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EARLY VIEW

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Assessment of Physicochemical Properties and Microbial Quality of Water on Broiler Farms in Bosnia and Herzegovina**

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Abstract

Water plays a critical role in broiler production. The use of contaminated water can result in infections, contamination of animal products, and is unsuitable for drug administration. This study assessed the physicochemical and microbiological quality of water used on poultry farms producing broilers in the northeastern region of Bosnia and Herzegovina. The majority of broiler farms had private wells (n =58) or public water supplies (n = 34). Mean values for turbidity (NTU), pH, KMnO4 consumption (mg/L), chlorides (mg/L), ammonia (mg/L), nitrates (mg/L), nitrites (mg/L) and, conductivity (µS/cm) were as follows: 0.16±0.04; 7.51±0.25; 1.37±0.64; 11.91±10.49; 0.09±0.31; 12.78±13.64; 0.06±0.09 and 612.61±216.40, respectively. Ammonia and nitrates exceeded the recommended standard in one sample. Total coliform, E. coli and Enterococcus sp. were detected in 41.30 %, 22.83% and 34.78% samples, respectively. We did not find significant differences in microbial load between poultry farms with public water service to private water wells. The water used on poultry farms has satisfactory physicochemical properties but a high microbial load and could represent a potential source of pathogens. This indicates that water from poultry farms needs more treatment to improve its microbiological quality. Regular monitoring of water utilized in poultry farms is essential for preventive measures.

Introduction

Over the past 30 years, meat production has doubled, and it is expected to double again by 2050 (Shahbandeh, 2019). One of the primary global sources of meat production is poultry (Savin *et al.,* 2021). In 2022, more than 69,094 tons of poultry, of which 67,007 tons from broilers and 2,087 tons from other poultry were processed in Bosnia and Herzegovina (Annual report MVTEO, 2023). Prioritizing hygiene and sanitation in poultry farms is essential for sustainable and disease-free production, ensuring the health of both the flock and the workers involved in the process. Water is a necessary component of blood and tissues as well as a vital physiological ingredient for digestion, absorption, enzyme activity, nutrition delivery, thermoregulation, and waste disposal (Abdullah, 2011). Because of its polarity and hydrogen bonding, water has unique chemical characteristics that enable it to dissolve, absorb, or suspend a variety of substances (Singh *et al.,* 2023). Water consumption has a significant influence on the health and productivity of broilers because it is roughly twice as much as feed intake (King, 1996; Maharjan *et al.*, 2016). Consequently, it is anticipated that poor-quality water will have a greater impact on hens than tainted or poor-quality food (Talha *et al.,* 2008). Broilers that are not provided with good qualitydrinking water tend to have poor feed conversion ratios, and overall performance (Boumedous et al., 2017). Poultry farms frequently encounter health issues with their broilers for unknown reasons. The majority of these instances involve issues with hygiene and water quality (Maharjan et al., 2017). Water quality regulations for drinking water for poultry in Bosnia and Herzegovina have been adopted from Bosnia and Herzegovina Regulation (40/10) and European Council Directive 98/83/EC on the quality of water designed for human use (European Commission 1998). The health and productivity of chickens are significantly impacted by the microbiological and physicochemical quality of their drinking water. Water's mineral and microbiological composition has an impact on chicken performance (King, 1996). This study's goals were to evaluate the main physicochemical water parameters and the presence of fecal coliforms (FC), *Escherichia coli* (EC), and fecal *enterococci* (FE) in water samples taken from 92 farms in the northeastern (NE) region of Bosnia and Herzegovina that had access to either a public or private water source.

Materials and Methods

Sample Collection

Water samples were collected between November and December 2023 at 92 poultry farms producing broilers in northeastern (NE) region of Bosnia and Herzegovina. Majority of broiler farms had private wells (n =58) or public water supplies (n = 34). Before they were aseptically collected into sterilized bottles, water was let to run for roughly 30 seconds. Water was brought to the lab at 4°C after being collected, leaving about 3 cm of space at the top for aeration. For microbiological analysis, samples were processed in 24 hours, and for physicochemical analysis, in 48 hours. An overview of the location of poultry farms included in the study is presented in Figure 1.

All parameters were analyzed using an appropriate ISO method accredited according to the requirements of BAS EN ISO/IEC 17025:2008. Following physicochemical parameters were analyzed: color, odor, taste, turbidity, pH value, KMNO4 consumption, chlorides, ammonia, nitrates, nitrites and conductivity. Analytical reagent-grade chemicals were employed for the preparation of all solutions.

The Platinum-Cobalt Scale was used for color measurement of water (BAS EN ISO 7887:2013), while determination of the threshold odor (TON) and taste number (TFN) was conducted according to BAS EN 1622:2008. The pH, turbidity and conductivity of the collected samples were determined using pH/turbidity/conductivity meter as described by appropriate ISO methods. Prior to analysis, all instruments were calibrated according to manufacturer's recommendations. The method used calculate $KMNO_4$ consumption involved heating a sample in a boiling water bath containing potassium permanganate and sulfuric acid for a predetermined amount of time. Part of the permanganate was then reduced by oxidizable material in the sample, and the amount of consumed permanganate was then determined by adding an excess of oxalate solution and titrating with permanganate. Using the Mohr's method, silver nitrate titration with chromate indicator was used to determine the chloride content. The manual spectrometric approach was used to determine the ammonium. The yellow chemical produced by the interaction of sulfosalicylic acid (made by adding sulfuric acid and sodium salicylate to a sample) with nitrate and then treating the mixture with alkali was used to quantify the nitrate ion in water using spectrometric measurement at the 655 nm wavelength. The amount of nitrite was determined using molecular absorption spectroscopy.

Following microbiological parameters were analyzed: detection and enumeration of *E. coli,* fecal coliforms and fecal streptococci *Enterococcus* spp. Analyzes were performed using membrane filtration technique according to BAS EN ISO 9308-1/A1:2018 and BAS EN ISO 7899-2:2003 thus 100 mL of the sample was filtered using 0.45 µm pore size membrane filter (Sartorius, Germany). The filter were then aseptically placed on Chromogenic Coliforms Agar (CCA) ISO (Condalab, Spain) and incubated at 37˚C for 21±3 h for detection of *E. coli* and fecal coliforms. *E. coli* count is presented as metallic blue to violet colonies while presumptive coliform colonies (pink to red in color) were confirmed through an oxidase-negative reaction. For the determination of the fecal *Enterococci*, the filter was placed on Slanetz and Bartley's agar (Condalab, Spain) and incubated at 37˚C for 24 – 48 h. Presumptive colonies of *enterococci* (red, maroon or pink color) were placed on Bile Esculin Agar (Remel, USA) and incubated at 44˚C for 4 hours. Plates were examined for blackening of medium around and under the colonies. A descriptive analysis was carried out (min, max, mean, and standard deviation). The median microbiological counts (FC, EC, and FE) for various water sources (public vs. private) were calculated compared using an Microsoft Excel tool, assessing the microbial load by the presence or absence of bacterial contamination. Statistical significance was established when the p-value was below 0.05, as determined by the t-test.

Results and Discussion

Physicochemical parameters of water samples are summarized in Table 1. The requirements for the physicochemical quality of the analyzed water samples were not met by 1 of the total 92 samples, for ammonia and nitrates quality parameters (Table 1). The results of microbiological analyses are presented in Table 2. Of the total of 92 examined samples. 21 samples (41.30%) did not meet the microbiological quality regarding the presence of *E. coli*. These samples had exceeded the allowed 0 cfu/100 ml, according to the recommendations from the Guidelines on Microbiological Criteria for Water in B&H, which are adapted to EU regulations Directive 98/83/EC. 38 samples had coliforms present (22.83%) and the presence of fecal *enterococci* was recorded in 32 (34.78%) samples.

We did not find significant differences ($p < 0.05$) in microbial load between poultry farms with public water service to private water wells**.** The physicochemical and microbiological results of this study were compared with those from the European Council Directive 98/83/EC on the quality of water meant for human consumption (European Commission 1998), as there is no special legislation for water used in animal production. Quality of drinking water is a crucial health determinant for animal health and production. Water which plays an important role in poultry farms can easily be contaminated with microorganisms and transmitted to the animals through water consumption. It is thus important to provide a microbial contamination free water source. These waterborne pathogens may cause infections and could also be responsible for development of antimicrobial resistance, which is a major public health issue (Jacobs *et al.*, 2008). The majority of tested poultry farms were dependent on private wells source of water. Physicochemical parameters assessed included color, odor, taste, turbidity, pH value, $KMNO_4$ consumption, chlorides, ammonia, nitrates, nitrites and conductivity of the water samples. The color of water indicates the presence of suspended particles or dissolved substances while the odor can result from various sources, including organic matter, minerals, or chemical contaminants. Factors such as high mineral content, chemical residues or microbial contamination can alter the taste of water.

The appearance, taste and odor of tested samples were satisfactory. Turbidity of water measures the presence of suspended particles that could potentially serve as reservoirs for bacteria and viruses (Mann *et al.,* 2007). All water samples used on poultry farms had turbidity values below 1 NTU with a mean of 0.16 NTU (Table 1). This implies that water used on broiler farms has low levels of suspended particles (Mann *et al.,* 2007). The degree of acidity or alkalinity caused by dissolved ions in water is measured by its pH. The pH of water is an important consideration; the optimal pH range for drinking water is between 5 and 7 (Saleh *et al.,* 2023). pH values below 5 can impact water consumption, potentially leading to parasitic infections due to altered conditions favoring certain organisms. Conversely, elevated pH values may suggest the presence of salt pollution, which could impact the assimilation of crucial nutrients such as calcium, phosphorus, potassium, and magnesium (Vermeulen *et al*., 2002). It's essential to maintain a balanced pH level for both water quality and health considerations. Most of the tested water samples were neutral to slightly alkaline with average values 7.51±0.25.

These findings are accordance to similar research (Abd-El-Kader *et al*. 2009, Mohebi *et al*., 2023, Haddad and Masoud, 2024). An essential water quality parameter, the permanganate index is used to calculate the amount of oxidizable materials (both organic and inorganic) in water. The values of this index were in range 0.30 to 2.89 mg/L and despite this variation remained lower than the permissible limit. In water and wastewater, one of the main inorganic anions is chloride, specifically the chloride (Cl^-) ion. Research has demonstrated that elevated amounts of chloride may cause disruptions in metabolism (Coetzee *et al.,* 2000). Elevated amounts of chloride have been shown to affect digestion, decrease feed consumption, and increase water intake (Ayoub *et al*., 2017). Concentrations of chloride in water in this study remained under the permissible limit and ranged between 0.71 and 77.81 %.

These results are similar to findings in well water in Nigeria (Taiwo *et al*. 2011). The presence of ammonia, nitrites and nitrates in groundwater is a criterion for water pollution with nitrogenous organic substances. It is likely that contamination of groundwater is from the farm as manure removed from the buildings is stored on the ground near the production buildings. In spring months, when the environment temperature is higher, more intensive are the processes of ammonification of organic matter in manure and the levels of ammonia, nitrites and nitrates in groundwater increase. High levels of agricultural activity are typically associated with elevated ammonia concentrations in groundwater (Liu *et al.,* 2020). Generally, nitrites and nitrates are natural components of water, originating from sources like fertilizers, organic matter decomposition but excessive levels can be harmful to poultry. For nitrites, concentrations above 10 ppm can be toxic to poultry, leading to conditions like methemoglobinemia which affects the bird's ability to transport oxygen (Casey *et al.,* 1998). Nitrate levels below 100 ppm are generally considered safe for poultry, although levels above this threshold may cause health issues such as reduced growth rates and reproductive problems (Saleh *et al,* 2023). Our results do not align with the study on water samples from broiler farms in Bulgaria, where the nitrate concentration was 71.6 mg/L (Stefanova *et al.,* 2012), which is above the reference standard. This suggests that sanitation measures in broiler farms in Bosnia and Herzegovina may be quite successful in controlling certain water quality issues. All water samples in our study had electrical conductivities within the acceptable range, with a mean value of 612.61 µS/cm. Our results are slightly higher compared to a study conducted in Ghana, where the conductivity of 100 samples ranged from 23.6 to 1114.0 µS/cm, with an average of 146.7 µS/cm (Osei *et al,* 2019). The lower conductivity values were likely due to low mineralization (Gray, 2004). Water is essential to chicken farms and is readily polluted with bacteria, which the animals can contract by drinking it. However, despite the high bacterial activity, nitrite and nitrate levels might be low if the microbial community is primarily composed of species that do not produce or consume significant amounts of these compounds. Waterborne gastroenteritis is most likely to occur when microorganisms are present in drinking water (Amaral *et al.,* 2004). (In the northeastern part of Bosnia and Herzegovina, we found that fecal *streptococci* (34.78%), *E. coli* (22.83%), and fecal coliforms (41.30%) polluted the water in broiler farms. These waterborne pathogens cause low body weight and high mortality in poultry (Amaral *et al.,* 2004). One bacterium connected to epidemics of colibacillosis disease is *E. coli.* It is important because 95% of the bacteria that comprise the most well-known and researched group of bacteria, fecal coliforms, are formed by it (Gama 2005; Cardozo *et al.,* 2015). Our findings are similar to those from Jordan in the context of the coliform pathogen patterns isolated (Haddad and Masoud, 2024). Both studies observed similar trends in coliform contamination, highlighting the importance of monitoring water quality to prevent potential health risks to poultry. Sewage treatment facilities nearby and insufficient waste management can also contribute to fecal bacteria contamination in well water samples. Fecal bacteria contamination in well water samples can arise from inadequate waste management and sewage treatment plants that are closely located to. To mitigate the risk of microbial contamination in wells located near surface drainage water, it's essential to implement proper well construction practices, including adequate casing depth, sealing, and placement away from potential contamination sources (Cronin *et al.,* 2006). From the above findings, there is an urgent need for the strict monitoring of the microbial quality and physicochemical properties of the various sources of water used in animal husbandry in Bosnia and Herzegovina.

Conclusion

Most poultry farms in the Northeastern region of Bosnia and Herzegovina rely on own well water as their main source of water, followed by public water sources. Most of the analyzed poultry farms has satisfactory physicochemical properties but a high microbial load that could represent a potential source of pathogenic organisms. Most often water samples were contaminated with coliform and *enterococci* bacteria with *Streptococcus* sp. being the predominant isolate. Ensuring access to clean, high-quality water is livestock and poultry production is of major importance to support optimal health, growth, and productivity. Water quality can impact animal health and performance, so it's crucial to monitor and maintain water sources to meet the specific needs of the animals. Regular monitoring of water used in poultry must be conducted.

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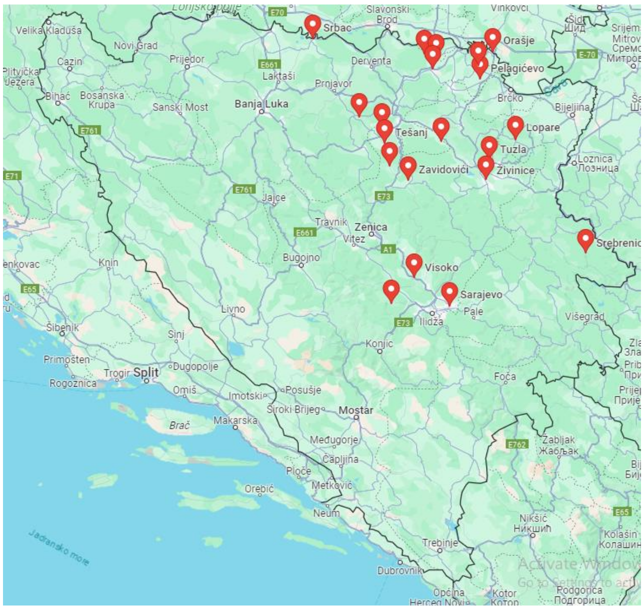

Figure 1. Location of study area and sampling poultry farms

Table 1. Physicochemical properties of water used on broiler farms

Physicochemical parameters	Ref.value	min	max	mean ± SD	median
Color	No change	ND	ND	ND	ND
Odor	No change	ND	ND	ND	ND
Taste	No change	ND	ND	ND	ND
Turbidity (NTU)	1.0	0.1	0.32	0.16±0.04	0.16
pH	≥ 6.5 ≤ 9.5	7.05	8.02	7.51±0.25	7.47
$KMNO_4$ consumption (mg/ L O_2)	5,0	0.30	2.89	1.37±0.64	1.20
Chlorides (mg/ L)	250	0.71	77.81	11.91±10.49	11.28
Ammonia (mg/ L)	0,50	<0.01	1.70	0.09±0.31	0.02
Nitrates (mg/ L)	50	0.97	80.56	12.78±13.64	7.73
Nitrites (mg/ L)	0.5	<0.002	0.34	0.06±0.09	0.01
Conductivity (µS/cm)	2500	208.00	1665.00	612.61±216.40	595.00

Table 2. Microbiological properties of water used on broiler farms

Microbiological properties	Ref. value	No of isolates	Public water	Private well
E. coli	0 cfu/100 ml	21	6	15
Total coliforms	0 cfu/100 ml	38	18	20
Fecal enterococci	0 cfu/100 ml	32	14	18