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Application of hydrogen-rich mineralized water on growth performance and meat quality of broiler chickens

M Asaduzzaman[1], MA Foysal[1], FT Zohora[1], S Khatun[1], S Halder[2], MA Hossian[3], B Dey[1]✉

[1]Department of Poultry Science, Bangladesh Agricultural University, Mymensingh 2202
[2]Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh 2202
[3]CP Bangladesh Company Limited, Bangladesh

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Correspondence:
bapon.dey@bau.edu.bd

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ABSTRACT

Hydrogen-rich mineralized water (HMW) containing a large amount of dissolved hydrogen gas has several beneficial properties, such as anti-inflammatory, antioxidant, and metabolic regulation. Regarding the ongoing concern for food safety, its unique biosafety makes remarkable appealing for use in the livestock industry. Under controlled experimental conditions, this study focused on determining the beneficial effects of drinking hydrogen-rich mineralized water on broiler growth efficiency, litter microbial load, and meat quality, thereby assessing its potential in broiler production. From day 1 to 35 of the study, one treatment group of broilers was administered hydrogen-rich mineral water and another group was administered normal tap water. At the end of the experiment, on day 35, five birds from each treatment group were selected for slaughter and subsequent dissection. The results indicated that the administration of hydrogen-rich mineralized water led to significant ($p<0.05$) differences in final body weight, and feed conversion ratio (FCR improved from 1.52 to 1.50). Microbial analysis regarding hydrogen mineralized water revealed a significant reduction of pathogenic bacteria in litter, such as *E. coli* and *Coliforms,* compared to normal tap water. Hydrogen mineralized water also shows a higher beneficial *Lactobacillus spp* in the litter, though not statistically significant*.* in the litter. No apparent shift in meat quality has been found between the two groups while using the hydrogen-rich water. While examining water quality,results revealed that normal tap water contained a significantly ($p<0.05$) higher microbial load than hydrogen-rich water. In summary, these findings suggest that application of HMW to enhance production efficiency and microbial safety without compromising the meat quality of broiler. Its application also supports a sustainable water-based intervention in poultry production. Future studies should emphasize caecum microbiome sequencing, molecular pathways and large-scale commercial validation.

Introduction

Hydrogen gas (H_2), the simplest and most common molecule, is biologically inert, being colorless, odorless, and tasteless under normal circumstances (Dole, Wilson, & Fife, 1975). This perception began to shift when Dole et al. (1975) explored its therapeutic potential in cancer, and later, Ohsawa et al. (2007) demonstrated that molecular hydrogen functions as a selective antioxidant capable of neutralizing highly reactive oxygen species (ROS), particularly hydroxyl radicals, in a rodent model of ischemia–reperfusion injury. Since these foundational studies, hydrogen has been shown to exert an assortment of biological processes, containing anti-inflammatory, anti-apoptotic, and gene-regulatory effects. These protective effects are partly mediated through key signaling pathways such as Nrf2 and NF-κB, which regulate antioxidant capacity and inflammatory responses (Kamimura, Nishimaki, Ohsawa, & Ohta, 2011).

Hydrogen can be administered by inhalation, injection of hydrogen-saturated solutions, or consumption of hydrogen-rich water (HRW). Among these, water-based supplementation is particularly attractive in livestock systems due to its simplicity, safety, and cost-effectiveness. In poultry, HRW or electrolyzed reduced water has shown promising outcomes. Broilers exposed to chronic heat stress and given electrolyzed reduced water exhibited decreased ROS production, lower lipid peroxidation, and improved growth performance (Azad, Kikusato,

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Zulkifli, & Toyomizu, 2013). Similarly, broilers provided hydrogenated water demonstrated increased antioxidant enzyme activity and improved feed conversion ratio (Shin, Cho, Bang, & Shim, 2016).

In recent times, hydrogen-rich mineralized water (HMW), which contains a remarkable stock of minerals and a substantial amount of dissolved molecular hydrogen, has enlightened its potential as an encouraging approach. The biological functions of hydrogen expand its antioxidant properties. It selectively neutralizes harmful ROS without disrupting physiological redox signaling (Ohta, 2014), activates Nrf2, suppresses NF-κB, and modulates apoptosis and cellular stress responses (Peng et al., 2022; Tian et al., 2021). Research in other livestock species further supports its benefits. In weaned piglets, hydrogen-rich water reduced diarrhea incidence, improved intestinal morphology, enhanced systemic antioxidant capacity, and modulated gut microbiota (Wang, Guo, & Zhao, 2025). In aquaculture, it has been shown to enhance growth performance and metabolic resilience in largemouth bass (Li et al., 2023).

Despite these promising findings, limited research has evaluated the effects of hydrogen-rich water in poultry beyond growth performance. Specifically, more people are not even conscious about its impacts on microbial ecology in litter, for instance, *Salmonella*, *Escherichia coli*, coliforms, and *Lactobacillus* spp. They highly influence the meat quality traits, known as significant variables for maintaining consumer acceptance and financial return. It is highly significant to understand how HMW may influence microbial ecology in maintaining litter quality and flock health. It is additionally demonstrated by the substantial relationship between overall flock health and the microbiological state of drinking water and litter materials.

Thus, the goal of the current study was to assess the effects of perpetual provision of hydrogen-rich mineralized water from day 1 to 35 on broiler growth performance, litter microbial quality, and breast meat characteristics.

Management and housing conditions for the experimental broiler chickens.

This experiment was conducted at BAU Poultry Farm, Bangladesh Agricultural University (BAU), Mymensingh, in an open-sided house that was divided into ten pens, each of 30 sq. ft.. After thorough cleaning and washing, bleaching powder was applied at 1 kg per 500 sq ft and left for 24 hours. The room and equipment were then disinfected using 1% and 0.5% TH4+ solutions, respectively. A total of 200-day-old Arbor Acres straight run commercial broiler chicks were used for this feeding trail and chicks were collected from Nourish Poultry and Hatchery Ltd. in Bhaluka, Mymensingh. The duration of the feeding trial was 35 days. The experimental broiler chicks were randomly divided into 2 dietary groups, with each group being replicated for five times. The chicks were brooded in four enclosures, each equipped with a 100-watt lamp. The temperature started at 35°C on day one and was gradually reduced every three days until one week of age. A thermo-hygrometer monitored room temperature and humidity. The relative humidity levels were maintained between 50-60% throughout the experimental period.

Experimental Diets

Each dietary group consisted of 100 chicks, divided into 5 replicated pens, each with 20 chicks. The hydrogen mineralized water (HMW) is a two-liquid mixing system (solution A and Solution B) developed to increase safety during production. The basic components of solution A are 75% water, 10% glacial acetic acid, 10% citric acid, 4.5% formic acid, and trace amounts of zinc, magnesium, calcium, and benzoic acid. Solution B contains 87% water, 12% NaClO and silicon in trace amounts. The chicks were fed standard starter (0–14 days) and grower (15–28 days) diets to meet their nutritional requirements (Table 1). Commercial ready feed (Kazi Farms Limited) was used for the starter and grower phases, while a hand-mixed finisher diet was provided from days 29–35. Hydrogen mineral sterilized water was measured for each treatment and mixed separately with drinking water for each replicate.

Materials and methods

Table 1: Ingredients and chemical composition of broiler starter and grower diet

Ingredients (kg/100kg)	*Starter diet (0-14 days)	Grower diet(15-28days)
Maize	50.32	53
Rice polish	8.00	10.0
Soybean	29.00	22.50
Protein concentrate (CP, 60%)	8.00	8.00
Oyster shell	1.00	1.00
Salt	0.30	0.25
DL-Methionine (99%)	0.20	0.18

Lysine (98.5%)	0.03	0.03
Vitamin-mineral premix**	0.25	0.25
Soybean oil	3.50	4.00
Di calcium phosphate	0.25	0.25
Choline chloride (50%)	0.10	0.10
Total	100	100
Chemical composition (calculated)		
ME Kcal/kg	3050	3125
CP%	22.75	20.75
DM %	90	90
Lys %	1.25	1.15
Met %	0.50	0.49
Met + Cys %	1.00	0.92
Ca%	1.2	1.2
Available P %	0.75	0.75

*Composition of fresh ingredients was collected from Quality Feeds Ltd., Bangladesh

***Vitamin-mineral premix supplied the following per kg of diet: Vit A, 12,000 IU; Vit D3, 4,000 IU; Vit E, 45 mg; Vit K3, 2.2 mg; Vit B1, 1.2 mg; Vit B2, 5.5 mg; Vit B6, 3 mg; Vit B12, 0.03 mg; Niacin, 50 mg; Panthothenic acid, 10 mg; Folic acid, 0.5 mg; Biotin, 0.08 mg, Mn, 70 mg; Fe, 48 mg; Cu, 5 mg; Zn, 60 mg; Se, 0.2 mg; I, 1 mg.*

Feed and water management

The day-old chicks were initially provided with a 5% glucose solution supplemented with vitamin C for the first five hours after arrival to minimize transport stress. During the first four days, feed was offered on newspaper, then on small trays, and from day seven onwards, small feeders were introduced to acclimate the chicks. Each pen was equipped with two feeders and a 7-liter drinker. Feeders were cleaned weekly and drinkers were cleaned twice daily.

A starter diet was provided from day 0 to 14, followed by a grower diet until day 28. Feed was supplied three times daily to ensure continuous availability, and all birds were maintained on *ad libitum* feeding schedule.

The Hydrogen mineral-sterilized water is a two-liquid mixing system (Solution A and Solution B) developed to increase safety during production. The product is developed by YONSEI University, South Korea. Hydrogen mineral-sterilized water was added to drinking water at 7.5 ml/L (days 1–7), 5 ml/L (days 8–14), and 3 ml/L (days 15–35), while the control group received plain water. Fresh water was provided at all times, and weekly feed refusals were recorded.

Litter, Lighting, Vaccination, and Biosecurity Management

Fresh rice husk (5–6 cm) was used as litter, with soiled portions replaced after two weeks and regularly turned to promote drying. During brooding, birds were exposed to 23 hours of light and 1 hour of darkness for the first four days, with the dark period gradually increased to 6 hours per day until week four and slightly reduced until the end of week five. The broiler birds received vaccinations against Infectious Bronchitis and Newcastle Disease (IB + ND) on day 3, followed by Infectious Bursal Disease (IBD) on day 10, and an IBD booster on day 17. All vaccines were administered via eye drops. Strict biosecurity was maintained, including controlled shed access, foot baths with TH4+ disinfectant solution, dedicated footwear and aprons, fencing, and measures to prevent intrusion by wild animals, rodents, and reptiles.

Sample Collection and Processing

At the end of the trial, from each treatment group, five birds of average weight were chosen at random. They were slaughtered, bled for 2 minutes, and semi-scalded in hot water (51–55°C for 120 s) for feather removal. Birds were weighed before and after feathering, and carcasses were dissected to record live weight, dressed weight, cut-up parts (breast, thigh, drumstick, wing), giblets (heart, liver, gizzard), head, neck, and abdominal fat

Growth Performance and Feed Utilization

Birds were weighed on the first day and weekly thereafter to calculate body weight gain (BWG = final weight − initial weight). Feed intake per replication was determined weekly by subtracting leftover feed from supplied feed, and the feed conversion ratio (FCR) was calculated as feed intake divided by body weight gain.

Meat Quality Parameters

Breast Meat Color

After 24 h of refrigeration, breast meat color was assessed using a Konica Chroma Meter CR-410 (Konica Minolta Inc., Tokyo, Japan. Three readings were taken per sample and averaged. Breast meat color was expressed in the CIE LAB dimensions of lightness (L*), redness (a*), and yellowness (b*).

Drip Loss Measurement

Approximately 25 g of breast muscle was excised from a consistent location on each bird 24 hours post-mortem and weighed (initial weight). Samples were suspended in airtight containers and refrigerated at 4°C for 24 hours. The final weight was recorded, and drip loss was calculated as:

$$\text{Drip loss}(\%) = \frac{\text{Initial weight of the sample} - \text{Final weight of the sample}}{\text{Initial weight of the sample}} \text{x100}$$

Water-Holding Capacity (WHC) Determination

Approximately 1 g of breast muscle was cut into small cubes from each sample and placed in a centrifuge tube. The samples were centrifuged at 10000 rpm for 10 minutes at 4°C, and WHC was calculated as the percentage of sample weight retained after centrifugation relative to the initial weight:

$$\text{WHC}(\%) = \frac{\text{Sample weight after centrifugation}}{\text{Sample weight before centrifugation}} \text{x100}$$

Determination of Water Quality

Water samples, including normal and hydrogen-mineralized water, were collected in sterile containers using aseptic techniques and transported on ice for analysis within 6 hours. Samples were centrifuged at 10,000 rpm for 10 minutes (repeated three times) to separate any particulates, and the supernatant was carefully decanted. For microbial analysis, aliquots were streaked onto agar plates using a standard quadrant streak method with a sterilized inoculating loop. Plates were incubated at 37°C for 24–48 hours, after which colony growth was observed, and isolated colonies from the final quadrant were used to obtain pure cultures.

Determination of litter microbial load

Taking and mixing all subsamples from the litter in a sterile container, a representative composite sample was obtained .10 g of litter was aseptically weighed into a sterile flask. After that, 90 ml of sterile buffered peptone water or 0.85% regular saline was added, and the mixture was rapidly agitated for five to ten minutes. This forms the 10^{-1} dilution. Transferring 1 mL from the 10^{-1} suspension into 9 mL sterile diluent to obtain a 10^{-2} dilution, and continued up to 10^{-6} or 10^{-7} depending on the estimated microbial density. The proper dilution (0.1 mL) was placed onto sterile Plate Count Agar (PCA) plates through spreading with a sterile spreader. After the Incubation at 37°C for 24–48 hours, some plates containing 30–300 colonies were selected. Microbial colonies were counted using a colony counter.

The microbial load is expressed as Colony Forming Units per gram (CFU/g) of litter.

CFU/g = (Number of colonies× Dilution factor) / Volume plated

Statistical analysis

The Analysis of Variance (ANOVA) method was used to statistically analyze all of the experiment's recorded and computed data, depending on the principles of the Completely Randomized Design (CRD). Duncan Multiple Range Test procedure was used to determine the significant differences among different means at 5% significance level (SAS, 2002).

Results and Discussion

GROWTH PERFORMANCE

The results showed (Table 2) significant differences ($p<0.05$) in weight gain and FCR between the HMW-treated and untreated groups. Other parameters did not show any statistically significant differences ($p>0.05$). This improvement in FCR aligns with findings of OUCI (2023), where hydrogen-rich water enhanced antioxidant status, which affected nutrient utilization efficiency in broilers.

Table 2: Overall growth performance of broilers supplemented with HMW

Parameters	T_1	T_2	P-value	LS
Initial wt. (g/bird)	41.69	41.69	-	NS
Final LW(g/bird)	2102±4.975	2132±14.652	0.056	*
Cum. LWG (g/bird)	2061±2.30	2091±2.302	1.18E-06	**
Cum FI (g/bird)	3153±1.843	3157±3.53	0.112	NS
FCR	1.52±0.003	1.50±0.002	0.0008	**
Survivability (%)	92	90	-	-

Where, LWG = Live weight gain, FI = Feed intake, FCR = Feed conversion ratio, LS = Level of significance, T_1= Normal tap water, T_2= Hydrogen mineralized water, ** = ($p<0.01$). Value indicates – Mean ± Standard Error (SE); LS = Level of significance.

Meat quality

The meat quality remains unaffected between the birds of the HMW and normal water-treated groups (Table 3). Jawasreh et al. (2019) found that the cooking loss of the Indian River broiler strain was 26.65% at 28 days (4th week), which was close to our current study. No significant ($p>0.05$) effects of treatment were observed on the color of breast meat among the treatment groups. The lightness (L*), redness (a*), and yellowness (b*) values reported by Hussein et al. (2020) are different from those in the current study. Li et al. (2025) reported that functional water systems can enhance meat tenderness and oxidative stability without altering color parameters.

Table 3: The quality parameters of breast meat of broilers supplemented with HMW

Parameters	T_1	T_2	P-value	LS
Cooking loss (%)	26.08±1.09	23.9±1.70	0.215	NS
Drip Loss (%)	2.143 ± 0.26	2.01 ± 0.29	0.785	NS
WHC (%)	94.763± 0.504	96.06 ± 1.005	0.306	NS
Lightness (L*)	41.35 ± 6.26	50.65± 3.81	0.44	NS
Redness (a*)	8.7 ± 1.36	8.39 ± 0.52	0.88	NS
Yellowness (b*)	6.64 ± 0.31	6.24 ± 0.74	0.507	NS

Where, LS = Level of significance, T_1= Normal tape water, T_2= Hydrogen mineralized water, Value indicates – Mean ± Standard Error (SE);

Litter and water microbial qualities

The microbial count of litter materials from the two treatment groups at the 5th week of age is shown in Figure 1. It is interesting to note that there were significant differences ($p>0.05$) between the treatment groups for *Salmonella, E. coli, coliform* and *Lactobacillus.* The number of microbes present in the litter was indicated in log10 CFU/g. The evidence of li et al. (2025) shows that hydrogen-based water systems can reduce planktonic bacteria by >99.99% and significantly inhibit biofilm formation in drinking lines. This refined microbial quality of litter and water helps to reduce ammonia quality and improve overall flock performance (Patel et al., 2025).

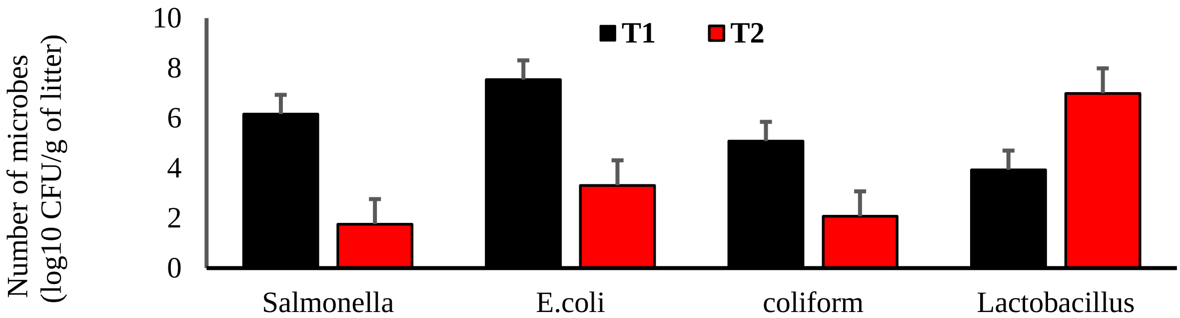

Figure 1: Microbes in the broiler litter

Microbial growth between the water of the two treatment groups is depicted in Figure 2. Here result shows the better quality of hydrogen-mineralized water compared to normal tap water based on the expansion of microbiota. Normal water often shows greater bacterial growth, while hydrogen mineral–sterilized water typically exhibits minimal or no growth. These images highlight the reduced microbial proliferation associated with hydrogen mineral–sterilized water.

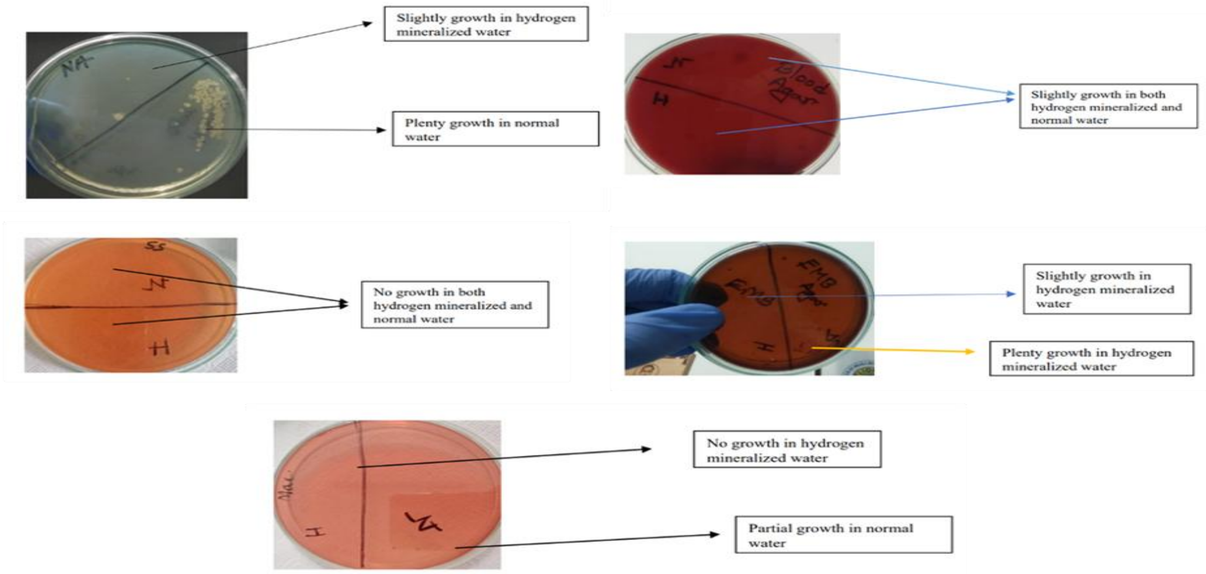

Figure 2: Comparison of microbial growth on culture media treated with normal water versus hydrogen mineral–sterilized water

Conclusion

Based on the findings of the study, it appears that the broiler industry can be exceptionally improved through the administration of hydrogen-rich mineralized water. It indicates broiler chickens can incredibly improve their efficiency without compromising meat quality as well as microbial safety from drinking hydrogen-rich water in a range of ways. The reduction of litter pathogenic microbiota essentially contributes to maintaining environmental hygiene, which is crucial for disease management and flock health in broiler production. By and large, using hydrogen-rich water in the broiler industry can be regarded as a viable, safe, practical, and promising strategy in sustainable broiler production.

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