

CONFERENCE PAPER

EFFICIENCY OF STABLE LIQUIDE CHLORINE DIOXIDE (ClO₂) IN SANITATION OF WATER DISTRIBUTION SYSTEM IN POULTRY PRODUCTION-SYNTHESIS OF THEORETICAL AND EXPERIMENTAL FINDINGS

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This paper was presented at the 10th International Congress on Advances in Veterinary and Technics (ICAVST 2025) in Sarajevo, Bosnia and Herzegovina.

How to cite this article:

Ališah A, Kapo-Dolan N, Pivić A, Bejdić P, Trifković J, Gagić A. 2025.

Efficiency of stable liquide chlorine dioxide (ClO₂) in sanitation of water distribution system in poultry production- synthesis of theoretical and experimental findings. Veterinaria, 74Suppl(1), 39-47.

ABSTRACT

Modern veterinary practice faces growing challenges in the field of biosecurity, especially in intensive livestock and poultry production. One of the most underestimated, yet critical, factors in maintaining the health status of animals is the quality of drinking water and the hygienic safety of the water distribution system. Although in everyday veterinary practice, the leading causes of health disorders are often attributed to diet, environmental factors, or genetic potential, numerous cases indicate that water is a key vector of microbial contamination, primarily through biofilm structures that form in internal water distribution systems. Due to the increasing incidence of resistant bacterial strains and the limited effectiveness of conventional disinfectants, such as chlorine, there is a growing need for new, safer, and more effective sanitation methods.

Stabilised liquid chlorine dioxide (ClO₂) is emerging as a potent alternative to conventional agents. This paper represents a synthesis of experimental and review findings related to the use of stabilised liquid chlorine dioxide for the rehabilitation of the drinking system in laying hens suffering from colisepticemia. Through a case study of a farm with a recorded problem of systemic infection caused by *Escherichia coli*, the causes of contamination, remediation measures, as well as the effects achieved in terms of biofilm reduction, mortality and microbiological load of water were analyzed. Along with the case report, the paper analyzes and expands the context of the effectiveness of stabilised liquid chlorine dioxide, based on the literature and previous studies. Stabilised ClO₂, in contrast to its gaseous form, offers safer handling, a prolonged residual protection effect, and minimal toxicity.

The paper emphasises the importance of microbiological control and improved sanitation of water supply systems in poultry production, and highlights stabilised liquid chlorine dioxide as a highly effective tool in the fight against biofilm and resistant bacterial strains.

Keywords: Animal production, disinfection, prevention, stable liquid chlorine dioxide

INTRODUCTION

Effective control of pathogenic microorganisms is essential for maintaining animal health and ensuring food safety in modern animal production (Gagić, 1988; Gagić, 2000; Asaj, 2003;). Within biosecurity measures, disinfection plays a crucial role, focusing on reducing microbiological contamination in facilities, equipment, water, and the environment. Despite its importance, disinfection is often treated as a routine and technically simple task, leading to an underestimation of its complexity and the key factors that determine its success. As a result, commonly used disinfectants, such as chlorine and its compounds, continue to dominate practice, even though evidence suggests their limited efficacy, particularly in the presence of biofilms and organic matter. (Gagić et al, 2013; Ališah, 2020.)

Biofilm represents a sophisticated organizational form of microbial communities that adhere to both biotic and abiotic surfaces, enveloped in a self-produced polysaccharide matrix. This structure enables microorganisms to survive extreme conditions and develop resistance to antimicrobials and the host's immune response. In poultry production, biofilm formation within water distribution systems creates a persistent source of contamination in drinking water, thereby directly jeopardizing animal health, increasing mortality rates, and impairing productivity. (Ališah, 2020; Pivić, 2025.) A significant challenge is that water, a key vector for pathogen transmission, is often neglected in veterinary diagnostics and therapy. Health issues in animals are more frequently attributed to factors like inadequate nutrition, housing, or hygiene, while water quality and the sanitation of drinking systems are not analyzed until chronic health problems arise. This oversight not only delays problem resolution but can also lead to unnecessary and ineffective use of antibiotics and additives, exacerbating the risk of antimicrobial resistance and leaving residues in animal products. (Gagić et al, 2013; Hadžiabdić et al, 2013, Mujaković et al, 2022.)

Given the limitations of conventional disinfectants

and the complexity of biofilm infections, modern research is increasingly focusing on stabilized liquid chlorine dioxide (ClO_2) - a broad-spectrum oxidant that, unlike its gaseous form, can be safely and effectively used in various sanitary applications. ClO_2 possesses exceptional antimicrobial properties, including the ability to eradicate biofilm and destroy spores and viruses resistant to other agents. Its selective oxidation mechanism, prolonged effectiveness in aquatic systems, and absence of toxic by-products make it an ideal candidate for contemporary disinfection practices in veterinary and livestock operations. (Gagić, 2000; Plavšić, 2011.)

This paper presents the results of a study conducted on a laying hen farm where a mass outbreak of colisepticemia occurred due to contamination in the feeding system. (Gagić et al, 2013.) After conventional measures, including water chlorination and antibiotic treatments, yielded little to no results, a remediation program utilizing stabilized liquid chlorine dioxide was implemented. The methodology includes diagnostics of the system (such as ATP luminometry and cultural isolation), application of ClO_2 at various concentrations and time intervals, and monitoring outcomes through measurement of microbial load and flock mortality.

Additionally, the paper offers a theoretical analysis of the properties of ClO_2 , its advantages over traditional disinfectants, and modern disinfection principles, including the stages of mechanical cleaning, sanitary washing, and proper application techniques (Ališah, 2020.). The goal is to provide an integrated model for disinfecting water supply systems that can be applied in real production conditions without disrupting the production process. Also, this work aims to enhance biosecurity practices in the poultry industry while addressing global challenges related to food safety, antimicrobial resistance, and public health.

Disinfection as a complex veterinary and hygienic procedure

Disinfection, defined as the set of measures aimed at destroying, reducing, or inactivating pathogenic

microorganisms on non-living surfaces, is a fundamental component of biosecurity in livestock and poultry production (Asaj, 2003.) Unlike sterilization, which involves the complete destruction of all microorganisms, disinfection seeks to reduce the microbiological burden to a level that does not jeopardize animal health or the safety of animal-origin products. (Gagić, 1988).

However, disinfection procedures are often carried out routinely without sufficient professional understanding of the prerequisites, limitations, and optimal methods of application. This approach significantly diminishes the effectiveness of disinfection measures, contributes to the development of microbial resistance, and leads to the inefficient use of disinfectants. (Ališah, 2020; Ališah et al, 2023.)

The classic process of disinfection consists of three main phases: mechanical cleaning, sanitary washing, and the application of disinfectants. Mechanical cleaning removes up to 90% of microorganisms from the target surface. Sanitary washing is performed with warm water and detergents, further reducing organic matter and making pathogenic microorganisms more susceptible to chemical agents. Skipping these preparatory steps in favor of “2-in-1” or “3-in-1” commercial products often results in ineffective disinfection, particularly in the presence of biofilm. (Asaj, 2003; Gagić, 2012.)

The Problem of Biofilm in Water Supply

A major challenge in modern water supply systems is the presence of biofilm: a complex microbial community that forms on the inner surfaces of pipelines, drinkers, and reservoirs. Biofilm consists of microorganisms (commonly bacteria from the Enterobacteriaceae family) (Ališah, 2020; Pivić, 2025), embedded in a self-produced polysaccharide matrix that shields them from disinfectants, antibiotics, and the host's immune system. Additionally, biofilm facilitates the horizontal transfer of genes, including those responsible for antimicrobial resistance, complicating treatment responses.

In poultry production, biofilm in animal watering systems can continuously introduce pathogenic microflora, such as *Escherichia coli*, *Salmonella spp.*, *Pseudomonas spp.*, and other opportunistic bacteria. These pathogens enter the body through drinking water, leading to respiratory, digestive, or systemic infections such as colisepticemia. This results in increased mortality, reduced egg production, and higher antibiotic usage. (Hadžiabdić et al, 2013.)

Limitations of Classic Disinfectants: Chlorine and Chloramine

Chlorine and its compounds (chlorine lime, caporite, chloramines) have been utilized for decades as primary disinfectants in livestock and community hygiene. While their application is simple, cost-effective, and efficient under certain conditions, they also come with several limitations. Chlorine is highly reactive in the presence of organic matter, leading to the formation of by-products such as trihalomethanes and chloramines, which are toxic and potentially carcinogenic. Moreover, at a pH greater than 7.5, chlorine's efficiency declines significantly, and its ability to eliminate biofilm is limited due to its inability to penetrate the polysaccharide matrix. (Plavšić, 2011; Gagić et al, 2013.)

When in contact with metals, chlorine can exhibit strong corrosive effects, posing additional technical challenges in systems with metal components. Its use in confined spaces also requires caution because of its potential toxicity to both operators and animals.

Stabilized Liquid Chlorine Dioxide (ClO₂): A Modern Broad-Spectrum Oxidant

In response to the shortcomings of conventional disinfectants, stabilized liquid chlorine dioxide (ClO₂) has been developed as an innovative solution that combines high biocidal efficacy with safety in application. Unlike the gaseous form of ClO₂, which is unstable and potentially explosive, the stabilized liquid formulation allows for easy storage, transport, and application without the need for additional protective measures. (Gagić et

al, 2013.)

ClO_2 acts as a selective oxidant, transferring one electron to electron-rich centers within the organic molecules of microorganisms. This reaction destroys cell membranes, denatures proteins, and inactivates enzymes, all without generating harmful by-products. (Mujaković et al, 2022).

ClO_2 is particularly effective at dismantling biofilm structures, as it can oxidize both the matrix and the cells within it. Its stability across a wide pH range (1.5-10), along with its non-toxicity and long-lasting residual action in drinking water, make it highly suitable for use in livestock facilities without disrupting production. Furthermore, ClO_2 does not produce chlorinated by-products or leave any unpleasant odor or taste in water and food. (Ališah et al, 2023.)

Additionally, ClO_2 demonstrates virucidal and sporicidal properties that exceed those of chlorine, and its application has been documented in various industries, from wastewater and swimming pool treatment to the decontamination of spaces contaminated with *Bacillus anthracis* spores. (Gagić, 2000; Hadžiabdić et al, 2013.)

Location of the Experiment and Research Objectives

The experimental study was conducted on a commercial farm dedicated to breeding laying hens for table eggs, located in the central part of Bosnia and Herzegovina. The facility is a closed-type operation, featuring two lines of automated six-storey cages manufactured by Salmat, with a total capacity of 7,800 Lohmann Brown laying hens. The feeding system utilizes nipple drinkers, providing a continuous flow of water ad libitum, while the ventilation system is automated and designed to accommodate a maximum capacity of 3.0 m³/h/kg of live weight.

The facility sources groundwater from its own catchment area, which includes a protective tank with a volume of 7,000 liters. Water from the reservoir is delivered to the building via a 75-meter-long supply line. A critical epidemiological concern was identified in the immediate vicinity,

as there were two pig fattening facilities located above the catchment area, which posed a risk of fecal contamination to the reservoir (Gagić et al, 2013.)

Experimental Subjects and Health Status

At the time of the observed health issues, the farm housed 7,800 three-month-old laying hens. By the end of February, their health and growth rates were above the standard for this genetic line. However, beginning on February 22, there was a sudden increase in daily mortality, with as many as 50 hens dying each day, exhibiting symptoms of systemic coli-septicemia. Initial treatment included antibiotic therapy (non-resorptive antibiotics) and vitamin supplementation (AD₃E and amino acids), but there was no clinical improvement.

Pathoanatomical and microbiological examinations confirmed the presence of *Escherichia coli* in the parenchymatous organs and a positive coli titer in the drinking water.

Methodology and Phases of the Experiment

The research protocol was divided into four phases with the following goals:

1. Confirming that the water and water distribution system were the source of the infection.
2. Sanitizing the system using stabilized ClO_2 .
3. Establishing a permanent disinfection program.
4. Implementing parallel anti-stress therapies.

Phase I – Diagnostic Evaluation

Water and biofilm samples were collected at three checkpoints:

- Accumulation tank
- Entrance shaft (external system)
- Ends of the pipes in each of the cages (internal network)

The following methods were employed:

- ATP luminometer (Charm Sciences Inc., USA) for bioluminescent quantification of contamination in relative light units (RLU).

- Microbiological culture on selective media for the identification of *E. coli*.

Samples were collected at the following times:

1. Before any treatment
2. 5 and 15 minutes after rinsing the system with clean water
3. After each treatment with ClO_2 .

Phase II – Initial Sanitation with Stabilized Liquid ClO_2

Double disinfection of the internal system was performed:

- Shock treatments at night with 4 ml of stabilized liquid ClO_2 per liter of water (4‰) for five consecutive nights.
- Daily prophylactic treatments with 2 ml/l (2‰) via water medication for five days.

After each shock treatment, the system was emptied and filled with a new solution.

Phase III – Sanitation Maintenance Program

A monthly and continuous regimen was established, including:

- Permanent disinfection of the accumulation tank (adding ClO_2 directly to the tank)
- Monthly shock treatments of both the external and internal systems (4‰)
- Continuous disinfection of incoming water into the building (2‰).

Phase IV – Shock Treatment

After each shock treatment, a three-day course of

vitamin C was administered per laying hen each day, through drinking water.

Instruments and Measurement Parameters

- ATP Luminometry: Measurements were taken at the sampling site within 5 seconds of swab contact with luciferase. Values above 20,000 RLU were considered unacceptable for sanitation.

- Microbiological Analyses: Standard methods of inoculation on selective media, with incubation at 37 °C for 24 hours.

- Mortality: The number of deaths recorded daily was analyzed in three-time intervals: before treatment, during treatment, and after the implementation of the rehabilitation program.

The effectiveness of treatment with stabilized liquid chlorine dioxide (ClO_2) was evaluated based on three key indicators: daily mortality of laying hens, the degree of microbiological contamination of water (measured by ATP luminometry), and the presence of pathogenic bacteria (*E. coli*) in the water supply. Analyzing data from various phases of the experiment revealed a significant difference between the initial conditions and the post-treatment period.

Mortality of Laying Hens

At the start of the study, the average daily mortality rate was 14.8 hens, which is more than ten times above the technological standard for the Lohmann Brown breed (1.4 hens per day). After completing the first two phases of sanitation, the average daily mortality decreased to 2.1 hens, and by the third month, it stabilized at 1.06 hens per day, which is below the standard.

Table 1 Overview of average mortality values of laying hens in four time periods

Remediation Phase	Mortality (individuals/day)
Before Treatment	14.8
During Shock Treatment	2.1
During Maintenance	1.06
Benchmark	1.4

Note: The reduction in mortality was noticeable within the first five days of shock treatment.

ATP Luminometry – Degree of Contamination

The readings from the ATP luminometer displayed a high degree of contamination in the internal water supply system prior to treatment, with values exceeding 450,000 RLU at the ends of

the pipes in the batteries. After applying ClO₂ at a concentration of 4‰, these values dropped to below 0.5 RLU.

Table 2 ATP Values at the ends of the tubes

Sample Location	Pre-treatment (RLU)	After Shock Treatment (RLU)
Battery 1 – Floor 1	465,000	0.00
Battery 2 – Floor 3	505,000	0.00
Battery 1 – Floor 5	487,000	0.32
Battery 2 – Floor 5	538,000	0.45

These results confirm that ClO₂ at a high concentration (4‰) effectively eliminated the biofilm and reduced the microbial burden to a sanitation-acceptable level ($\leq 20,000$ RLU).

Microbiological Analysis

Microbiological tests on selective media for *E. coli* indicated that water samples from the ends of the pipes before treatment were significantly contaminated, often showing overgrown colonies ($>10^2$ CFU/mL). After treatment, all samples from the internal system tested negative for *E. coli*.

Table 3 Presence of *E. coli* at defined control points

Sample Location	Before Treatment	After Treatment
End of Pipe – Battery 1	+++ (overgrown)	Negative
End of Pipe – Battery 2	+++ (overgrown)	Negative
Accumulation Pool	Negative	Negative
Manhole at the Entrance to the Facility	Negative	Negative

*Note: The absence of *E. coli* in the external system further confirms that the source of infection was localised within the internal distribution system of the facility, where biofilm had formed.

Presence of Residual ClO₂

Test strips confirmed the presence of residual ClO₂ at all ends of the system during daily treatment (2‰), indicating that the disinfecting potential of the water was sustained throughout the day.

Table 4 Detection of residual ClO₂ during prophylactic treatment

Location	Residual ClO ₂ present
Pipe – Floor 1, Battery 1	Yes
Pipe – Floor 5, Battery 2	Yes
Pipe – Floor 3, Battery 1	Yes
Pipe – Floor 2, Battery 2	Yes

CONCLUSION

This study provides a comprehensive evaluation of the efficacy of stabilized liquid chlorine dioxide (ClO₂) as a sanitation agent for farm water supply systems, with particular emphasis on biofilm elimination and the control of colisepticemia caused by *Escherichia coli*. The results unequivocally demonstrated that ClO₂ represents a superior alternative to conventional disinfectants such as chlorine and chloramines, especially in the context of eradicating microbiological contaminants and biofilms in drinking water systems. Application of stabilized ClO₂ at concentrations of 4‰ for nocturnal shock treatments and 2‰ for daily prophylactic disinfection resulted in a significant reduction in ATP luminometry readings, from over 450,000 RLU to below 1 RLU. This indicates effective biofilm removal from the internal surfaces of the pipes, an outcome that conventional chlorination could not achieve. Mortality in laying hens decreased from 14.8 to 2.1 birds per day in the initial days of ClO₂ treatment, with further stabilization to 1.06 after three months, which is below the technical standard for this genetic line. This substantial reduction in mortality reflects improved flock health and the elimination of infection sources. Microbiological analyses following treatment confirmed the complete elimination of *E. coli* and other pathogenic bacteria from the water supply system, confirming ClO₂'s successful suppression of contamination in drinking water. Stabilized liquid ClO₂ does not produce harmful byproducts such as trihalomethanes or chloramines, making it safe for use in food production. Its long-lasting residual effect in water systems ensures continuous protection, reducing the need for frequent disinfectant application. Stabilized liquid ClO₂ offers several operational advantages: it is safe to handle, requires no special storage conditions, leaves no unpleasant odor, and does not affect the taste of water or eggs. Its proven residual activity, as demonstrated in this study, provides extended microbiological safety in closed water systems that are often difficult to access for cleaning. The use

of ClO₂ does not necessitate interruptions in the production cycle, which is crucial for commercial farms where each non-productive day results in economic loss. This makes ClO₂ application cost-effective, particularly when compared to expenses associated with treatment, production losses, and potential public health implications from antibiotic residues and pathogenic microorganisms in eggs. Given its proven biofilm elimination capability, broad-spectrum antimicrobial activity, and technical advantages, ClO₂ has clear potential for wider application in the livestock and food industries—from sanitation of water and ventilation systems to treatment of working surfaces and packaging materials. Its established efficacy in dentistry, dairy processing, and foodservice disinfection further underscores its versatility and safety. The most direct indicator of sanitation success was the sharp decrease in flock mortality—from 14.8 to just 2.1 birds per day within the first five days of treatment, followed by stabilization at 1.06, below the technological norm. This change suggests not only the elimination of the infection source (*E. coli*) but also an overall improvement in flock health. Concurrently, the need for further antibiotic use was eliminated, aligning with modern principles of reducing antimicrobial usage in livestock production. Additionally, the supplementary administration of vitamin C as an anti-stress therapy following shock treatments had a positive effect, supporting the immune response of the birds during recovery. This synergy between chemical sanitation and nutritional support provides a practical and scalable model for implementation in intensive poultry production systems.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: AA, AG; Design: NKD, AP, PB, JT; Supervision: AA, AG; Materials: AA, AP, JT; Data Collection and/or Processing: AA; Analysis

and/or Interpretation of the Data: AA, NKD; Literature Review: AP, NKD, JT; Writing: AA, AG, PB; Critical Review: AG

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EFIKASNOST STABILIZOVANOG TEČNOG HLOR-DIOKSIDA (ClO_2) U DEZINFEKCIJI SISTEMA ZA DISTRIBUCIJU VODE U PERADARSKOJ PROIZVODNJI – SINTEZA TEORIJSKIH I EKSPERIMENTALNIH NALAZA

SAŽETAK

Savremena veterinarska praksa suočava se s rastućim izazovima u oblasti biosigurnosti, posebno u intenzivnoj proizvodnji stoke i peradi. Jedan od najpotencijelnijih, a ipak ključnih faktora u održavanju zdravstvenog statusa životinja jeste kvalitet pitke vode i higijenska sigurnost vododistributivnih sistema. Iako se u svakodnevnoj veterinarskoj praksi glavni uzroci zdravstvenih problema često pripisuju ishrani, faktorima sredine ili genetskom potencijalu, brojni slučajevi ukazuju na to da je voda ključni vektor mikrobne kontaminacije, prvenstveno putem biofilm struktura koje se formiraju u unutrašnjim sistemima za distribuciju vode. Zbog sve češće pojave rezistentnih bakterijskih sojeva i ograničene efikasnosti konvencionalnih dezinfekcionih sredstava, poput hlora, raste potreba za novim, sigurnijim i efikasnijim metodama sanacije.

Stabilni tečni hlor dioksid (ClO_2) pojavljuje se kao snažna alternativa konvencionalnim sredstvima. Ovaj rad predstavlja sintezu eksperimentalnih i preglednih nalaza vezanih za primjenu stabilnog tečnog hlora dioksida u rehabilitaciji sistema za piće kod koka nosilja oboljelih od koliseptikemije. Kroz studiju slučaja na farmi sa zabilježenim problemom sistemske infekcije izazvane bakterijom *Escherichia coli*, analizirani su uzroci kontaminacije, mjere remedijacije, kao i postignuti efekti u pogledu smanjenja biofilma, smrtnosti i mikrobiološkog opterećenja vode. Pored izvještaja o slučaju, rad analizira i širi kontekst efikasnosti stabilnog tečnog hlora dioksida na osnovu literature i prethodnih istraživanja. Stabilni ClO_2 , za razliku od svog gasovitog oblika, omogućava sigurnije rukovanje, produžen efekat rezidualne zaštite i minimalnu toksičnost.

Rad naglašava važnost mikrobiološke kontrole i unapređenje sanitacije sistema za vodosnabdijevanje u peradarskoj proizvodnji, te ističe stabilni tečni hlor dioksid kao izuzetno efikasno sredstvo u borbi protiv biofilma i rezistentnih bakterijskih sojeva.

Ključne riječi: Animalna proizvodnja, dezinfekcija, prevencija, stabilizirani tečni hlor dioksid