

Research Article



Efficacy of Feed Sterilization with Ozone in Reducing Microorganisms and Mycotoxins and Improving Some Productive Traits in Broilers

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Abstract | This study investigated the effect of sterilizing feed with different concentrations of ozone (O₃) gas on the presence of microorganisms, mycotoxin concentrations, and various feed characteristics. A total of 225 one-day-old unsexed broiler chicks (Ross-308) were used, distributed randomly into five experimental groups with 45 chicks per group, and each group was further subdivided into three replicates of 15 birds each. The experimental treatments were as follows: T1 served as the control (feed not sterilized with ozone), while T2, T3, T4, and T5 involved feed sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for durations of 5, 10, 15, and 20 minutes, respectively. The results demonstrated that ozone gas effectively inhibited microorganisms in the feed, as indicated by a significant reduction ($P \leq 0.05$) in the logarithm of total bacterial counts and *E. coli* counts in the ozone-treated feeds compared to the control. Furthermore, ozone treatments T4 and T5 resulted in a significant decrease ($P \leq 0.05$) in the concentrations of aflatoxin and ochratoxin compared to the other treatments. Additionally, all ozone gas treatments led to a significant reduction ($P \leq 0.05$) in T-2 toxin concentration compared to the control. In terms of production traits, birds fed with T3 feed showed a significant improvement ($P \leq 0.05$) in average live body weight and weight gain rate compared to the control group (T1). Moreover, a significant improvement ($P \leq 0.05$) in feed conversion efficiency was observed for all ozone treatment groups compared to the control.

Keywords | Poultry, Production, Contamination bacteria, Mycotoxins, Ozone

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INTRODUCTION

Poultry feeds are susceptible to contamination with microorganisms (bacteria or fungi) from a variety of sources including feed ingredients contaminated with plant or animal origin, during processing, transportation or storage or from the poultry fields themselves (Maciorowski et al., 2007). Many fungal species are known to produce mycotoxins in feed during crop growth or after harvest. Contamination with mycotoxins negatively affects a large number of raw materials and finished feeds intended for animal production. Among these, the economic impact on animal production includes the cost of disposing of contaminated feed and the decrease in animal productivity,

where the pollution affects the nutritional value and degree of acceptability of the contaminated feeds (Magnoli et al., 2019). Therefore, this is accompanied by many negative health effects on birds, including the decreases in the rate of feed consumption, weight gain due to feed conversion rate weak, egg production, and resistance weak to infectious diseases, that increasing the mortality rate (Nazarizadeh and Pourreza, 2019; Sineque et al., 2017).

Microorganism-free feed is crucial to maintaining poultry health and ensuring optimal bird performance. Contaminated feeds may cause pathogenic bacteria, fungi, protozoa and viruses to enter the digestive system of poultry, leading to various diseases and infections.

Microbial contamination of feed poses a potential risk to human health as well, which underscores the importance of feed safety for both animal health and public health (Mgbeahuruike et al., 2023).

Generally, different physical, chemical and biological methods are used to protect feed from microbial activities and mycotoxin contamination (Ricke et al., 2019; Conte et al., 2020). One of these methods is sterilization with ozone (O₃) due to the rare properties that ozone possesses in sterilization and a continuous clean reaction (Meher et al., 2023; Valiati et al., 2023). Ozone has gained great attention as a promising technology for food preservation due to its strong oxidizing properties, its spontaneous decomposition into oxygen, and most importantly, it does not leave any residue in food materials (Sarron et al., 2021; Sivaranjani et al., 2021). In recent times, food processing techniques have witnessed a continuous development in the use of ozone as it has been proven to be effective against various microorganisms and a means of extending the shelf life of many foodstuffs (Pandiselvam et al., 2022). Ozone is one of the most powerful oxidizing disinfectants, with a redox potential of 2.07 V. It is 1.5 and 1.3 times stronger than chlorine and hydrogen peroxide, respectively, in its effectiveness against bacteria, viruses, algae, and fungi (Trombete et al., 2016). It has been demonstrated that O₃ is capable of breaking down various cellular components in microorganisms, including the cell wall, cell membrane, and other cellular structures, through oxidation, thereby exerting a lethal effect (Aslam et al., 2020; More and Rao, 2022). Ozone gas has been employed in a multitude of applications within the food sector, including the preservation of fruits, vegetables, liquid foods (such as beverages), spices, grains, meat, poultry, and seafood, as well as the reduction of pesticide residues (Aslam et al., 2022; Khanashyam et al., 2022). The disinfection of food factory equipment (Heacox, 2013), the removal of toxins and micropollutants from wastewater (Bradú et al., 2017; Stylianou et al., 2018), and the disinfection of drinking water (Rosenblum et al., 2012) are further applications of ozone gas. As an alternative to many chemical and therapeutic products, which can sometimes be harmful to human health (Godfray et al., 2010; Winter and Davis, 2006). The ozone sterilization process, which is free of chemicals, is considered an environmentally safe sterilization process and one of the green sterilization techniques (Remondino and Valdenass, 2018). Ozone exhibits strong oxidizing, bacteria- and virus-inhibiting properties and has been widely used in the food industry to eliminate pesticide residues from fresh produce and grains (De Ávila et al., 2017; Savi et al., 2020) and to inhibit toxin-producing molds and degrade mycotoxins (McDonough et al., 2011; de Oliveira et al., 2020). In addition to the possibility of using ozone due to its ability to protect contaminated food and feed and improve food safety (Pandiselvam et al., 2019; Sivaranjani et al., 2021).

This also includes the ability of ozone as an effective grain disinfectant and its efficiency in decomposing mycotoxins and inhibiting microorganisms (Freitas-Silva and Venâncio, 2010). On the other hand, ozone can also be used as a fumigator to prevent insects and pests in stored products with minimal impact on grain quality (Pandiselvam et al., 2019). According to Al-Emara et al. (2021), ozone gas can be a suitable alternative to pesticides, especially fumigants, for the control of storage insects, as ozone can be used as a component of integrated management of storage insect pests. Ozone is also used to inhibit the growth of bacteria and fungi in cereal products (Obadi et al., 2018). Ozone penetrates the surface of the grain by diffusing through the seed coat or through small cracks in the grain (White et al., 2013). Therefore, this study aimed to evaluate the effect of sterilizing feed with different concentrations of ozone gas on the presence of microorganisms (indicated by total and *E. coli* bacteria) and concentrations of mycotoxins in the feed and studying the ozone treatments effect on some productivity traits in broilers.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was conducted for 28 days in the poultry field in the Department of Animal Production, College of Agriculture, University of Basrah during the period from 2022/11/18 to 2022/12/17. A total of 225 broiler birds (Ross-308), unsexed, with average age of one day, were used in this study. Initial body weight was 42 gm. The chicks were raised on a wire floor raised above the ground, where the birds were distributed into 5 experimental treatments (each group containing 45 birds) with 3 replicates, each group 15 birds/replicate. The birds were raised under ideal conditions according to the Ross company manual and were fed on the starter and growth diets (supplied from local markets). The protein and energy content was 23% and 2950 kCal/kg of feed for the starter diet and 21% and 3200 kCal/kg for the growth diet, respectively. The treatments were distributed to be ration not sterilized (T1 control) or four ration sterilized with ozone at Various concentrations.

FEED STERILIZATION WITH OZONE

The ration (starter and growth) used in the experiment was sterilized using an ozone gas generating device (Multifunctional Ozonizer and Fruits and Vegetables Detoxification Washer) with a production capacity of 400 mg/hour. The feed was placed in a closed space (large sterile polyethylene bags), and the tube connected to the device was placed to deliver ozone gas into the tightly closed bags. The sterilization duration was adjusted in accordance with the concentration and time specified in each transaction, as detailed below.

T1: Control group (non-ozonized diets).
T2: The feed was sterilized at a concentration of 33.3 mg/5 kg of ozone for a period of 5 minutes in closed polyethylene bags.
T3: The feed was sterilized at a concentration of 66.6 mg/5 kg of ozone for a period of 10 minutes in closed polyethylene bags.
T4: The feed was sterilized at a concentration of 99.9 mg/5 kg of ozone for a period of 15 minutes in closed polyethylene bags.
T5: The feed was sterilized at a concentration of 133.3 mg/5 kg of ozone for a period of 20 minutes in closed polyethylene bags.

MICROBIAL TESTS

These tests were conducted in the laboratories for graduate studies in the Department of Food Science and the Microbiology Laboratory in the Department of Animal Production, College of Agriculture, University of Basrah. Bacterial enumeration was performed using the Petrifilm method as described by Karomy et al. (2019), which is known to be more accurate and time-efficient compared to traditional enumeration methods, for both total bacteria and *Escherichia coli*. The numbers of total bacteria and *E. coli* were estimated for the feed used in the different treatments using Petrifilm3M membranes according to the instructions of the manufacturer (Oxoid/England). A 1ml of the prepared mixture of Peptone was transferred using a micropipette, planted on Petrifilm membranes and spread over the membranes with light pressure in the middle of the concavity, left for a while to ensure even spreading, then incubated at 37°C for 24-48 hours. The growing-colored colonies were then counted (Blackburn and Mccarthy,2000).

ESTIMATION OF FEED CONTENT OF MYCOTOXINS

As for feed contamination with fungal toxins, mycotoxins (T-2, Ochratoxin, Aflatoxin) concentrations were estimated in the laboratory of the Ministry of Science and Technology in Baghdad. The presence of mycotoxins were estimated using ELISA technology in three feed samples from each experimental treatment (Maqbool et al., 2004).

STATISTICAL ANALYSIS

The experimental data were analyzed by performing (One-way ANOVA) using the computed SPSS (2019) program. Significant differences between the means were evaluated at a significance level (0.05) by Duncan's (1955) multinomial test.

RESULTS AND DISCUSSION

The results shown in Table 1 showed the effect of feed sterilization with different concentrations of ozone gas on the total number of *E. coli* bacteria. There was a significant decrease ($P\leq0.05$) in the average logarithm of the total

bacterial counts in dishes grown with feed samples sterilized with ozone compared to the non-sterilized control treatment. The sterilized sample of T5 with the highest concentration of ozone (133.3 mg/5 kg) recorded the lowest average of the total bacterial numbers i.e., 4.13 cfu/g, which increased with a decrease in the concentration of ozone used in sterilization. Treatments T4, T3, and T2 recorded the average logarithm of the bacterial numbers (4.33, 4.57, and 5. 57 cfu/g), respectively compared to 7.76 cfu/g in the non-sterile (control). The results (Table 1) also showed a significant decrease ($P\leq0.05$) in the average logarithm of the number of *E. coli* bacteria, which also decreased with an increase in the concentration of ozone used. The ozone treatments recorded a clear decrease in the average numbers of *E. coli* bacteria compared to the control treatment, which recorded 3.33 cfu/g, while, the minimum average number was recorded in T5, which allowed only 1.20 cfu/g and did not differ from T3 and the T4 treatments.

Table 1: Effect of sterilizing poultry feed with different concentrations of ozone on the average logarithm of the numbers of total bacteria and *E. coli* bacteria (mean ± standard error).

Treatments (Feed sterilized with ozone)#	Total count of bacteria cfu/g	No. of <i>E. coli</i> cfu/g
T1 (Control)	7.67 ± 0.17 ^a	3.33 ± 0.12 ^a
T2	5.57 ± 0.03 ^b	2.17 ± 0.09 ^b
T3	4.57 ± 0.07 ^c	1.47 ± 0.09 ^c
T4	4.33 ± 0.09 ^{cd}	1.33 ± 0.09 ^c
T5	4.13 ± 0.09 ^d	1.20 ± 0.1 ^c
Significance	*	*

* Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. #T2, T3, T4, and T5 feeds were sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes, respectively.

On the other hand, the use of ozone to sterilize poultry feed led to a significant reduction ($P\leq0.05$) in the percentage of mycotoxins (Aflatoxin, Ochratoxin, and T-2) in the feed content compared to non-sterilized feed (Table 2). The highest percentage of reduction in aflatoxin concentration was recorded with the two highest concentrations of ozone in laboratories T4 and T5 compared to the rest of the experiment's laboratories. The table also shows a significant decrease ($P\leq0.05$) in ochratoxin in the fifth treatment (T5) compared to the rest treatments, being decreased from 0.57 ppb in control treatment to reached 0.03 ppb in T5. Generally, all sterilization treatments also led to a significant decrease ($P\leq0.05$) in the concentration of mycotoxin T-2 compared to the control treatment.

Table 2: Effect of sterilizing poultry feed with different concentrations of ozone on feed content of mycotoxins (mean ± standard error).

Treatments (Feed sterilized with ozone) [#]	Aflatoxin ppb	Ochratoxin ppb	T2 ppb
T1 (Control)	4.77±0.09 ^a	0.57±0.03 ^a	20.90 ^a ±0.15 ^a
T2	4.53±0.12 ^a	0.43± 0.09 ^{ab}	17.73±0.27 ^b
T3	4.50±0.12 ^a	0.37±0.03 ^b	15.50±0.31 ^c
T4	3.93±0.09 ^b	0.30±0.00 ^b	16.00±0.15 ^c
T5	3.40±0.06 ^c	0.03±0.03 ^c	15.97±0.09 ^c
Significance	*	*	*

* Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. [#]T2, T3, T4, and T5 feeds were sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes, respectively.

As for the effect of feed sterilization treatments on bird growth and health indicators, the results (Table 3) showed a clear effect of treating feed with different concentrations of ozone gas on the average live body weight (grams/bird). The treatments did not differ in average live body weight during the first week, while significant differences ($P\leq0.05$) were recorded in the second week of the experiment, where

the T3 treatment was significantly superior ($P\leq0.05$) in compared to the control treatment, while the other sterilization treatments did not differ from the control treatment. A significant superiority ($P\leq0.05$) was observed for the T3 treatment in the third and fourth weeks over the control treatment, with a body weight rate of (1001 vs. 959 and 1793.33 vs. 1695 gm/bird), and it did not differ from the rest sterilization treatments, in compared to control.

There were no clear effects for treatments on the weekly and total weight gain rate (g/bird) of broilers (Table 4) for the first, third, and fourth weeks. While, results showed that the sterilization treatment of 66.6 mg/5 kg ozone (T3) led to a significant increase in the rate of weekly weight gain in the second week, being 334.33 g/bird, as compared with ($P\leq0.05$) the control treatment (302.89 g/bird) and the treatment T4 (298.33 g/bird). While, the rest treatments did not show a significant difference in the rate of weekly weight gain compared to the third treatment and the control treatment. A significant superiority ($P\leq0.05$) was also recorded for the T3 treatment in the rate of total weight gain, which recorded 1751.33 g/bird compared to the control, which resulted in 1653 g/bird, while the other treatments did not differ compared to the T3 or the control treatments.

Table 3: Effect of feed sterilization with ozone on broilers live body weight (g/bird) (means ± standard error).

Treatments (Feed sterilized with ozone) [#]	Week 1	Week 2	Week 3	Week 4
T1 (Control)	193.11±2.18	496.00±14.11 ^b	959.00±4.58 ^b	1695.00±30.55 ^b
T2	184.22±4.87	494.99±6.86 ^b	986.67±17.14 ^{ab}	1735.00±27.84 ^{ab}
T3	193.89±5.46	528.22±11.23 ^a	1001.00±9.54 ^a	1793.33±42.26 ^a
T4	186.33±2.85	484.67±2.40 ^b	967.00±6.08 ^{ab}	1730.00±16.07 ^{ab}
T5	185.22±1.44	501.55±5.39 ^{ab}	990.00±16.86 ^{ab}	1710.00±18.03 ^{ab}
Significance	N. S	*	*	*

Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. [#]T2, T3, T4, and T5 feeds were sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes, respectively.

Table 4: Effect of feed sterilization with different concentrations of ozone on weekly and total broiler weight gain, g/ bird (means ± standard error).

Treatments (Feed sterilized with ozone) [#]	Weekly weight gain g/bird				Total weight gain
	Week 1	Week 2	Week 3	Week 4	
T1 (Control)	151.11± 2.19	302.89±11.95 ^b	463.00±12.17	736.00±31.53	1653.00±30.55 ^b
T2	142.22±4.87	310.78±2.51 ^{ab}	491.67±12.34	748.33±40.81	1693.00±27.84 ^{ab}
T3	151.89 5.46	334.33± 9.84 ^a	472.78±20.07	792.33±51.66	1751.33±42.26 ^a
T4	144.33±2.85	298.33±2.96 ^b	482.33±3.76	763.00±10.02	1688.00±16.07 ^{ab}
T5	143.22±1.44	316.34±3.95 ^{ab}	488.45±11.57	720.00±23.12	1668.00±18.03 ^{ab}
Significance	N. S	*	N. S	N. S	*

*Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. [#]T2, T3, T4, and T5 feeds were sterilized with ozosne at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes, respectively.

Table 5: Effect of feed sterilization with different concentrations of ozone on weekly and total broiler feed consumption, g feed/bird (means ± standard error).

Treatments (Feed sterilized with ozone) [#]	Weekly feed consumption g feed/bird				Total consumed feed (g feed/bird)
	Week 1	Week 2	Week 3	Week 4	
T1 (Control)	181.72±5.94 ^{ab}	558.33±11.35 ^a	640.20±13.25	864.00±35.02	2244.25±61.86
T2	174.97±5.06 ^{ab}	377.67±7.17 ^b	659.67±2.19	893.17±44.48	2105.47±45.58
T3	182.92± 5.61 ^a	398.67± 4.84 ^b	659.07±7.53	892.93±64.14	2133.59±73.60
T4	160.24±11.06 ^b	380.00±10.69 ^b	657.00±2.65	884.33±12.20	2081.58±36.33
T5	181.64±1.88 ^{ab}	394.66±10.17 ^b	659.23±0.96	876.33±24.06	2111.87±16.77
Significance	*	*	N. S	N. S	N. S

*Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. [#]T2, T3, T4, and T5 feeds were sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes respectively.

Table 6: Effect of feed sterilization with different concentrations of ozone on weekly and total broiler feed conversion efficiency (g feed/g weight gain) (means ± standard error).

Treatments (Feed sterilized with ozone) [#]	Weekly feed conversion efficiency g feed/g weight gain				Total feed conversion efficiency g feed/g weight gain
	Week 1	Week 2	Week 3	Week 4	
T1 (Control)	1.20±0.03	1.85±0.04 ^a	1.35±0.04	1.19±0.05	1.36±0.02 ^a
T2	1.23±0.02	1.22±0.03 ^b	1.27±0.03	1.17±0.07	1.24±0.01 ^b
T3	1.20±0.01	1.19± 0.04 ^b	1.33±.06	1.11±0.07	1.22±0.02 ^b
T4	1.11±0.01	1.27±0.03 ^b	1.30±0.01	1.14±0.02	1.23±0.02 ^b
T5	1.27±0.02	1.25±0.03 ^b	1.28±0.03	1.21±0.04	1.27±0.01 ^b
Significance	N. S	*	N. S	N. S	*

*Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($P\leq0.05$). N.S: Not significant ($P>0.05$). Values are means of 3 replications (±) standard deviation. [#]T2, T3, T4, and T5 feeds were sterilized with ozone at concentrations of 33.3, 66.6, 99.9, and 133.3 mg/5 kg, for 5, 10, 15, and 20 minutes, respectively.

Regarding the effect of sterilizing feed with ozone on the weekly and total amount of feed consumed (g feed/bird), the results indicate that there are significant differences ($P\leq 0.05$) in the average amount of feed consumed during the first week of the experiment (Table 5), where the T3 treatment recorded the highest amount of consumed feed (182.92 g feed/bird) compared to the T4 treatment, which consumed the least amount of feed (160.24 g feeds/bird). The other experimental treatments did not differ in the average amount of feed consumed compared to the T3 and T4 treatments at the same period. However, in the second week, it was noted that the control treatment recorded the highest amount of feed consumption with significant differences ($P\leq0.05$) compared to all other treatments, (consuming feed of 558.33 g feed/bird). All treatments at the third and fourth weeks did not differ significantly for the average of total amount of feed consumed.

The results of Table 6 showed the effect of feed sterilization with different ozone concentrations on the average of weekly and total feed conversion efficiency (g feed/g weight gain) of broilers. In general, all treatments did not differ during the first, third, and fourth weeks, while a significant improvement ($P\leq0.05$) was recorded in sthe second week for all feed ozone sterilization treatments compared to the

control group. The feed conversion efficiency value was the highest for the control treatment being 1.85 g feed/g weight gain during the second week and 1.36 g feed/g weight gain for the total period of the experiment.

The results indicate that ozone is effective in inhibiting both total bacterial counts and *E. coli* present in the feed. Ozone acts as a potent and versatile oxidizing agent capable of destroying microbial cells through several key mechanisms. Specifically, ozone oxidizes intracellular proteins, including enzymes, peptides, biosynthetic proteins, and carrier proteins that facilitate intercellular transport, breaking them down into smaller peptides. Additionally, ozone oxidizes polyunsaturated fatty acids, which are essential components of the lipopolysaccharide layer in the cell membrane of Gram-negative bacteria, converting them into acidic peroxides. This oxidative damage compromises the integrity of the cell membrane by forming pores, leading to the leakage of vital cellular components and ultimately resulting in cell death (Bhati et al., 2024).

The present study also showed that the effectiveness of ozone in destroying fungal toxins, which is attributed to the strong oxidizing properties of ozone that enable it to destroy

fungal toxins through the oxidation process. When ozone comes into contact with mycotoxins, it interacts with the chemical bonds in the toxin molecules and causes them to break down. This oxidation process involves the transfer of oxygen atoms from ozone to mycotoxin molecules, leading to structural changes that decompose the mycotoxins into smaller, less toxic compounds. Oxidative reactions initiated by ozone can break double bonds, disrupt aromatic rings, and modify functional groups within mycotoxin molecules, making the latter less harmful or toxic (Conte et al., 2020). The antimicrobial properties of ozone can also target fungi and molds responsible for producing mycotoxins and inhibit the growth and activity of these microorganisms, thus reducing mycotoxin levels in feed (Torlak et al., 2016). These results are consistent with Celik et al. (2021) which indicated the effectiveness of ozone gas in inhibiting the microbial presence in poultry feed. They also explained that the greater concentration and duration of ozone, has greater effectiveness in sterilization. Exposure to ozone for a longer period at a higher concentration led to more effective inhibition of microorganisms (Brodowska et al., 2018).

The improvement of productive characteristics (body weight, weight gain, feed conversion efficiency) in the ozone treatments in the current study can be attributed to the role of ozone in inhibiting microorganisms in feed (Celik et al., 2021; Torlak et al., 2016). Consequently, this led to improve the balance of microorganisms in the intestine, which led to the competitive exclusion of harmful microorganisms present in the feed that absorb nutrients. It also led to enhancing the presence of beneficial microorganisms, thus increasing the birds' benefit from the amount of feed consumed and improving the process of digestion, metabolism and absorption. This is evident in the improvement of body weight, weight gain, and food conversion efficiency in the ozone treatments under study. In line with the results of current study El-Badawi et al. (2015) found an improvement in the average final body weight, average daily weight gain, and the efficiency of feed conversion when sterilizing the drinking water with ozone gas for New Zealand rabbits. However, this is contrary to previous results of Amwele (2004), who did not record any improve in the productive characteristics of broilers when sterilizing the drinking water with ozone gas. On the other hand, McKenzie et al. (1998) indicated an improvement in the quality of corn contaminated with aflatoxin after sterilization with ozone, but there were no significant differences in total weight gain in turkey birds between the control treatment and groups treated with ozone gas. However, the total weight gain of the treatments that consumed corn contaminated with mycotoxins decreased, which proves the effectiveness of ozone in inhibiting mycotoxins and its lack of impact on bird production. Eshak et al. (2013) indicated that

the use of ozone-sterilized diets for feeding animals and conducting biological examinations on these animals is still very limited or has not yet been used in practice. Therefore, this study may be considered one of the limited studies that dealt with the use of ozone gas to sterilize broiler feed and its impact on public health and the birds' productive performance.

CONCLUSIONS AND RECOMMENDATIONS

This study concluded that treating poultry feed with ozone effectively inhibits microorganisms that may contaminate the feed at any stage of poultry production. Ozone treatments also demonstrated efficacy in degrading mycotoxins and reducing their concentrations in the treated diet compared to the untreated control. Furthermore, the study found that ozone gas treatment improved the productive performance of broiler chickens, as evidenced by increases in live body weight, weight gain, and feed conversion efficiency. These results suggest that ozone gas is a viable, environmentally friendly technology for sterilizing poultry feed without adversely affecting poultry health.

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AUTHOR'S CONTRIBUTION

Alfred Sulaka and Qutiaba Jasim: Designed the study and collected the data.
Zahraa Ahmed: Completed the statistical analysis of the data and wrote the research paper.

ETHICAL APPROVAL

Ethical approval (Approval no. AEC :4372024) of study was obtained by the Animal Ethical Committee of the Faculty of Veterinary Medicine, University of Basrah, Iraq.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Al-Emara, M.S., Alyousuf, A.A., Abass, M.H. (2021). Efficacy of ozone gas against all stages of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) at different temperatures and exposure periods. *Basrah J. Agric. Sci.*, 34(2): 240-252. <https://doi.org/10.37077/25200860.2021>.

34.2.18

- Amwele, H.R. (2004). The effect of ozone on the production of broiler (Doctoral dissertation, Nelson Mandela Metropolitan University).
- Aslam, R., Alam, M.S., Pandiselvam, R. (2022). Aqueous ozone sanitization system for fresh produce: Design, development, and optimization of process parameters for minimally processed onion. *Ozone: Sci. Eng.*, 44(1): 3-16. <https://doi.org/10.1080/01919512.2021.1984206>
- Aslam, R., Alam, M.S., Saeed, P.A. (2020). Sanitization potential of ozone and its role in postharvest quality management of fruits and vegetables. *Food Eng. Rev.*, 12: 48-67. <https://doi.org/10.1007/s12393-019-09204-0>
- Bhati, D., Singh, A., Kaur, G. (2024). Ozone technology in food disinfection. In *Emerging Techniques for Food Processing and Preservation* (pp. 83-120). CRC Press. <https://doi.org/10.1201/9781003217138-4>
- Blackburn, C.D.E., McCarthy, J.D. (2000). Modifications to methods for the enumeration and detection of injure *Escherichia coli* O157:H7 in foods. *Int. J. Food Microbiol.*, 55: 285-290. [https://doi.org/10.1016/S0168-1605\(00\)00205-1](https://doi.org/10.1016/S0168-1605(00)00205-1)
- Bradru, C., Magureanu, M., Parvulescu, V.I. (2017). Degradation of the chlorophenoxyacetic herbicide 2, 4-D by plasma-ozonation system. *J. Hazard. Mater.*, 336: 52-56. <https://doi.org/10.1016/j.jhazmat.2017.04.050>
- Brodowska, A.J., Nowak, A., Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Crit. Rev. Food Sci. Nutr.*, 58(13): 2176-2201. <https://doi.org/10.1080/10408398.2017.1308313>
- Celik, O., Tirpanci S.G., Okur, A.A. (2021). Gaseous ozone application on microbial properties of broiler feeds. *Ital. J. Anim. Sci.*, 20(1): 1094-1102. <https://doi.org/10.1080/1828051X.2021.1945960>
- Conte, G., Fontanelli, M., Galli, F., Cotrozzi, L., Pagni, L., Pellegrini, E. (2020). Mycotoxins in feed and food and the role of ozone in their detoxification and degradation: An update. *Toxins*, 12(8): 486. <https://doi.org/10.3390/toxins12080486>
- De Ávila, M.B., Faroni, L.R.A., Heleno, F.F., de Queiroz, M.E.L., Costa, L.P. (2017). Ozone as degradation agent of pesticide residues in stored rice grains. *J. Food Sci. Technol.*, 54: 4092-4099. <https://doi.org/10.1007/s13197-017-2884-1>
- de Oliveira, J.M., de Alencar, E.R., Blum, L.E.B., de Souza Ferreira, W.F., Botelho, S.D.C.C., Racanicci, A.M.C., da Silva, C.R. (2020). Ozonation of Brazil nuts: Decomposition kinetics, control of *Aspergillus flavus* and the effect on color and on raw oil quality. *LWT*, 123: 109106. <https://doi.org/10.1016/j.lwt.2020.109106>
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1): 1-42. <https://doi.org/10.2307/3001478>
- El-Badawi, A.Y., Yacout, M.H.M., Hafsa, S.H.A., Hassan, A.A. (2015). Application of ozone treatments on growing male rabbits.
- Eshak, M.G., Deabes, M.M., Farrag, A.H., Farag, I.M., Stino, F.K. (2013). Effect of ozone-treated aflatoxin contaminated diets on DNA damage, expression of androgen and androgen receptor genes, and histopathological changes in Japanese quail. *Glob. Vet.*, 11(1): 01-13.
- Freitas-Silva, O., Venâncio, A. (2010). Ozone applications to prevent and degrade mycotoxins: A review. *Drug Metab. Rev.*, 42(4): 612-620. <https://doi.org/10.3109/03602532.2010.484461>
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, 327(5967): 812-818. <https://doi.org/10.1126/science.1185383>
- Heacox, D. (2013). U.S. Patent No. 8,609,120. Washington, DC: U.S. Patent and Trademark Office.
- Karomy, A.S., Gheni, Q.J., George, S.S. (2019). Effect of the frequency of the microsafe spraying on the production performance and the microbial presence in mouth and vent of broiler chickens. *J. Pure Appl. Microbiol.*, 13(3): 1543-1548. <https://doi.org/10.22207/JPAM.13.3.26>
- Khanashyam, A.C., Shanker, M.A., Kothakota, A., Mahanti, N.K., Pandiselvam, R. (2022). Ozone applications in milk and meat industry. *Ozone: Sci. Eng.*, 44(1): 50-65. <https://doi.org/10.1080/01919512.2021.1947776>
- Maciorowski, K.G., Herrera, P., Jones, F.T., Pillai, S.D., Ricke, S.C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. *Anim. Feed Sci. Technol.*, 133(1-2): 109-136. <https://doi.org/10.1016/j.anifeedsci.2006.08.006>
- Magnoli, A.P., Poloni, V.L., Cavaglieri, L. (2019). Impact of mycotoxin contamination in the animal feed industry. *Curr. Opin. Food Sci.*, 29: 99-108. <https://doi.org/10.1016/j.cofs.2019.08.009>
- Maqbool, U., Ahmad, M., Anwar-ul-Haq, Iqbal, M.M. (2004). Determination of aflatoxin-B1 in poultry feed and its components employing enzyme-linked immunosorbent assay (ELISA). *Toxicol. Environ. Chem.*, 86(4): 213-218. <https://doi.org/10.1080/02772240400007138>
- McDonough, M.X., Mason, L.J., Woloshuk, C.P. (2011). Susceptibility of stored product insects to high concentrations of ozone at different exposure intervals. *J. Stored Prod. Res.*, 47(4): 306-310. <https://doi.org/10.1016/j.jspr.2011.04.003>
- McKenzie, K.S., Kubena, L.F., Denvir, A.J., Rogers, T.D., Hitchens, G.D., Bailey, R.H., Phillips, T.D. (1998). Aflatoxicosis in turkey poults is prevented by treatment of naturally contaminated corn with ozone generated by electrolysis. *Poult. Sci.*, 77(8): 1094-1102. <https://doi.org/10.1093/ps/77.8.1094>
- Meher, P., Deshmukh, N., Mashalkar, A., Kumar, D. (2023). Ozone (O3) generation and its applications: A review. In *AIP Conf. Proc.* AIP Publishing. 2764(1). <https://doi.org/10.1063/5.0144316>
- Mgbeahuruike, A.C., Agina, O.A., Anyanwu, M.U., Emejuo, N.T., Ekere, S.O., Ugwu, P.C., Andong, F.A. (2023). Microbial contamination of poultry feed and the effects on birds' performance. *Anim. Res. Int.*, 20(1): 4834-4861. <https://www.ajol.info/index.php/ari/article/view/247059>
- More, S., Rao, T.R. (2022). Photosensitization mediated retention of nutritional quality of banana during its postharvest storage. *J. Hortic. Sci. Biotechnol.*, 97(3): 361-375. <https://doi.org/10.1080/14620316.2021.1997099>
- Nazarizadeh, H., Pourreza, J. (2019). Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B1 in growing broilers. *J. Appl. Anim. Res.*, 47(1): 135-139. <https://doi.org/10.1080/09712119.2019.1584106>
- Obadi, M., Zhu, K.X., Peng, W., Sulieman, A.A., Mohammed, K., Zhou, H.M. (2018). Effects of ozone treatment on the physicochemical and functional properties of whole-grain flour. *J. Cereal Sci.*, 81: 127-132. <https://doi.org/10.1016/j.jcs.2018.04.008>

- Pandiselvam, R., Singh, A., Agriopoulou, S., Sachadyn-Krol, M., Aslam, R., Lima, C.M.G., Khaneghah, A.M. (2022). A comprehensive review of impacts of ozone treatment on textural properties in different food products. *Trends Food Sci. Technol.*, 127: 74-86. <https://doi.org/10.1016/j.tifs.2022.06.008>
- Pandiselvam, R., Subhashini, S., Banuu Priya, E.P., Kothakota, A., Ramesh, S.V., Shahir, S. (2019). Ozone based food preservation: A promising green technology for enhanced food safety. *Ozone: Sci. Eng.*, 41(1): 17-34. <https://doi.org/10.1080/01919512.2018.1490636>
- Remondino, M., Valdenassi, L. (2018). Different uses of ozone: environmental and corporate sustainability. *Literature review and case study. Sustainability*, 10(12): 4783. <https://doi.org/10.3390/su10124783>
- Ricke, S.C., Richardson, K., Dittoe, D.K. (2019). Formaldehydes in feed and their potential interaction with the poultry gastrointestinal tract microbial community. A review. *Front. Vet. Sci.*, 6: 188. <https://doi.org/10.3389/fvets.2019.00188>
- Rosenblum, J., Ge, C., Bohrerova, Z., Yousef, A., Lee, J. (2012). Ozonation as a clean technology for fresh produce industry and environment: Sanitizer efficiency and wastewater quality. *J. appl. Microbiol.*, 113(4): 837-845. <https://doi.org/10.1111/j.1365-2672.2012.05393.x>
- Sarron, E., Gadonna-Widehem, P., Aussenac, T. (2021). Ozone treatments for preserving fresh vegetables quality: A critical review. *Foods*, 10(3): 605. <https://doi.org/10.3390/foods10030605>
- Savi, G.D., Gomes, T., Canever, S.B., Feltrin, A.C., Piacentini, K.C., Scussel, R., Angioletto, E. (2020). Application of ozone on rice storage: A mathematical modeling of the ozone spread, effects in the decontamination of filamentous fungi and quality attributes. *J. Stored Prod. Res.*, 87: 101605. <https://doi.org/10.1016/j.jspr.2020.101605>
- Sineque, A.R., Macuamule, C.L., Dos Anjos, F.R. (2017). Aflatoxin B1 contamination in chicken livers and gizzards from industrial and small abattoirs, measured by ELISA technique in Maputo, Mozambique. *Int. J. Environ. Res. Publ. Health*, 14(9): 951. <https://doi.org/10.3390/ijerph14090951>
- Sivaranjani, S., Prasath, V.A., Pandiselvam, R., Kothakota, A., Khaneghah, A.M. (2021). Recent advances in applications of ozone in the cereal industry. *LWT*, 146: 111412. <https://doi.org/10.1016/j.lwt.2021.111412>
- SPSS, I. (2019). *Statistics for Macintosh (Version 26.0) [Computer Software]*. IBM Corp, Armonk, N.Y.
- Stylianou, S.K., Katsoyiannis, I.A., Mitrakas, M., Zouboulis, A.I. (2018). Application of a ceramic membrane contacting process for ozone and peroxone treatment of micropollutant contaminated surface water. *J. Hazard. Mater.*, 358: 129-135. <https://doi.org/10.1016/j.jhazmat.2018.06.060>
- Torlak, E., Akata, I., Erci, F., Uncu, A.T. (2016). Use of gaseous ozone to reduce aflatoxin B1 and microorganisms in poultry feed. *J. Stored Prod. Res.*, 68: 44-49. <https://doi.org/10.1016/j.jspr.2016.04.003>
- Trombete, F., Freitas-Silva, O., Saldanha, T., Venâncio, A., Fraga, M.E. (2016). Ozone against mycotoxins and pesticide residues in food: Current applications and perspectives.
- Valiati, B.S., Domingos, M.M., Lepaus, B.M., Faria-Silva, L., de São José, J.F.B. (2023). Emerging green technologies for decontamination of fresh produce. *Green Prod. Food Saf.*, Academic Press. pp. 179-224. <https://doi.org/10.1016/B978-0-323-95590-4.00008-4>
- White, S.D., Murphy, P.T., Leandro, L.F., Bern, C.J., Beattie, S.E., van Leeuwen, J.H. (2013). Mycoflora of high-moisture maize treated with ozone. *J. Stored Prod. Res.*, 55: 84-89. <https://doi.org/10.1016/j.jspr.2013.08.006>
- Winter, C.K., Davis, S.F. (2006). Organic foods. *J. Food Sci.*, 71(9): R117-R124. <https://doi.org/10.1111/j.1750-3841.2006.00196.x>