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Nanoborate Based Decontamination of Poultry Carcasses

Hakan Tavşanlı¹  | Seda Beyaz²  | Gülşah Çelik Gül³ ¹Department of Public Health, Faculty of Veterinary Medicine, University of Balıkesir, Balıkesir, Türkiye | ²Department of Chemistry, Faculty of Science and Art, University of Balıkesir, Balıkesir, Türkiye | ³Department of Veterinary, Savaştepe Vocational School, University of Balıkesir, Balıkesir, Türkiye**Correspondence:** Hakan Tavşanlı (tavsanli@balikesir.edu.tr)**Received:** 23 February 2026 | **Revised:** 23 February 2026 | **Accepted:** 4 March 2026**Keywords:** antimicrobial | carcass quality | decontamination | nanoborate solution

ABSTRACT

Despite the implementation of strict biosecurity and food safety systems in broiler production, poultry products continue to be a significant source of foodborne pathogens. Traditional decontaminants, including chlorine dioxide (ClO₂), have shown limited effectiveness in completely reducing this risk. We evaluated the antimicrobial activity of novel nanoborate solutions prepared from polyborates using a nanotechnological approach compared with chlorine dioxide. The optimized nanoborate formulation demonstrated superior antimicrobial activity by achieving approximately 10⁵ CFU/mL bactericidal activity at 1 min and 10⁶ CFU/mL at 30 min, approaching TS EN 1656 standards. Remarkable reductions in the concentrations of TAMB, *Pseudomonas* spp., lactic acid bacteria, and *Enterococcus* spp. were observed in poultry carcasses treated with the nanoborate solution by day 12 ($p < 0.05$). On Day 12, carcasses treated with nanoborate exhibited reductions of 2.5 log CFU/carcass in TAMB, 1.5 log CFU/carcass in *Pseudomonas* spp., 2.96 log CFU/carcass in lactic acid bacteria on MRS agar, 2.85 log CFU/carcass on M17 agar, and 4.29 log CFU/carcass on KE agar ($p < 0.05$). Poultry carcasses treated with nanoborate displayed enhanced microbiological quality and exhibited an approximate 50% increase in shelf life relative to the control group. Results from sensory examination demonstrated that nanoborate-treated carcasses preserved acceptable quality on Day 12. Elemental analysis verified the existence of very trace amounts of boron residue. Upon assessing the application of a nanoborate solution regarding public health, it could potentially be an appropriate alternative decontaminant for poultry carcass disinfection.

1 | Introduction

Poultry carcasses and products continue to be a major cause of foodborne diseases in the US even with the strict application of biosecurity and food safety management systems in broiler farms, slaughterhouses, and transportation (Cano et al. 2021). The most commonly encountered microorganisms in raw poultry meat products are *Campylobacter* spp. and *Salmonella* spp. species. In the United States, it is estimated that approximately one million people fall ill each year due to the consumption of contaminated chicken meat (CDC 2022). Furthermore, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) reported that between 2018 and 2019, the prevalence of *Salmonella* spp. and *Campylobacter* spp. in chicken carcass parts was 8.77% and

17.60%, respectively, while in whole carcasses, the prevalence was 3.62% and 21.15%, respectively (USDA-FSIS 2020). The stages with the highest microbial contamination during poultry slaughtering are scalding, defeathering, and evisceration. In addition, contamination can also originate from plant air, processing equipment, and personnel. To reduce these risks on poultry carcasses, a decontamination process is applied immediately after the point of highest microbial contamination (Perez-Arnedo et al. 2021). Although these critical areas may vary depending on the facility's machinery and equipment structure, decontamination is generally carried out after defeathering and evisceration and in the chilling tunnels where the poultry carcasses leave the slaughtering area and enter a less contaminated zone (Takeuchi-Storm et al. 2024). In the United States and other countries, many decontaminants are

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used at legally permitted concentrations. According to the directives issued by the USDA and FSIS in 2024, the use of decontaminants such as peroxyacetic acid, hydrogen peroxide, acetic acid, optionally sulfuric acid, optionally 1-hydroxyethylidene-1,1-diphosphonic acid, optionally dipicolinic acid, dimethylpolysiloxane, polysorbate 65, polyethylene glycol, sorbitan monostearate, sodium hydroxide, polyacrylic acid (sodium salt), and formaldehyde is permitted in specific stages of the process for poultry carcasses and parts, provided that they are used within acceptable limits. In the European Union (EU), regulations regarding the treatment of poultry carcasses with antimicrobial substances are more stringent. The EU takes a cautious approach to the use of antimicrobial agents such as chlorine dioxide and restricts their application (Del Río et al. 2007). Generally, these substances are used in spraying and immersion water at concentrations of 30 mg/L, resulting in residues of 5 mg/kg/L in the final product. However, the European legislation does not include a statement accepting levels as high as 0.5 ppm (Wang et al. 2018). Furthermore, chlorine can react with organic matter present on the surface of carcasses and with dissolved organic matter in water, leading to the formation of toxic and potentially carcinogenic chlorinated disinfection by-products (DBPs) such as trihalomethanes-4 (THM4) and haloacetic acids-5 (HAA5). Moreover, chlorine, which is widely used in slaughterhouses, can enter the environment through wastewater and react with nonylphenol ethoxylates (NPE) commonly used as surfactants in detergents, cleaning agents, and textile processing resulting in the formation of even higher levels of DBPs (Qadafi et al. 2023). This, in turn, contributes to environmental pollution and contamination of water resources. In addition, since the decontaminants used are unable to form an antimicrobial film, they may be insufficient in preventing microbial growth on the carcass, particularly during summer months or in outdoor activities (barbecue) where the cold chain is disrupted (Göçmez and İlhak 2025; Ji et al. 2025).

Borax or boric acid is a chemical nutrient essential for the growth of plants and animals. In humans, the boron element ingested daily through food and beverages is utilized in numerous metabolic reactions, primarily in the immune system and cell formation (Cengiz et al. 2023). Due to these properties, the United States Food and Drug Administration (FDA) granted Generally Recognized as Safe (GRAS) status to borax and boric acid in 1948 (Hernandez-Patlan et al. 2019). Moreover, these substances have also been used as low-efficacy antiseptics for nearly a century (Zan et al. 2013). For example, boric acid is being investigated in medical research for its antimicrobial properties as an alternative treatment for fungal and protozoal vaginal infections (Coghi et al. 2021). As with many antimicrobial compounds, borax and boric acid exhibit a non-specific mechanism of action by targeting multiple sites within microorganisms. However, for these compounds to exhibit biocidal effects, they must remain in contact with microorganisms for extended periods.

The limited solubility of boric acid (4.90 g/100 mL at 20°C) and borax (4.7 g/100 mL at 20°C) poses challenges in improving their antimicrobial effectiveness (Etimine USA Inc 2019a; Etimine USA Inc. 2019b; Karabacak et al. 2022). Converting boric acid and borax into nanoborates may enhance their water solubility and intracellular concentrations, resulting in

increased antimicrobial activity. Furthermore, nanoagents that can more effectively penetrate microbial cell membranes have the potential to act as next-generation disinfectants (Makabenta et al. 2021). As a result, producing stable nanosized borate solutions may greatly improve the antibacterial capabilities of these compounds. Based on this concept, the impact of nanoborate solutions on poultry carcasses was studied here.

This study produced the nanoborate solutions exhibiting significant antibacterial activity and examined their potential application for decontaminating poultry carcasses in comparison with chlorine dioxide. The efficacy of decontamination in carcasses treated with the nanoborate solution was assessed using the physical attributes of the carcasses, sensory quality metrics, microbiological load levels, and residual elemental boron concentration.

2 | Materials and Methods

2.1 | Preparation and Characterization of Nanoborate Solutions

Boric acid and borax decahydrate have been provided by Eti Mining Operations General Directorate. Polyvinylpyrrolidone (PVP) and hydroxyethyl cellulose (HEC) were obtained from Ashland Global Holding Inc. Lialette-8 and Lialette-7 were supplied by Sasol Ltd. Carbomer and PEG 400 were purchased from ZAG Chemistry Ltd. Tween 80 and xanthan gum were bought from Ataman Company.

The nanoborate solutions were prepared following the previously outlined methodology (Tsuyumoto et al. 2007) at elevated temperatures. The procedure commenced with the dissolution of boric acid in hot water, followed by the addition of borax at temperatures exceeding 80°C. The essential aspect of this solution is the Na/B ratio, which falls within the range of 0.19–0.40. The surfactants and polymers were incorporated as the solution cooled to room temperature (25°C) and were homogenized with a magnetic stirrer for a duration of 20 min. This solution was classified as stock (100%). The nanoborate solutions were prepared at various concentrations, including 20%, 30%, 50%, 60%, 70%, and 80% v/v, through the addition of distilled water to the stock solution. A total of 48 preparations were made, consisting of 8 distinct types of polymers/surfactants and 6 varying concentrations of nanoborate solutions. The surfactant concentrations in the nanoborate solutions were varied at 0.25%, 0.5%, 1%, and 2%. The particle size distributions of nanoborate solutions (stock and 50% (v/v)) were assessed at room temperature utilizing the dynamic light scattering technique with a Malvern Nano ZS 90 Zetasizer.

2.2 | Determination of Antimicrobial Activity of Nanoborate Solutions

In the study, test microorganisms *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus hirae* ATCC 10541, and foodborne pathogens *Salmonella mixi* (*S. typhimurium* ATCC 14028 and NCTC 12416, *S. enteritidis* ATCC 13076) and *L. monocytogenes*

mixi (ATCC 7644, 13932, and N 7144) mentioned in the TS EN 1656 standard were used (TS EN 1656 2019). Test microorganisms were incubated separately in 10 mL of Tryptic Soy Broth (TSB, Merck) for 24 h at 37°C. At the end of incubation, the test microorganisms, approximately 10^9 CFU/mL, were centrifuged at 5000 rpm for 5 min. The upper liquid was discarded, and the bacterial pellet was resuspended in 10 mL of Maximum Recovery Diluent (MRD, Merck). It was then transferred to tubes containing 9 mL of MRD at approximately 10^6 CFU/mL. Test bacterial counts were confirmed on Tryptic Soy Agar (TSA, Merck). In the dilution/neutralization method, the same protocol was applied to nanoborate solutions and test bacteria. In the protocol, nanoborate solutions were tested with test bacteria in two different media. One medium was prepared using sterile distilled water to represent low organic matter contamination, while the other was prepared using sterile bovine albumin to represent high organic matter contamination. In the test, 1 mL of sterile organic matter (low: distilled water; high: bovine albumin) was mixed with 1 mL of test bacteria at approximately 10^6 CFU/mL in a sterile 15 mL tube. Then 8 mL of nanoborate solution was added into the tube and vortexed. Nanoborate solution and test bacteria were allowed to stand for 1- and 30-min contact times. At the end of the contact time, 1 mL was taken from the tube and transferred to a blank tube. To this tube, 8 mL of Maximum Recovery Diluent (MRD) and 1 mL of sterile distilled water were added for neutralization, and the mixture was allowed to neutralize for 30 min. After the neutralization process, 0.1 mL was taken from the neutralization tube and plated on Tryptic Soy Agar (TSA, Merck) to determine the antimicrobial activity of the nanoborate solutions. Following incubation at 37°C for 24 h, the colonies were evaluated. A reduction of more than 5 log in test bacteria based on colony counts on Tryptic Soy Agar (TSA, Merck) indicates the antimicrobial efficacy of the nanoborate solution (TS EN 1656). The antimicrobial activity of approximately 50 different formulations of nanoborate solutions, including ethoxylates (Lialette 7 and Lialette 8) (0.25, 0.5, 1, 2%) and Tween 80 (0.25, 0.5, 1, 2%) at four different concentrations (20, 30, 40, 50%), was determined using the dilution-neutralization method.

2.3 | Decontamination of Poultry Carcasses With Nanoborate Solutions

At this stage, a 50% nanoborate solution, which demonstrated the most effective antimicrobial activity against the test microorganisms specified in the TS EN 1656 standard as well as foodborne pathogens, was selected for the decontamination of poultry carcasses. Broiler carcasses obtained from the same poultry house were used due to their similar microbiota.

To ensure food safety, a total of 48 carcasses were taken from the slaughterhouse after the plucking stage and subjected to spraying with either 3 ppm chlorine dioxide (ClO_2) or 50% nanoborate solution in the R&D facility.

2.3.1 | Treatment Groups

2.3.1.1 | Chlorine Dioxide Group ($n=12$). In accordance with the broiler slaughterhouse process, carcasses were sprayed with 3 ppm chlorine dioxide (ClO_2) at three different stages

of the processing line: after plucking, after evisceration, and at the exit of the cooling tunnel. This application resulted in a final product residue level of 0.5 ppm.

2.3.1.2 | Nanoborate Groups. Unlike ClO_2 , the nanoborate solution was applied at a single point in the slaughtering process to achieve a lower residue level. The groups were coded according to the application stage. Approximately 5 mL of 50% nanoborate solution was used for each carcass.

2.3.1.3 | NB Plucking ($n=12$). Carcasses were sprayed with nanoborate solution after the plucking stage.

2.3.1.4 | NB Evisceration ($n=12$). Carcasses were sprayed with nanoborate solution after evisceration.

2.3.1.5 | NB Cooling ($n=12$). Carcasses were sprayed with nanoborate solution at the exit of the cooling tunnel.

A total of 48 carcasses from both the chlorine dioxide and nanoborate groups were placed in sterile packages and stored under cold chain conditions until the designated analysis days. Analyses were conducted on days 1, 7, and 12 after the application. On each analysis day, 4 carcasses from each group were examined.

2.4 | Microbial Analysis of Poultry Carcasses

Each carcass was placed in a sterile bag containing 400 mL MRD, shaken for 2 min to prepare a 10^{-1} dilution, and then serial dilutions were made up to 10^{-7} in glass tubes containing 9 mL MRD (Thames et al. 2022). From the appropriate dilutions, total aerobic mesophilic bacteria (TAMB) counts were cultured on PCA agar for 24 h at 37°C. Lactic acid bacteria counts were cultured on M17 Agar (Merck), MRS Agar (Merck), and KE Agar (Merck) for 48 h at 30°C; presumptive colonies were phenotypically confirmed as lactic acid bacteria based on Gram staining and catalase test results. *Pseudomonas* spp. were cultured on *Pseudomonas* CFC Agar (Merck) containing *Pseudomonas* Selective Supplement, and colonies were evaluated after 48 h of incubation at 25°C.

2.5 | Sensory Analysis of Poultry Carcasses

Sensory analyses of poultry carcasses were conducted on days 1, 7, and 12 of storage by eight panelists who were final-year veterinary students informed about meat inspection and hygiene. The panelists evaluated the carcasses based on odor, color, and overall acceptability using a quantitative descriptive analysis (QDA) where 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = very poor (Pateiro et al. 2022). All participants received training on the test format and evaluation scale prior to the sensory assessment.

2.6 | Color and pH Analysis of Poultry Carcasses

Color measurements were performed using a Lovibond SV 100 colorimeter (Amesbury, UK) according to the Commission Internationale d'Eclairage (CIE, 1978) parameters (L value for

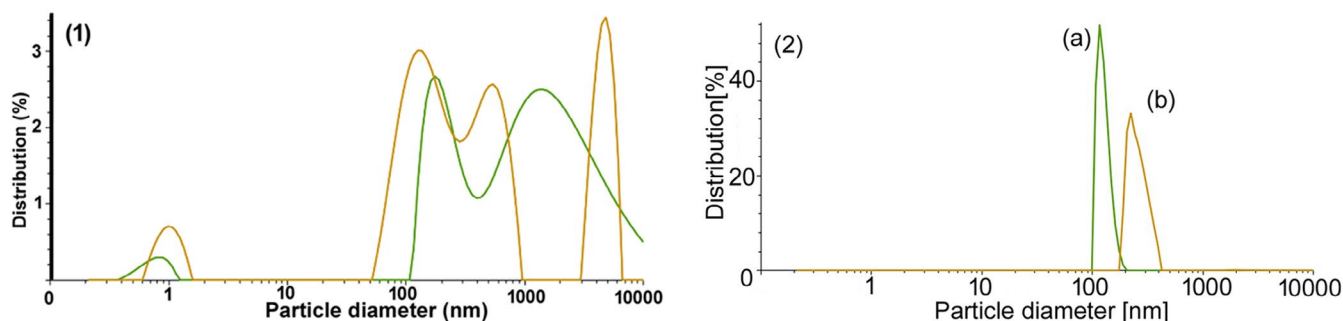


FIGURE 1 | Particle size distributions of the polyborate solutions without surfactant (1) and the nanoborate solutions (2) include 1% of Tween 80 (a) 134.0 nm and 1% of Tween 80 (b) 290.1 nm. (Brown line and green line indicate 50%, v/v and stock solution, respectively).

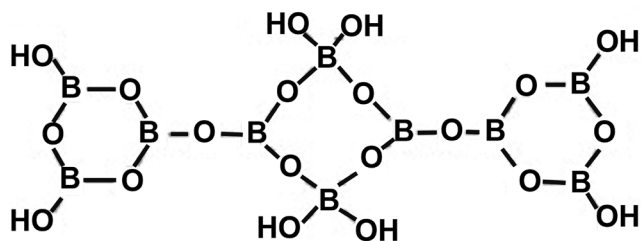


FIGURE 2 | One possible structure suggested for $B_{10}O_{12}(OH)_8^{2-}$ (Tsuyumoto et al. 2007).

lightness, a for red/green, and b for yellow/blue). Results were calculated as the average of five measurements taken at 5-min intervals in three replicates. The 7th and 12th day color analysis results of the chlorine dioxide group carcass samples were not shown in the table because they were outside of acceptable values. The pH of the chlorine dioxide and nanoborate groups was measured using a Hanna pH meter (Nederland).

2.7 | Boron Inheritance Levels in Poultry Carcasses

Tissue samples of 50 g, including skin and muscle mass, were collected from broiler carcasses and homogenized. From each sample, 0.5 g was weighed and digested using a microwave digestion system (Milestone Ethos UP) with the addition of 5 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 . The digested samples were diluted to 25 mL with deionized water and filtered through a 0.45 μm PTFE membrane. Boron determination was performed using an ICP-OES device (ICP-OES Optima, 7300, PerkinElmer USA) at a wavelength of 249.677 nm in radial view mode. The calibration curve was prepared in the range of 0.1–2.0 mg/L, and measurements were conducted in triplicate (Choi and Jun 2008).

2.8 | Statistical Analysis

The results of the analysis were analyzed with the SPSS 26 (SPSS IBM, USA) statistical package program. The normality of the data was assessed using the Kolmogorov–Smirnov test. For normally distributed data, differences between groups were evaluated using one-way analysis of variance (one-way ANOVA) followed by Duncan's multiple range test. A

significance level of $p < 0.05$ was considered statistically significant in all analyses.

3 | Results

This study identified that Tween 80 and ethoxylate polymers (Lialette) are successful for the preparation of stable nanoborate solutions. Furthermore, the nanoborate solutions demonstrated highly effective antibacterial properties. Nonetheless, ethoxylate polymers have not received approval for application in food products. Consequently, Tween 80, recognized as a food additive (E433), was chosen as the surfactant for this study.

Figure 1 presents the particle size distribution (PSD) graphics derived from dynamic light scattering analysis (DLS). Borate ions (2–3 nm) and their aggregates or polyborate ions (1–10 μm) were identified in the polyborate solutions without of surfactant as illustrated on PSD graph (1) in Figure 1. The PSD graph (2) presented in Figure 1 displays the particle distributions within the nanoborate solutions (50% and stock) containing 1% of Tween 80 surfactant. Consequently, the inclusion of surfactant resulted in a particle size distribution that exhibited a more monodisperse structure (Figure 2).

The nanoborate solutions had a bactericidal effect against approximately 10^5 CFU/mL within a 1 min contact time and roughly 10^6 CFU/mL within a 30 min contact time for all tested microorganisms, according to the TS EN 1656 standard for bio-cidal products.

Tables 1 and 2 indicate the microbiological analysis findings for poultry carcasses decontaminated using the nanoborate solution. Statistically significant differences in TAMB counts were detected between the chlorine dioxide and nanoborate groups on Days 1, 7, and 12 ($p < 0.05$). Particularly, by Day 12, the nanoborate-treated group demonstrated an approximate drop of 2.5 log CFU/carcass in TAMB counts. Likewise, for *Pseudomonas* spp., notable differences were identified between the chlorine dioxide and nanoborate groups on day 1 ($p < 0.05$), however no statistically significant difference was detected on Day 7. On Day 12, *Pseudomonas* spp. counts in the nanoborate group decreased to roughly 1.5 log CFU/carcass ($p < 0.05$). Furthermore, intra-group analyses for both TAMB and *Pseudomonas* spp. revealed substantial alterations between Days 7 and 12 ($p < 0.05$). No significant change in lactic acid bacteria counts was seen between

TABLE 1 | TAMB and *Pseudomonas* spp. counts on cold storage days (log CFU/carcass).

	TAMB/day			Pseudomonas spp./day		
	1	7	12	1	7	12
ClO ₂ group	5.65 ± 0.17 ^{aX}	6.66 ± 0.08 ^{aX}	7.95 ± 0.55 ^{aY}	6.48 ± 0.16 ^{aX}	8.57 ± 0.27 ^{aY}	10.07 ± 0.12 ^{aZ}
NB plucking	3.94 ± 0.32 ^{bX}	4.55 ± 0.22 ^{bX}	5.66 ± 0.11 ^{bY}	5.68 ± 0.11 ^{bX}	8.55 ± 0.11 ^{aY}	8.81 ± 0.08 ^{bY}
NB evisceration	4.25 ± 0.13 ^{bX}	4.47 ± 0.33 ^{bX}	5.46 ± 0.5 ^{bY}	5.15 ± 0.03 ^{cX}	7.96 ± 0.94 ^{aY}	8.39 ± 0.45 ^{bY}
NB cooling	4.66 ± 0.9 ^{bX}	4.50 ± 0.26 ^{bX}	5.48 ± 0.13 ^{bY}	5.69 ± 0.01 ^{bX}	8.52 ± 0.22 ^{aY}	8.06 ± 0.02 ^{bY}

Note: ^{a-c}Columns show differences between groups on analysis days, ^{X-Z}Rows show differences within a group on storage days ($p < 0.01$). NB, 50% concentration of nanoborate solution.

the groups on Day 1 when examined on MRS agar. Notable changes were seen between the chlorine dioxide and nanoborate groups on M17 and KE agars on the same day ($p < 0.05$). The Day 12 analysis indicated that the chlorine dioxide group exhibited the greatest lactic acid bacteria count at 6.20 log CFU/carcass, whereas the nanoborate cooling group displayed the lowest count at 3.35 log CFU/carcass. On Day 12, decreases in lactic acid bacteria counts were observed as 2.96 log CFU/carcass on MRS agar, 2.85 log CFU/carcass on M17 agar (both in the nanoborate cooling group), and 4.29 log CFU/carcass on KE agar (in the nanoborate evisceration group).

The results of sensory analysis of poultry carcasses are shown in Table 3. The table shows that there was a statistically significant difference in the sensory attributes tested during the storage period in the chlorine dioxide group ($p < 0.05$). On the last day of storage, the samples in the chlorine dioxide group were rated as 1 (bad) by the panelists for all three sensory criteria. In the samples decontaminated with nanoborate solution, the statistical difference ($p < 0.05$) was especially significant between the 7th and 12th day analysis. Carcasses in this group were evaluated as 3 (medium) in terms of sensory characteristics by the panelists on the 12th day analysis. When the differences between the groups were evaluated within the framework of color, odor, and general acceptability criteria, no significant difference was observed between the chlorine dioxide and nanoborate groups on Day 1 analysis ($p > 0.05$), while statistically significant differences were detected between the chlorine dioxide group and nanoborate group on Days 7 and 12 analysis ($p < 0.05$). According to the sensory evaluation results, the most effective spraying stage was determined to be the cooling outlet.

Color analysis and pH values of carcass samples are presented in Table 4. When Table 4 was examined, it was determined that there was a statistically significant difference in the “L” value of the carcasses on the day of the nanoborate group compared to the chlorine dioxide group ($p < 0.05$). It was determined that the “L” value of the carcasses sprayed with nanoborate solution was closer to the whiteness value of 100. However, no statistically significant difference was observed between the chlorine dioxide group and the nanoborate group in terms of a and b values. No significant differences were found in pH measurements between the groups on Day 1 of cold storage. The pH values varied between 6.29 and 6.44. There was also no difference between the pH measurements taken on the 1st, 7th, and 12th days of cold storage between the groups (not shown in the table). In the chlorine dioxide group, due to the spoilage process, different

colors were observed on the carcasses on the 7th day and especially on the 12th day, so color measurements could not be made meaningfully and only the results for the 1st day were shown in Table 4.

Elemental boron analysis revealed that the boron level in the carcasses with chlorine dioxide group was 0.19 ± 0.06 mg/kg, however the boron level in the carcasses sprayed with the nanoborate group was found as 0.20 ± 0.07 mg/kg (Figure 3). This result shows that negligible amounts of boron penetrated the tissue of the poultry carcasses with the spraying method.

4 | Discussion

The concentrated sodium borate solutions far above the solubility limits of boric acid and borax have polyborate ions such as $B_9O_{10}(OH)_9^{2-}$, $B_{10}O_{12}(OH)_8^{2-}$, $B_{11}O_{14}(OH)_7^{2-}$, $B_{12}O_{16}(OH)_6^{2-}$ as seen in Figure 2 (Tsuyumoto et al. 2007). Condensed borate species form complexes at concentrations greater than approximately 0.1 mol/L, even though $B(OH)_3$ and $B(OH)_4^-$ are monomeric in diluted solutions (Novak and Taylor 1951). However, these species are highly unstable and have polydisperse particle size distributions, as seen on PSD graph (1) in Figure 1. Here, the polyborates were converted into nanoborates in the presence of some surfactants. Thus, two important properties were obtained. The first is a monodisperse size distribution (PSD graph (2) in Figure 1), and the second is stable sols (non-collapsed). Moreover, the nanoborate solutions with high antimicrobial activity were obtained when ethoxylates or polysorbates were used as surfactants. Thus, for the first time, boric acid and borax were used together to obtain a strong antiseptic, and a patent application was submitted (Patent No: 2023/014487). As a result, a new decontamination solution that has high antimicrobial activity, can be used in food, and can remain in water for years without precipitation has been developed for poultry carcasses.

In our country, as in the rest of the world, poultry farming is primarily focused on meat and egg production. As in every year, the USA was the leading country in world poultry meat production in 2022 with 20.9 million tons of production. China ranked second with 14.2 million tons of poultry meat production, and Brazil ranked third with 14.0 million tons. It is seen that almost half of the poultry meat production in 2022 (48.8%) was carried out by these three countries. Turkey ranked 8th in world production in 2022 with 2.4 million tons of poultry meat production (TUIK 2022). The Food and Agriculture Organization (FAO) of

TABLE 2 | Lactic acid bacteria counts on cold storage days (log CFU/carass).

	MRS agar			M17 agar			KE agar		
	1	7	12	1	7	12	1	7	12
ClO ₂ group	4.55 ± 0.48 ^{aXY}	3.58 ± 0.08 ^{aY}	5.79 ± 0.13 ^{aX}	4.90 ± 0.09 ^{aX}	4.77 ± 0.17 ^{aX}	6.20 ± 0.45 ^{aY}	4.16 ± 0.09 ^{aX}	4.23 ± 0.31 ^{aY}	6.48 ± 0.7 ^{aZ}
NB plucking	3.83 ± 0.16 ^{aX}	3.07 ± 0.24 ^{aY}	4.29 ± 0.36 ^{bX}	3.76 ± 0.39 ^{bX}	3.99 ± 0.14 ^{abX}	4.53 ± 0.14 ^{bX}	2.05 ± 0.02 ^{cX}	3.01 ± 0.1 ^{bY}	2.70 ± 0.1 ^{bY}
NB evisceration	3.42 ± 0.20 ^{aXY}	2.83 ± 0.29 ^{aY}	3.83 ± 0.11 ^{bX}	3.62 ± 0.07 ^{bXY}	3.27 ± 0.17 ^{bY}	4.10 ± 0.29 ^{bcX}	3.49 ± 0.15 ^{bX}	2.23 ± 0.18 ^{cY}	2.19 ± 0.26 ^{bY}
NB cooling	3.40 ± 0.48 ^{aX}	3.55 ± 0.47 ^{aX}	2.83 ± 0.85 ^{cX}	4.38 ± 0.24 ^{abX}	4.40 ± 0.26 ^{aX}	3.35 ± 0.09 ^{cY}	2.17 ± 0.04 ^{cX}	4.11 ± 0.28 ^{aY}	2.22 ± 0.4 ^{bX}

Note: ^{a-c}Columns show differences between groups on analysis days, ^{x-z}Rows show differences within a group on storage days ($p < 0.01$). NB, 50% concentration of nanoborate solution.

TABLE 3 | Sensory analysis of carcasses on storage days.

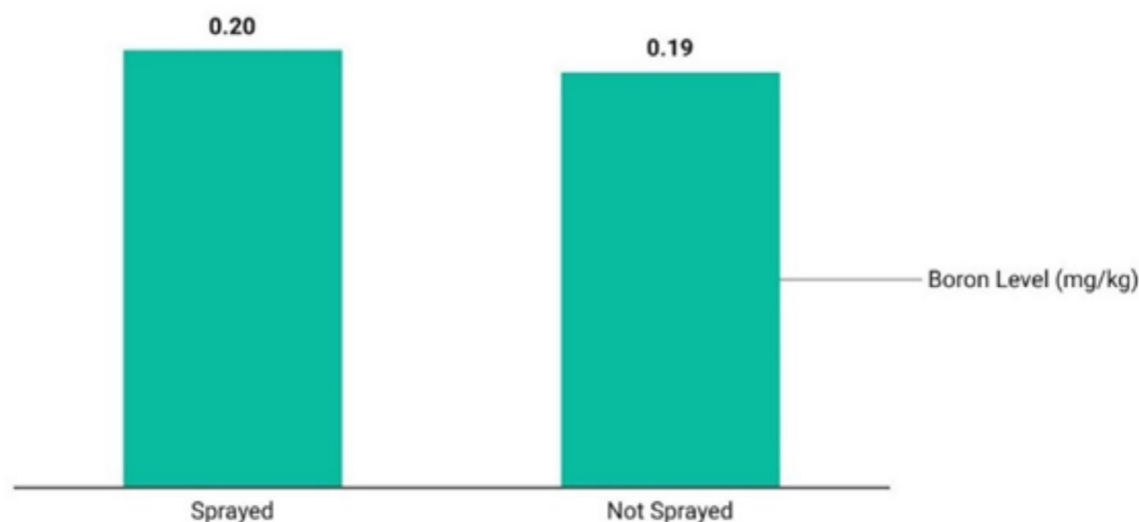
Analysis days	Color			Smell			Acceptability		
	1	7	12	1	7	12	1	7	12
ClO ₂ group	4.3 ± 0.3 ^{aX}	2.7 ± 0.5 ^{aY}	1 ± 0.0 ^{aZ}	4.4 ± 0.2 ^{abX}	3.3 ± 0.3 ^{aY}	1 ± 0.0 ^{aZ}	4.4 ± 0.2 ^{aX}	3.1 ± 0.2 ^{aY}	1 ± 0.0 ^{aZ}
NB plucking	4.6 ± 0.2 ^{aX}	4 ± 0.3 ^{bY}	3.7 ± 0.5 ^{bY}	4 ± 0.2 ^{aX}	3.7 ± 0.5 ^{abY}	3.6 ± 0.2 ^{bY}	4.6 ± 0.2 ^{aX}	4.2 ± 0.5 ^{bX}	3.3 ± 0.3 ^{bY}
NB evisceration	4.2 ± 0.5 ^{aX}	4.2 ± 0.6 ^{bX}	3.5 ± 0.2 ^{bX}	4.7 ± 0.3 ^{bX}	4.2 ± 0.5 ^{bX}	3.5 ± 0.3 ^{BX}	4.7 ± 0.2 ^{aX}	4 ± 0.0 ^{bY}	3.5 ± 0.2 ^{bZ}
NB cooling	4.4 ± 0.2 ^{aX}	4 ± 0.2 ^{bX}	3.6 ± 0.4 ^{bX}	4.2 ± 0.5 ^{aX}	4 ± 0.6 ^{abX}	3.7 ± 0.5 ^{bX}	4.4 ± 0.3 ^{aX}	3.6 ± 0.4 ^{abY}	3.6 ± 0.2 ^{bY}

Note: ^{a-c}Columns show differences between groups on analysis days, ^{x-z}Rows show differences within a group on storage days ($p < 0.01$). Sensory evaluation, 5: excellent, 4: good, 3: moderate, 1-2: poor. NB, 50% concentration of nanoborate solution.

TABLE 4 | Color analysis of carcasses 24 h after nanoborate solution application.

	L	a	b	pH
ClO ₂ group	70.52 ± 1.52 ^a	0.91 ± 0.37 ^a	12.31 ± 1.21 ^a	6.33 ± 0.25 ^a
NB plucking	79.49 ± 1.49 ^b	1.18 ± 0.09 ^a	13.84 ± 2.11 ^a	6.44 ± 0.14 ^a
NB evisceration	76.28 ± 2.03 ^b	1.94 ± 0.45 ^a	13.68 ± 1.44 ^a	6.35 ± 0.14 ^a
NB cooling	76.84 ± 1.27 ^b	0.57 ± 0.06 ^a	14.04 ± 2.95 ^a	6.29 ± 0.09 ^a

Note: ^{a-c}Columns show differences between groups. NB, 50% concentration of Nanoborate solution.

**FIGURE 3** | Boron residue levels sprayed and not sprayed of poultry carcasses tissues.

the United Nations predicted in a 2018 report that by the 2016–2025 period, half of the total meat consumption per capita worldwide would be poultry meat. The same report forecasted that global poultry meat production would reach 132 million tons by 2026 (OECD/FAO 2018). However, the demands of the global market and high-capacity production have introduced several problems, the most significant being the microbial load of poultry carcasses. The nutrient content, high water activity, and pH of poultry meat provide a favorable environment for microbial growth. Additionally, contamination during processing stages such as skin retention, evisceration, and feather removal poses a threat to both the shelf life and public health (Paulus 2005; Cantalejo et al. 2016). These threats necessitate the strict implementation of biosecurity and safety management systems in broiler production and slaughterhouses. The broiler meat industry is known for the effective application of comprehensive food safety management systems, such as “farm to fork” or “field to table”. Despite these measures, poultry meat remains a major source of foodborne illnesses (Gonçalves-Tenório et al. 2018). In response to foodborne illnesses and shelf-life threats, some countries use decontaminant substances in carcass washing, though this may not be legal in all regions. In Turkey, regulations stipulate that the residual chlorine in water or ice that encounters carcasses or meat should not exceed 0.5 ppm, and this amount must be regularly monitored.

In the slaughterhouse where the study was conducted, 3 ppm chlorine dioxide was sprayed on each carcass at three separate

points during the slaughtering process. The nanoborate solution was sprayed at only one point of the process. According to the data obtained, deterioration began to be observed in the carcasses sprayed with chlorine dioxide at three separate points on the 7th day of cold storage, according to both sensory and microbial analysis results. In contrast, no deterioration was observed in the carcasses sprayed with nanoborate solutions at a single point, even on the 12th day. In addition, the antimicrobial activity of the nanoborate solution increased during the storage period. This may be attributed to the bacteriostatic effect of boric acid, as reported by Meers in 1990 (Meers and Chow 1990). A decontaminant with a bacteriostatic effect at low concentrations may have a bactericidal effect at higher concentrations (Gürler 2003). The increased antimicrobial activity in long-term cold storage may be due to the inhibition caused by nanoborate solution for more than 48 h and cell death in microorganisms. In addition, it may also be due to the formation of an antimicrobial film on the carcass surface, depending on the properties such as solvent evaporation and particle size of nanomaterials (Savrik et al. 2018). In our study, the application of nanoborate solution caused a 1.5 log CFU/carcass decrease in TAMB and *Pseudomonas* spp. counts compared to the chlorine dioxide group on the first day of application and a 2 log CFU/carcass decrease on the other analysis days. This decrease for lactic acid bacteria counts was 1 log CFU/carcass on average. Literature review revealed that there is no previous study on the use of nanoborate solution for the decontamination of poultry carcasses. Therefore, in our discussion, we compare these results with the

results of other decontamination formulations used for similar purposes. Chousalkar et al. (2019) reported reductions in TAMB counts of 0.1, 0.1, and 0.5 log CFU/cm² after immersing poultry carcasses in 50 ppm chlorine for 20 min at 4°C, 15°C, and 22°C, respectively. Using peracetic acid (200 ppm, 20 min), they observed reductions in TAMB counts of 0.0, 0.0, and 0.1 log CFU/cm². Guastalli et al. (2016) reported average reductions in TAMB counts of 0.4 and 0.2 log CFU/g after a 25-min immersion in 50 ppm acidic sodium chlorite and chlorine dioxide, respectively. Wang et al. (2018) found a reduction of 1 log CFU/cm² in TAMB counts after spraying carcasses with slightly acidic electrolyzed water (SAEW) for 15 s, but the study did not report if this effect continued on other storage days. Another study reported a reduction of 0.1 log CFU/g in TAMB counts after a 30-s spray application of 6 ppm chlorine dioxide (Purnell et al. 2014). The antimicrobial effects observed in these studies were generally lower than the results from our study. However, Purnell et al. (2014) reported a reduction of 1.3 log CFU/g in TAMB counts after spraying poultry carcasses with 1000 ppm acidic sodium chlorite for 30 s, like our findings. Another study on the use of organic acids as decontaminants reported lower antimicrobial activity compared to the nanoborate solution used in our study. Habeeb et al. (2021) immersed poultry thighs in 1% sodium lactate, 1.5% lactic acid, 1.5% acetic acid, and their mixtures for 5 min and evaluated their antimicrobial activities immediately after application and on days 3, 5, and 8 of cold storage at +4°C, focusing on lactic acid bacteria and *Pseudomonas* spp. They found that only acetic acid extended the shelf life of the product by 2 days compared to the chlorine dioxide group. In addition, Hernandez-Patlan et al. (2019) reported a study that oral administration of boric acid to chicks significantly reduced the colonization of *Salmonella Enteritidis* in the cecum, did not affect lactic acid bacteria, and reduced intestinal inflammation and intestinal IgA.

In the literature review, no study was found on the use of nanoborate solution as a decontaminant in poultry carcasses. Accordingly, no discussion could be made on sensory qualities. However, it was observed that the analysis results were parallel to the scores given by the panelists to the carcasses using the nanoborate solution. While the panelist scores on the 12th day were 1 bad in the chlorine dioxide group, they were above the average value in the nanoborate groups.

Several factors influence the color of poultry meat, including environmental factors before slaughter, rearing conditions, transportation, stress, and decontaminants used during slaughter. The animals used in this study came from the same flock, eliminating many variables affecting meat color and leaving only the slaughter process to account for color changes in the carcass samples (Güney and Toplu 2017). Color changes and defects in poultry meat that are imperceptible to the human eye can be detected using colorimetric devices. This process measures the color quality attributes of meat in terms of L* (lightness), a* (redness), and b* (yellowness) values. The color data obtained in our study cannot be compared directly with those from other literature due to the many factors affecting this quality criterion. When some borates come into contact with water, they release hydrogen peroxide (H₂O₂) and oxidize organic stains and pigments, causing color to lighten (Deary 2023). In addition, the released H₂O₂ supports the antimicrobial effect (Tran et al. 2017).

In support of this, in sensory analyses parallel to color analyses in nanoborate groups, nanoborate groups were scored higher in terms of color criteria. Additionally, the absence of differences in pH values between groups indicates that the nanoborate solutions did not induce chemical reactions in the poultry meat. When the nanoborate groups were evaluated among themselves, the lowest microbial load in terms of TAMB and *Pseudomonas* spp. counts was observed in the nanoborate evisceration group, making it the most effective application in terms of microbiological performance. However, based on the sensory analysis results, the nanoborate cooling group received higher scores compared to the other groups on Day 12 in terms of color (3.6), odor (3.7), and overall acceptability (3.6). Additionally, on Day 12, the *Pseudomonas* spp. load in the nanoborate cooling group was lower (8.06 log CFU/carcass) than in the other nanoborate groups, indicating that it was able to maintain both microbiological and sensory quality. These findings suggest that the nanoborate cooling group has greater potential to positively influence consumer perception due to its ability to preserve sensory attributes throughout shelf life.

Boron is taken in daily with food and water and is an essential nutrient and is involved in many metabolic and physiological processes. In eukaryotes, boron plays an important role in mineral metabolism, immune response, endocrine system, and cellular structure (Hunt 2003). In addition, boron plays a fundamental role in the homeostatic biology of living organisms and was registered as a generally recognized as safe (GRAS) pesticide in the United States in 1948 (Kabu and Akosman 2013). Balıkesir Province, where the study was conducted, contains 73% of the boron reserves in Türkiye. Accordingly, high boron levels are found in the products grown in this region. In our study, no statistical difference was detected in terms of boron content in the chlorine dioxide group and boron residue levels in the carcasses sprayed with the nanoborate solution. This situation can be explained by the fact that the decontaminant is a nanomaterial and is used in a very low amount of 5 mL per carcass. In another study conducted in Türkiye, boron levels in breast meat samples were reported to be much lower than the findings of our study (0.16 µg/g). This may be due to the fact that the Balıkesir region is very rich in terms of boron mines and the high boron levels in soil and water resources.

5 | Conclusion

The nanoborate solution showed higher antimicrobial activity than chlorine dioxide, both immediately after application and during cold storage at +4°C, as evidenced by microbial analysis results. In addition, when the results of microbial and sensory analysis on the 12th day of broiler carcasses were evaluated, it provided a shelf-life gain of approximately 50%, with an average of 5 days, compared to the chlorine dioxide group, which started to show signs of spoilage on the 7th day. Given its more natural structure than many other decontaminants and its low residue, nanoborate solution has the potential to be employed as a decontaminant in chicken slaughterhouses if the consequences of the applied dosage on public health are thoroughly explored. Moreover, the use of nanoborate solution as an alternative to chlorine in water and food disinfection processes may significantly reduce the formation of chlorine-derived disinfection

by-products (DBPs) in water sources, thereby contributing to improved environmental and public health outcomes.

Author Contributions

Hakan Tavşanlı: methodology, validation, software, formal analysis, project administration, data curation, resources, conceptualization, writing – original draft. **Seda Beyaz:** conceptualization, Data curation, Formal analysis. **Gülşah Çelik Gül:** writing, original draft, data curation, Resources.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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