concentration of 0.03 ppm (30 μg/L) in drinking water [34], although that relationship was not found in another study [35].

Water Intake

Overall water intake did not differ among water treatments; however, we were not able to record intake at the pen level; thus, potential differences between pens within treatments could not be quantified. Average water consumption in our study was higher than the numbers reported by Tabler et al. [36]. For instance, on day 30, water intake was 326 L per 1,000 birds in our study, compared with an average of 238 L and a maximum of 290 L per 1,000 birds in their data set from 12 broiler flocks. However, the lack of replication in our intake data and lack of information on feed intake from their reported flocks makes comparison difficult.

Drinking behavior differed among treatments. The low number of drinking bouts in the Iodine treatment could indicate taste aversion, likely due to low pH (2.89). Accordingly, pH levels between 5 and 6, 6 and 6.8, or 6.5 and 7.8 are recommended for broiler production depending on the source [36–38]. A pH less than 5 was theorized to result in unpalatable water [36]. The ClO_2 water treatments and control treatment did meet the pH recommendations, which could have played a role in frequent drinking compared with number of bouts in the Iodine treatment.

The Iodine treatment resulted in numerically lower body weights, although not significantly different. This finding is in line with the results of Grizzle et al. [39], who reported reduced broiler chicken growth in the treatment groups with a lowest water pH of 5.75.

Foot and Leg Health

Moderate and severe footpad lesions are painful for poultry [40]. In all treatments, foot and leg health was good, with low scores for FPD and hock burns, and as expected, all scores worsened with age [6, 13, 41]. However, there is a strain component, with commercial fast-growing strains as used in the present study more likely to develop FPD than slow-growing (or dual-purpose) strains [42].

Wet litter is also an important factor contributing to FPD and hock burns [6, 12, 13, 43], and the water treatments could have played a role in this factor. Water treatments affected FPD, with lowest scores found in the Iodine treatment. Possibly, reduced water intake may have resulted in reduced moisture content of litter in the Iodine treatment, in turn reducing moisture in the litter. This argument is supported by the litter moisture content on day 35, which was 4–5% lower in the Iodine treatment than in others (observational data).

Chronic Stress Response

Adrenal gland weights can be used as a measure to quantify chronic stress response as glands respond with hyperplasia and hypertrophy to chronic stress in rats [16] and broilers [18]. In the present study, variation in weight asymmetry was used to indicate levels of experienced chronic stress, with water treatments resulting in differences in adrenal gland asymmetry. We found less adrenal asymmetry in the Iodine treatment and Full-ClO_2 treatment than in the control treatment, suggesting less chronic stress experienced in the former 2 treatments than in the control treatment. Drinking water may have been unpalatable in Iodine-treated birds, but this taste aversion does not seem to have caused chronic stress because that group showed the lowest asymmetry (suggesting less chronic stress). This treatment did result in fewer drinking bouts and reduced body weight, although weight differences diminished at day 35. In a previous broiler chicken study, more chronic stress (heavier glands, with left glands responding more than right glands) was linked to broilers with an impaired gait, with birds having lower body weights [18]. We were not able to establish a relationship between body weight and chronic stress response.

CONCLUSIONS AND APPLICATIONS

1. The impact of water sanitation did not differ when birds were in a challenging environment (used litter) compared with a cleaner environment.
2. Water sanitation did provide some benefits for production (body weight, ADG) and welfare outcomes (FPD and chronic stress response). Although the Iodine treatment resulted in improved footpad condition (lower FPD scores) and a lower chronic stress response, the reduced intake and numerically lower body weights indicate that long-term application of iodine at the tested concentration may not be an appropriate sanitation treatment throughout production.
3. Fully activated ClO_2 did show potential to be applied throughout broiler production, with its positive impact on body weight and chronic stress response.
4. Birds did not show aversion to the ClO_2 treatments, and under more challenging circumstances, it may provide benefits greater than those established in the present study.

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