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Research Article

Effect of Chlorine Dioxide on the Performance, Meat Quality, Gut Health, Microbial Load, Noxious Gas Emission and Immune Responses of Broiler Chicken

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Abstract

Objective: The experiment was conducted to study the effects of chlorine dioxide (ClO_2) on the growth performance, meat quality, gut health, microbial load, noxious gas emissions and immune responses of broiler chickens. **Methodology:** The birds were divided into 6 treatments with 5 replications in each treatment. Birds under the T_1 and T_2 treatments received only clean drinking water, whereas in the T_3 and T_4 treatments, 2 ml of ClO_2 was added in 3-liter water for 12 and 24 hrs, respectively. On the other hand, in T_5 and T_6 treatments, 1 ml ClO_2 was added in 6-litre water for 12 and 24 hrs, respectively. **Results:** The inclusion of chlorine dioxide in drinking water did not significantly affect the growth performance and meat quality of the broiler chickens, but the emission of noxious gases from excreta was significantly reduced in different treatments, especially the T_4 treatments. In T_4 treatment, hydrogen sulfide (H_2S), ammonia (NH_3) and methane (CH_4) were significantly reduced. The faces mineral contents, such as nitrogen (N), phosphorus (P), potassium (K), sulfur (S) and zinc (Zn), were significantly reduced compared to the T_1 treatment. ClO_2 also did not significantly affect the blood properties. The bactericidal effects of disinfectant using 60, 100 and 300 ppm were observed to be effective in 60 sec. **Conclusion:** Chlorine dioxide (ClO_2) is an alternative disinfectant which has no effect on broiler chicken production but significantly reduces noxious gases and microbial load from the environment, which may help to reduce pollution.

Key words: Broiler chicken, chlorine dioxide, disinfectant, poultry, virus zero

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bangladesh is a south-east Asian developing country based on agriculture. Poultry is one of the most significant agricultural subsectors in the country, with an average flock size of 6.9 birds being reared by around 87% of rural households. The commercial poultry sector achieved a significant annual average growth rate of 15-20%. In Bangladesh, industrial and backyard poultry sectors are approximately 60:40 for meat production[1]. Therefore, broiler plays an important role in fulfilling the meat requirement and economic growth due to the advantage of a quick return. Currently, per capita meat consumption in Bangladesh is 4.5 kg per year[2]. According to vision 2021, the target is to increase poultry meat consumption from 4.5 kilograms to 7.5 kg[3]. Bangladesh, use AGP unlawfully to improve growth and feed efficiency. Antibiotics are readily available to people in local drug stores and approximately 80% of all food-producing animals receive AGP[4]. Food safety has been recognized as important issue; therefore, many poultry meat consumers avoid poultry meat and egg by being fed diets containing AGP. The AGP prohibition necessitates the search for more acceptable and safer antibiotic replacements that will boost growth while having no negative impact on chicken products. Chlorine dioxide is an oxygen-based oxidant, not chlorine. It eliminates viruses such as CORONA, SARS, Ebola, Norovirus, Influenza, Bird Flu and others. It also kills bacteria such as anthrax, *E. coli*, *Staphylococcus aureus*, fungi and protozoa. Per ppm, it is 2.5 times more potent than chlorine. It does not include any hazardous by-products. WHO, US EPA, KFDA, FDA and EU has approved this product. The USDA-Food Safety Inspection Service has shown that chlorine dioxide is effective in decreasing bacterial contamination of broiler carcasses[5], washing water[6] and surfaces[7]. When exposed to a wide variety of pH levels, it is also thought to be an effective bactericide that produces fewer chlorinated organic compounds than aqueous chlorine[8]. Moreover, bacteria do not acquire resistance to chlorine dioxide because it reacts with biological thiols, which are essential to all living organisms[9]. Chlorine dioxide kills microorganisms by acting directly on the 2-cell membrane and disrupting basic cellular processes[10]. To minimise foodborne pathogens in the gastrointestinal system Barnhart *et al.*[11] recommended that suitable disinfectants might be given orally during pre-slaughter feed withdrawal. In chickens and pigs, oral administration of chlorine dioxide up to 500 ppm (0.05%) exhibited no harmful effects on diet or feed intake[12,13], although having high bactericidal and virucidal action. In addition, a case study of chlorine dioxide administered with drinking water showed an improved feed conversion ratio and lower mortality in broilers[14]. However, no research has been conducted to investigate the effects of chlorine dioxide injection on the broiler diet. Ahmed *et al.*[15] evaluated the effects of two different amounts of chlorine dioxide on growth, intestinal microbiota and pH, excreta microbiota and pH and odorous gas emissions from broiler excreta[15]. Therefore, the present study was undertaken to evaluate the effects of chlorine dioxide on the growth performance, meat quality, gut health, microbial load, noxious gas emission and immune responses of broiler chicken.

MATERIALS AND METHODS

Birds, treatments and management: To execute this research program, birds were obtained from commercial hatcheries/farms. Standard commercial diet and water were provided *ad libitum*. The temperature, ventilation and humidity were maintained as per the requirement of the bird. According to the treatments, diets were formulated to meet the nutrient requirements of the bird[16]. Different kinds of feed additives and disinfectants were inoculated with different levels to the basal diet. A total of 600, 1-day-old broiler chicks were procured from commercial hatcheries/farms, weighed without arousing and put into 30 cages. Chicks were randomly separated into 6 groups, each with 20 birds and were randomised to disinfectants containing (0,1 and 2 ppm) and time (1 and 2 times per day) in a 3×2 factorial arrangement of treatments (Table 1). The disinfectant virus zero (ClO_2 6000 ppm) was dissolved in water and was given once or twice a day, depending on the therapy. The room temperature was kept at 33°C for the first week, then gradually decreased

Table 1: Experimental design

	Treatments (3×2 factorial arrangement)					
Replications	T_1 (A1×X1)	T_2 (A1×X2)	T_3 (A2×X1)	T_4 (A2×X2)	T_5 (A3×X1)	T_6 (A3×X2)
R_1	20	20	20	20	20	20
R_2	20	20	20	20	20	20
R_3	20	20	20	20	20	20
R_4	20	20	20	20	20	20
R_5	20	20	20	20	20	20

NB: Treatment A is the factor 1 with 3 concentration level and X is the factor 2 with two times

by 2-3°C per week until it reached 22°C, which was kept at by a circulating fan until the experiment was completed. Throughout the experiment, the mean relative humidity was kept between 60 and 65%.

Measurement of faces gas emission and litter quality: At the conclusion of the feeding trial, ten birds from each treatment were chosen at random and assigned to their own cage. Fresh excreta from broiler chicks were collected after a three-day adaptation period to determine excreta noxious gas emission. Excreta samples (1000 g) were kept in a 10L plastic bucket and fermented at room temperature for one day. The gases generated (NH_3, CO_2, O_2, H_2S) were measured using a BOSEAN (BH-4S, portable multi-gas detector) from about 5 cm above the excreta samples after the fermentation time. Based on the procedure described by Gogavekar *et al.*[17], various litter parameters such as moisture, pH and nitrogen were estimated. At the second, fourth and fifth weeks, three samples from each replication of the various treatments were evaluated. The Kjeldahl method was used to determine the nitrogen content of the litter sample.

Measurement of blood properties: At 4-5 weeks of age, peripheral blood samples (2-3 mL) were obtained by wing vein puncture and allowed to clot for 2 hrs at room temperature, centrifuged at 1500 rpm for 15 min at 4° C and the serum was collected and stored at -20°C until analyses. Serum triglycerides (TG), total cholesterol (TCL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose, serum creatinine, albumin, total protein, ESR, PCV, Hemoglobin, RBC, Immunoglobulin A, Immunoglobulin G, calcium, phosphorus and Iron content were measured through outsourcing using a turbidimetric method as described by the manufacturer.

Meat quality and carcass characteristics: At the end of the feeding trial, birds were slaughtered and samples were collected for analysis. The pH value of each pectoral muscle sample was determined using a digital pH meter. Cooking loss was determined based on the following formula:

$$\text{Cooking loss} = \frac{\text{Initial weight-cooked weight}}{\text{Initial weight}} \times 100$$

Meat colour, CIE L* (lightness), a* (redness) and b* (yellowness) colour space values were determined using a Minolta colorimeter.

Determine the microbial load in meat:

Bactericidal activities of the tested solution (Virus-Zero) against *Salmonella enteritidis*, *Salmonella typhimurium*, and *Escherichia coli* (strain 1 and 2) in liquid reaction: Bactericidal activities in an aqueous phase were investigated at one reaction temperature (RT) with two different contact durations (30 sec and 1 min) towards *Salmonella enteritidis*, *Salmonella typhimurium*, and *Escherichia coli* (strain 1 and 2). Four hundred micro-litters of each tested solution (60, 100 and 300 ppm) were mixed with 100 µl of each culture bacteria in a microtube, and incubated at the indicated time (30 sec and 1 min) at RT. Following incubation, the bactericidal inactivation was stopped by adding 500 µl of the blocking solution[18]. The viable bacteria in each sample, including the positive control, were counted (log10 CFU/ml) by plating 25 µl portions on a plate count agar plate after making a serial ten-fold dilution with sterile phosphate buffered saline (PBS), followed by 24 hr incubation at 37° C. For the positive control, 100 µl of each culture bacteria was mixed in 400 µl of PBS, and then 500 µl of the blocking solution was added (final 50% FBS and 0.5 M Tris-HC); subsequently, serial 10-fold dilutions were made. The viable bacteria in each sample was counted (log10 CFU/ml), as described above. Each test was carried out three times in triplicate as shown in the suspension test, data were shown in the result section, after statistical analyses.

Statistical analysis: All the data were analyzed using the Mixed Models procedures of Statistical Analysis System (SAS)[19] and the difference was be determined by the Duncan Multiple Range Test[20]. P-value 0.05 was considered significant.

RESULTS AND DISCUSSION

The current experiment was carried out to see how varying amounts of disinfectant in drinking water affected broiler chicken development, meat quality, blood characteristics, microbiological growth and noxious gas emission.

No significant differences in body weight, weight gain, feed intake and FCR were found after 30 days trial (Table 2). However, numerically higher body weight (1835.17 g) and weight gain (1796.68 g) were attained in the T_4 group which was treated with 1 mL ClO_2 per 3L of drinking water for 24 hrs. Similarly, relatively lower feed intake (2354.04 g) and better FCR was found in the T_4 treatment group, where birds received the same dose of ClO_2 for 24 hrs. Body weight gain was not significantly different from those of the treatment and control group. Sultan *et al.*[21], used chlorine dioxide and recorded improved body weight. Similar results were reported in some

Table 2: Effects of ClO_2 in drinking water on the performance of broiler chicken (0-30 day)

	Treatments							
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	p-value
Body weight (g)								
7 day	171.6	163.68	164.45	157.54	161.34	168.66	0.153	0.089
21 day	918.74	909.31	921.56	904.73	900.55	905.12	4.773	0.631
30 day	1783.15	1796.32	1794.45	1835.17	1825.53	1778.43	8.294	0.562
Weight gain (g)								
0-7 day	132.85	124.86	125.3	119.05	122.97	131.07	1.44	0.069
8-21 day	747.14	745.63	757.11	747.19	739.21	736.46	4.22	0.814
22-30 day	826.2	848.43	833.75	891.81	886.74	835.72	8.28	0.237
0-30 day	1744.4	1757.5	1755.3	1796.68	1787.16	1740.84	8.17	0.360
Feed intake (g)								
0-7 day	147.02^{a}	137.38^{ab}	127.82^{b}	132.94^{ab}	136.02^{ab}	139.162^{ab}	1.562	0.041
8-21 day	889.64	878.23	889.58	851.64	883.12	873.48	11.02	0.503
22-30 day	1361.73	1356.34	1396.73	1369.49	1379.02	1360.83	13.35	0.951
0-30 day	2398.39	2371.95	2414.13	2354.07	2395.08	2373.472	25.932	0.941
FCR								
0-7 day	1.106661648	1.1002723	1.02011173	1.11667367	1.10612344	1.061738	0.006	0.302
8-21 day	1.190727307	1.17783619	1.17496797	1.1397	1.1946	1.1860522	0.004	0.963
22-30 day	1.64818	1.5984	1.6752	1.5356	1.5551	1.6283	0.017	0.533
0-30 day	1.3749	1.3496	1.3753	1.3102	1.3402	1.3634	0.012	0.685
Mortality (%)	2	0	1	0	0	1		

Table 3: Effects of virus zero in drinking water on noxious gas emission of broiler chicken

	Treatments							
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	p-value
O_2 (%)	19.50	19.50	21.43	23.34	17.67	21.60	1.750	0.964
CH_4 (%)	14.35	19.57	13.40	9.35	5.80	12.83	2.300	0.669
H_2S (ppm)	45.75^{a}	29.93^{ab}	30.10^{ab}	22.60^{b}	33.56^{ab}	30.69^{ab}	3.250	0.046
CO (ppm)	786.00	843.75	793.30	741.60	763.85	695.45	54.660	0.986
NH_3 (ppm)	82.63^{a}	79.17^{a}	72.46^{ab}	59.72^{b}	84.68^{a}	71.32^{ab}	5.260	0.038
CH_4S (ppm)	76.97^{a}	54.85^{b}	52.35^{b}	49.75^{c}	50.70^{b}	40.90^{ab}	4.230	0.029
NO_2 (ppm)	0.21	0.21	0.14	0.11	0.21	0.25	0.020	0.651
CO_2 (%)	3.89	4.58	4.42	4.72	4.47	4.55	0.163	0.792
SO_2 (ppm)	15.09	17.14	15.93	16.52	15.37	15.07	0.775	0.972
pH	6.70	6.29	6.22	5.78	6.46	6.10	0.211	0.901
Temperature (°C)	34.92	35.17	35.42	34.75	35.00	34.67	0.356	0.995

previous studies[22-25]. WHO[26] also found that chlorine dioxide did not adversely affect water intake, so no significant change was found in body weight and weight gain. In another experiment, Ahmed *et al.*[15], used chlorine dioxide and observed that the body weight and weight gain of broiler chicks was not different significantly. Demeckova *et al.*[12], reported that 300 ppm chlorine dioxide (ClO_2) in an oral supplement had no negative impact. In present study, a significant difference was found in the first 7 days treatment trial. But on the other hand, there was no significant difference in control and other treatment using chlorine dioxide. According to Islam *et al.*[27], adding disinfectant in drinking water can reduce the FCR. Ahmed *et al.*[15], reported that dietary chlorine dioxide (0.05 and 1%) significantly reduced the FCR without a negative impact on the growth rate of broiler. But Sultan *et al.*[21]., mentioned that chlorine dioxide did not alter FCR.

After the feeding trial, ten birds from each treatment were selected randomly and assigned to each cage. After a three-day adaptation period, fresh excreta from birds were collected in order to determine the 22 noxious gases they emit. Excreta samples (1000 g) were placed in a 10 L plastic bucket and left to ferment at room temperature for one day. After the fermentation, the gases generated (NH_3, CO_2, O_2, H_2S, SO_2, CH_4S, CO, Cl_2) were measured using a Geotech (Biogas 5000) and multi-gas detector (BH-4S) about 5 cm above the excreta samples. ClO_2 has a significant impact on reducing H_2S, NH_3 and CH_4S gas emissions when it ferments, while others have a negligible impact (Table 3). H_2S and NH_3 gas emissions were the lowest in T_4 group (22.60 and 59.72 ppm, respectively), whereas CH_4S emissions were considerably lower in T_6 group treated with 1 ml of chlorine per 6L of drinking water for 24 hrs. Ahmed *et al.*[15], reported that 0.05% chlorine dioxide

Table 4: Effects of virus zero in drinking water on mineral content in the faeces

	Treatments							
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	p-value
Nitrogen (%)	2.940^{a}	2.800^{a}	2.240^{ab}	1.260^{b}	2.660^{a}	2.800^{a}	0.589	0.028
Phosphorus (%)	1.220^{a}	0.530^{b}	0.760^{ab}	0.510^{b}	0.700^{ab}	0.540^{b}	0.358	0.019
Potassium (%)	6.640^{a}	5.810^{a}	4.560^{ab}	3.320^{c}	4.150^{bc}	3.320^{c}	0.874	0.003
Sulfur (%)	2.590^{a}	1.350^{bc}	1.980^{ab}	1.050^{c}	1.300^{bc}	1.650^{b}	0.781	0.012
Zinc (%)	0.004	0.005	0.002	0.003	0.005	0.003	0.001	0.087

Table 5: Effects of virus zero in drinking water on meat quality of broiler chicken

	Treatments							
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	p-value
Carcass characteristics								
Body weight (g)	1773.26	1786.38	1784.42	1826.24	1816.51	1769.32	17.480	0.124
Carcass weight (g)	1250.44	1276.64	1295.59	1356.03	1338.11	1252.58	13.590	0.587
Dressing (%)	70.50^{b}	71.57^{ab}	72.70^{ab}	74.20^{a}	73.80^{a}	70.79^{b}	2.430	0.038
Breast meat (%)	23.86	22.20	21.00	23.50	23.30	23.18	0.910	0.249
Thigh meat (%)	19.26	19.29	20.13	19.89	20.25	19.41	0.870	0.247
Drumstick (%)	12.56	11.60	12.26	12.91	13.22	12.87	0.690	0.359
Wing (%)	12.26	12.60	11.92	11.89	12.56	12.38	0.640	0.289
Liver (%)	2.36	2.30	2.20	2.50	2.41	2.41	0.160	0.817
Heart (%)	0.52	0.55	0.53	0.63	0.64	0.53	0.030	0.598
Fat pad (%)	2.80	2.83	2.77	2.97	2.41	2.82	0.170	0.378
Gizzard (%)	2.46	2.19	2.21	2.65	2.11	2.31	0.150	0.591
Spleen (%)	0.11	0.11	0.09	0.11	0.16	0.11	0.010	0.428
Shank (%)	5.06	5.08	4.18	5.28	5.69	5.11	0.380	0.241
Bursa (%)	0.08	0.07	0.06	0.09	0.11	0.08	0.004	0.127
Meat quality								
Cooking loss (%)	12.01	10.65	10.30	9.07	11.26	11.47	0.580	0.421
Meat color (CIE)								
L*	48.83	49.55	47.88	47.37	49.53	48.24	0.520	0.663
a*	1.64^{b}	2.68^{ab}	2.99^{ab}	3.64^{a}	3.53^{a}	2.64^{ab}	0.197	0.003
b*	6.95	7.82	7.03	8.37	7.40	6.97	0.305	0.565
pH	5.69^{b}	6.05^{a}	6.05^{a}	6.0^{a}	6.09^{a}	5.94^{ab}	0.013	0.030

significantly increased H_2S emissions from broiler faeces compared to the non-additive group (T_4>T_2> T_3>T_6> T_5>T_1). The range of NH_3 was found as T_4> T_3> T_6> T_2> T_1> T_5.

The percentage of Nitrogen, Phosphorus, Potassium and Sulfur content in poultry faeces was found to be significantly lower (1.26, 0.51, 3.32 and 1.05%, respectively) in T_4 while the non-significant result was observed in the case of Zinc percentage (Table 4). ClO_2 in drinking water significantly reduced the mineral content in faces. The major nutrient such as nitrogen, phosphorus, potassium, sulfur was significantly reduced in faces. The result indicated that chlorine dioxide has reduced the adverse impact on faeces by using chlorine dioxide (ClO_2).

Table 5 shows the carcass characteristics of broiler chickens. The presence of disinfectants in drinking water did not affect the features of carcasses. T_4 and T_5 had much greater dressing percentages than those of the other treatments. The disinfectant treatments did not affect carcass characteristics (breast, thigh, drumstick, wings, fat pad, spleen, shank and bursa weight percentage). The T_4 and T_5 considerably enhanced muscle pH and colour (CIE) (a*redness) compared to the other treatments. Disinfectant treatments did not affect cooking loss, CIE (L*lightness and b*yellowness). Results of the current study showed no significant differences in meat quality. Similarly, Sultan *et al.*[21], reported that chlorine dioxide (ClO_2) therapy did not affect relative weight, liver, heart, or gizzard. In contrast, Maćkiewicz and Dziubek[28] found that carcass output was much higher and Cengiz *et al.*[23], observed that gizzard weight increased due to ClO_2 being a disinfectant.

On the other hand, various levels of ClO_2 significantly affected different blood properties (HDL-cholesterol, LDL-cholesterol, total protein and Phosphorus) of broiler chicken (Table 6). Significantly higher amount of HDL-cholesterol (135.00 mg/dL), Phosphorus (1.93 mg/dL) and lower amount of LDL-cholesterol (2.75 mg/dL) were found in T_4 group. Levels of IgA in blood was significantly ($p<0.05$) higher in T_3, T_4 and T_6 treatments than those of T_1 and T_2 treatment. However, serum IgG levels were not significantly influenced by the addition of disinfectant in drinking water, instead, this value numerically increased as compared to T_3 and T_4 treatments.

Table 6: Effect of virus zero on the blood properties of broiler chicken

Treatments	Glucose (mmo1/L)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Iron (mg/dL)
T_1	10.170	130.000	47.750	68.3300^{c}	47.0000^{a}	2.5700^{b}	1.450	6.300	1.550^{b}	120.250
T_2	11.760	140.330	59.660	115.0000^{b}	13.6600^{b}	2.9000^{a}	1.480	6.400	1.820^{ab}	122.660
T_3	9.430	134.660	55.330	107.3300^{b}	16.6600^{b}	2.8000^{a}	1.530	5.900	1.660^{ab}	121.000
T_4	10.650	143.000	55.500	135.0000^{a}	2.7500^{c}	2.5000^{bc}	1.500	6.170	1.930^{a}	146.330
T_5	11.950	118.750	74.500	97.0000^{b}	8.7500^{bc}	2.4700^{bc}	1.350	6.970	1.550^{b}	127.000
T_6	10.300	131.000	60.330	73.2500^{c}	43.3300^{a}	2.4000^{c}	1.600	6.660	1.600^{b}	134.250
SEM	1.710	32.950	8.840	9.3100	4.0200	0.8400	0.480	1.400	0.680	12.580
p-value	0.272	0.060	0.092	0.0001	0.0001	0.0001	0.734	0.522	0.043	0.102

Table 7: Effect of virus zero on humoral immune responses of broiler chicken

	Treatments							
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	p-value
IgA (mg/dL)	10.69^{b}	10.87^{b}	22.40^{a}	20.18^{a}	18.19^{ab}	20.59^{a}	1.457	0.043
IgG (mg/dL)	2.08	2.18	3.25	3.24	2.77	2.35	0.169	0.152

Table 8: Bactericidal effect of virus zero (ClO_2) solution against some bacteria (60 ppm)

		Bacterial titer [log_{10} (CFU/mL)]		
			Contact time/treatment time	
ClO_2 solution	Tested bacteria	Positive control	30 sec	60 sec
60 PPM	*E. coli*-1	8.73±0.34	6.73±0.48	5.71±0.46*
	E. coli-2	8.91±0.45	6.93±0.17	5.79±0.13*
	S. enteritidis	8.87±0.27	6.80±0.28	5.63±0.17*
	S. typhimurium	8.49±0.13	6.24±0.14	5.41±0.58*

Blood properties such as HDL, LDL and Total protein were significantly differ in different treatments but other parameters did not alter significantly compared with control treatment. Which indicated that ClO_2 did not badly impact on the chickens' health properties.

We measured total serum antibody levels (IgG and IgA) at 30-days of age (Table 7). A significant ($p<0.05$) increase in IgA levels in blood was observed in T_3, T_4 and T_6 treatments when disinfectant was mixed with drinking water compared to the T_1 and T_2 treatments. Nevertheless, the mixing of disinfectant in drinking water did not affect serum IgG levels significantly but T_3 and T_4 treatments numerically increased them. IgA and IgG levels did not alter within the control treatment, respectively, revealing that ClO_2 treatment did not affect the humoral immune system.

We also evaluated the bactericidal efficacy of chlorine dioxide against *Salmonella enteritidis*, *Salmonella typhimurium* and *Escherichia coli* (strain 1 and 2) in liquid reaction and found that 60 PPM ClO_2 solution could not able to inactivate our tested bacteria at an adequate level [>3 log_{10} colony-forming units (CFU)/mL] within 30 sec contact time but it could be able to inactivate the tested bacteria (>2 log_{10} CFU/mL) within 30 and 60 sec contact time (Table 8). On the other hand, 100 and 300 PPM ClO_2 solution could be able to inactivate our tested bacteria at a sufficient level (>3 log_{10} CFU/mL) in both contact times, even in the presence of organic materials (nutrient broth) (Table 8). In control bacteria, we found a high titer (8.8 log_{10} CFU/mL) but after treatment with ClO_2 solution, we found greater than >5 log_{10} CFU/mL bacterial reduction at 60 sec contact time.

A study conducted by Sultan *et al.*[21], found pathogenic microorganisms (*Salmonella* and *E. coli*) on day 21 (2.63 and 3.73 CFU/g) and day 28 (2.58 and 3.53 CFU/g). Chlorine dioxide significantly disrupted the permeability of the outer membrane of the pathogens[29]. similar results were reported by Dibner and Buttin[29], who used organic acidulants and observed reduction of pathogenic load in the intestinal tract of broilers. Results of the present study are consistent with Isabel and Santos[30] who used plant-derived essential oils and other acidulants and observed the reduction of pathogenic microorganisms in the broiler gut. Similar results were reported by Khan *et al.*[31] and Fronte *et al.*[25], who used acidulants in poultry drinking water and observed significant reduction in *Salmonella* and *E. coli* . An increase in villus height (920.03 µm) and goblet cell number (80.25) was also noted in this study. This effect is possibly due to the prevention of bacterial colonization as these microorganisms cause sloughing of the intestinal mucosa through pathogenic inflammation, thereby impairing the digestion, goblet cell function and nutrient absorption[32]. The intestinal mucosa is

Table 9: Bactericidal effect of virus zero (ClO_2) solution against some bacteria (100 ppm)

		Bacterial titer [log_{10}(CFU/mL)]		
			Contact time/treatment time	
ClO_2 solution	Tested bacteria	Positive control	30 sec	60 sec
100 PPM	*E. coli*-1	8.73±0.34	5.70±0.26*	4.91±0.17*
	E .coli-2	8.91±0.45	6.13±0.11	4.79±0.26*
	S. enteritidis	8.87±0.27	5.81±0.21*	4.63±0.14*
	S. typhimurium	8.49±0.13	6.04±0.29	4.47±0.18*

Table 10: Bactericidal effect of virus zero (ClO_2) solution against some bacteria (300 ppm)

		Bacterial titer [log_{10} (CFU/mL)]		
			Contact time/treatment time	
ClO_2 solution	Tested bacteria	Positive control	30 sec	60 sec
300 PPM	*E. coli*-1	8.73±0.34	4.77±0.29*	2.83±0.11*
	E .coli-2	8.91±0.45	5.03±0.21*	3.07±0.17*
	S. enteritidis	8.87±0.27	4.33±0.34*	2.93±0.26*
	S. typhimurium	8.49±0.13	4.94±0.29*	3.49±0.18*

Bacterial titer (log_{10} CFU/mL), *Asterisk indicates effective bacterial reduction ($\geq$3 log_{10} CFU/mL), Bacterial titer $\leq$2.6 log_{10} CFU/mL indicates bacterial counts under detection limit, Both effective and under detection limit are significantly different ($p<0.05$) from bacterial positive control titer

inhabited by many microorganisms including both beneficial and pathogenic bacteria. Pathogens often cause infections, impair bird health and cause necrotizing enterocolitis. Necrotizing enterocolitis impairs growth[33] and it can be prevented by using high-quality acidulants[21,23]. The basic mode of bacterial inactivation by chlorine dioxide (ClO_2) is the inhibition of bacterial protein synthesis[34]. In particular, chlorine dioxide (ClO_2) directly affects the cell membrane by altering (at high concentrations) or destroying (at low concentrations)[10] the permeability of the cell membrane, penetrating the cell and interfering with protein synthesis. However, no significant effect of chlorine dioxide was found on Gram-positive *Lactobacillus* and *Bacillus*, except for a significant reduction in *cecal bacillus* levels. This may be because gram-positive bacteria have thicker walls than gram-negative bacteria[15,35]. Ko *et al.*[36], also reported that chlorine dioxide alters the properties of microorganisms and reduces the growing population of microorganisms. During treatment, 3.0, 50 and 100 ppm chlorine dioxide (ClO_2) was used and, as a result, 6.1, 5.8 and 4.5 log CFU/g mold or yeast, 6.7, 6, 5.3 log CFU/g total bacterial counts and 5.6, 5.2 and 4.2 CFU/g coliforms were found, respectively. This result showed a declining trend in the microbial population. Current study also showed that chlorine dioxide treatment also reduced microbial growth. There was a significant decrease in the growth rate of *E. coli*-1, 2 and *S. enteritidis* and *S. Typhimurium* when 60, 100 and 300 ppm ClO_2 solutions were used.

The *E. coli*-1, *E. coli*-2, *S. enteritidis*, *S. typhimurium* were reduced in 60 sec when 60 and 100 ppm ClO_2 was used (Table 9 and 10). The reduction levels were 4.91±0.17, 4.79±0.26, 4.63±0.14, 4.47±0.18 CFU/mL, respectively when 100 ppm ClO_2 was used. The reduction levels were lower when 300 ppm ClO_2 was used (Table 10). It was indicated that ClO_2 reduced bacteria significantly, which were more harmful. So, it was an effective disinfectant for reducing microbial load in broilers.

CONCLUSION

This study indicated that T_4 treatment group (1 mL chlorine dioxide in 3-litre water for 24 hrs) was better than other treatments. It reduced the noxious gas emission (NH_3, H_2S), which caused terrible odour and also harmful to the environment. It also reduced the microbial load effectively by using 300 ppm ClO_2 within a minute. Chlorine dioxide is an excellent alternative disinfectant which has no effect on the production performance of broiler chicken but has significantly reduced noxious gases and microbial load from the environment, which may help to reduce pollution.

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