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Effects of oxygenated or hydrogenated water on growth performance, blood parameters, and antioxidant enzyme activity of broiler chickens FREE

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Abstract

This study was conducted to investigate the effects of providing oxygenated and hydrogenated water on the growth performance, blood biochemical parameters, and immunoglobulin concentrations and antioxidant enzyme activity of broiler chickens. In our investigation, 144 Ross × Ross broiler chicks were randomly allotted to three different treatment groups with four replicates (treatment × replicate × bird = 3 × 4 × 12). All chicks were given one of the following types of water for five weeks: tap water (CON), hydrogenated water (HNW), and oxygenated water (ONW). ONW supplementation increased the final body weight and weight gain and also improved both feed intake and feed conversion of broiler chickens as compared to those of CON broiler chickens ($P < 0.05$). The abdominal fat and its ratio to the final body weight showed that fat accumulation in the broiler chicken abdomen was reduced when broiler chickens drank only ONW for five weeks ($P < 0.05$). ONW supplementation

improved blood parameters, including triacylglyceride, total cholesterol, and low-density lipoprotein-cholesterol. Additionally, in accordance with a globulin increase in broiler chickens, both IgG and IgM generation were significantly enhanced when ONW was supplied to broiler chickens ($P < 0.05$) but only a numerical advance was observed in the HNW group ($P > 0.05$). Both oxygenated and hydrogenated water supplementation significantly improved the antioxidant effects ($P < 0.05$), and it seems that superoxide dismutase refinement was completed due to oxygen and/or hydrogen enhancement of drinking water. These results indicate that oxygen enhancement of drinking water may be recommended to improve growth performance by increasing immunoglobulins mainly IgG and IgM.

Subject: Physiology and Reproduction

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INTRODUCTION

Reactive oxygen species (**ROS**) formed in mitochondria are removed in the form of water due to the activity of superoxide dismutase (**SOD**) and glutathione peroxidase. An excess of ROS can overwhelm antioxidant capacities, resulting in stimulation of nuclear factor-kB (NF-kB), which is a key transcription factor for IgG production (Zhu et al., 2015). Importantly, exogenous supply of oxygen and/or hydrogen supply may influence IgM and IgG production, which are produced by B cells. When hydrogen and oxygen are reinforced through drinking water, individual hydrogen and oxygen atoms can be linked to oxygen and hydrogen in water, respectively, which may supply more hydrogen and oxygen to broiler chickens. In addition, it is possible that drinking hydrogenated and oxygenated water may enhance the available oxygen and hydrogen in the active tissues of the broiler chicken, and hydrogen and oxygen may improve both vitality and immune parameters (Sommer et al., 2007).

A number of oxygenated water products containing 30 to 120 mg/L of oxygen, 7 to 10 times higher than tap and fountain water, are commercially available (Choi et al., 1982; Gruber et al., 2005; Jung et al., 2012a,b); the importance of both are important for the creation of adenosine triphosphate for cellular energy. Indeed, about 97 to 98% of hemoglobin is saturated in healthy humans (Willmert, 2001), which prompts the question does a greater oxygen supply obtained from drinking water can create physiological and immunological differences in broiler chickens.

Therefore, to estimate the improvement of broiler chicken immune system parameters and to identify any enhancement of broiler chicken performance, oxygenated, hydrogenated, and control drinking water was administered to Ross × Ross broiler chickens for five weeks, and immune system and performance enhancement among the groups receiving different water supplies were compared.

MATERIALS AND METHODS

Hydrogenated and Oxygenated Water Generation

The stoma of bamboo stems supply water and nutrients to the plant's side face, and bamboo stems with stomas 10 nm in diameter were selected for generation of hydrogen and oxygen nano-bubbles in this study. Specifically, to produce hydrogenated and oxygenated water, a 250 mm long bamboo stem with a 50 mm diameter was irradiated under 50 kGy to eliminate any hemicellulose from the stomas. Next, normal tap water was bubbled with either pure hydrogen or oxygen via the bamboo stem. Addition of the hydrogen and oxygen supply at 3 bars was completed three times per day for 2 h at room temperature, and the resulting hydrogenated water (**HNW**) or oxygenated water (**ONW**) was supplied to broiler chicks for five weeks. The hydrogen and oxygen content in HNW and ONW were routinely confirmed as containing 1 to 1.5 ppm of hydrogen (HACH Inc., CO) and 40 to 60 ppm of oxygen (YSI Inc., OH), respectively, which are significantly higher than the values for the same substances in tap water (hydrogen 2 to 3 ppb and oxygen 1 to 8 ppm, respectively). Bubbled water has a greater capacity to transfer oxygen and hydrogen than dissolved water, and bubble size and number are important in specifying the quality of nano-bubbled water. The average diameter and number per milliliter of nano-bubbles for hydrogen and oxygen were approximately 250 nm and 2.05×10^8, and 173 nm and 1.75×10^8, respectively (LM10-HS Model Nano Particle Tracking Analyzer, NanoSight Ltd., Amesbury, United Kingdom).

Experimental Design and Birds Management

One-day-old male Ross × Ross male broiler chicks (*Gallus gallus domesticus*) of body weight ranging from 45 to 46 g were used for the study. The chicks were maintained in the chicken house, Department of Animal Biotechnology, Chonbuk National University. They were individually housed under hygienic conditions (25 to 28°C) under a 12 h light/12 h dark cycle. The chicks were allowed free access to commercial diet containing corn and soybean based on the NRC (1994) recommendation. The experimental procedures were approved by the Institutional Animal Care and Use Committee, Chonbuk National University, (No.2012-

1-0014) in accordance with the Korean National Law on Animal Care and Use.

After acclimatization for a period of 1 week, the chicks were divided into the following groups and maintained for a total period of five weeks.

Group 1: **CON**, chicks received the normal tap water (pH 7.3 to 7.4) by nipple drinker for 5 weeks.

Group 2: **HNW**, chicks received hydrogenated treated water (pH 7.3 to 7.4) by nipple drinker for 5 weeks.

Group 3: **ONW**, chicks received oxygenated treated water (pH 7.3 to 7.4) by nipple drinker for 5 weeks.

Water was provided by nipple drinker ad libitum to the chicks and the study consisted of four independent replicate with 12 birds per replicates (12 × 4 × 3 = birds × replications × treatments).

Sample Collection

At the end of the rearing period, all feeds were withdrawn for 12 h and the birds were transported to a pilot processing plant for slaughter. All birds were then shackled, stunned, and sacrificed by cutting the jugular and carotid veins, after which further slaughter processing was conducted. During the slaughter process, 3 mL of blood as well as tissue from the breast muscle were collected and delivered to the laboratory for biochemical analysis. To determine blood biochemical parameters and immune system enhancement, each blood sample collected during the slaughter process was allowed to coagulate for 30 min at room temperature followed by centrifugation at 3,000 rpm for 10 min to separate the serum. All serum samples were then stored at −70°C until analysis. Breast muscles were washed with pH 7.4 phosphate-buffered saline and homogenized in a solution of pH 7.4 phosphate-buffered saline containing ethylene diamine tetra acetic acid. Each homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was collected and used to determine antioxidant enzyme activity.

Growth Performance

At the end of the rearing period, liver, abdominal fat, and spleen material were collected and weighed. To assess the growth performance of broiler chicks, both their initial and final body weights were measured and used to determine the amount of weight gained. The feed intake per chicken pen was recorded at the end of the experiment, and the feed conversion

ratio was calculated based on both the feed intake and weight gain.

Blood Biochemical Parameters, Serum IgG and IgM Concentration

Serum samples from individual animals were used for blood biochemical parameters measurements. The levels of glucose, triacylglyceride (**TAG**), total cholesterol (**TCL**), high density lipoprotein cholesterol (**HDL**-cholesterol), low density lipoprotein cholesterol (**LDL**-cholesterol), total protein, albumin, and globulin were determined using a Hitachi 7600 automatic analyzer (Hitachi Co., Tokyo, Japan). LDL-cholesterol levels were calculated based on the following equation.

$$\begin{aligned}\text{LDL} - \text{cholesterol} = {} & \text{Total cholesterol} \\ & - (\text{HDL} - \text{cholesterol} + \text{TAG}/5)\end{aligned}$$

To determine serum immunoglobulin (Ig) G and M levels, serum immunoglobulin was measured using respective chicken IgG and IgM ELISA kits (Bethyl Laboratories, Montgomery, TX) following the manufacturer's guidelines. Each IgG and IgM level was calculated based on measurement of the absorbance at 450 nm.

Antioxidant Enzyme Activity Analysis

Both serum and breast muscle samples were used to measure catalase, superoxide dismutase (**SOD**), and glutathione peroxidase (**GSH-Px**) enzyme activity and antioxidant activity [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), **ABTS**] using a commercial kit from the Cayman Chemical Company (Cayman Chemical Co., Ann Arbor, MI). Protein quantification for breast muscle was measured using a kit from Bio-Rad (Bio-Rad, Hercules, CA).

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SAS program (SAS, 2002). The level of statistical significance for all data was set at $P < 0.05$, which was determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

There was a significant difference in final body weight and weight gain among the different

groups (Table 1). Specifically, birds supplied with oxygenated water weighed on average 156 g more than birds in the CON group ($P < 0.05$). On the other hand, broiler chicks in the HNW group exhibited an intermediate increase in final body weight and weight gain compared to birds in the CON and ONW groups ($P > 0.05$). Both feed intake and feed conversion effects were improved in birds reared on only HNW and ONW, which also resulted in numerically and significantly altered abdominal fat accumulation in broiler chicks ($P < 0.05$). Weight gain was observed in all groups at the end of the experimental period. However, the final body weight, weight gain, feed intake, and feed conversion were increased in hydrogenated and oxygenated groups than control groups. The abdominal fat and its ratio to the final body weight showed that fat accumulation in the broiler chicken abdomen was reduced when broiler chickens drank only ONW for five weeks. Sommer et al. (2007) reported that supplying ONW to 3 to 6 months old female mice for 22 weeks significantly increases their body weight for the first two weeks, but that this difference is no longer significant after this time period. Thus, weight gain for the first two weeks may be due to the higher concentration of oxygen, as high oxygen concentrations lead to an enhanced rate of oxygen absorption by the body, resulting in increased glycolysis and/or mitochondrial protein synthesis (Willmert et al., 2002; Attaix et al., 2005; Bibby et al., 2005). Increased glycolysis and mitochondrial protein synthesis rates tend to increase myofibrillar protein synthesis, and such increased muscle protein mass results in a higher weight gain and lower amount of feed intake (Balagopal et al., 1997), which may explain the changes in abdominal fat deposition of broiler chicks.

Table 1.

Effects of hydrogenated and oxygenated water supplementation on broiler chicken performance.[1]

	Treatment[2]		
Items	**CON**	**HNW**	**ONW**
Initial body weight (g/bird)	45.6 ± 0.16	46.4 ± 0.53	46.0 ± 0.90
Final body weight (g/bird)	2,167 ± 32^{b}	2,274 ± 38^{a,b}	2,323 ± 44^{a}
Weight gain (g/bird)	2,121 ± 32^{b}	2,227 ± 38^{a,b}	2,277 ± 44^{a}
Feed intake (g/bird)	3,540 ± 19^{a}	3,315 ± 10^{b}	3,381 ± 59^{b}
Feed conversion	1.67 ± 0.04^{a}	1.49 ± 0.04^{b}	1.48 ± 0.04^{b}

Liver (g)	50.2 ± 1.90	47.6 ± 1.61	50.3 ± 1.49
Spleen (g)	2.29 ± 0.19	2.14 ± 0.14	2.51 ± 0.20
Abdominal fat (g)	36.4 ± 3.08[a]	31.2 ± 4.28[a,b]	27.4 ± 1.96[b]
Liver/Final body weight	2.00 ± 0.08	1.92 ± 0.07	2.02 ± 0.07
Spleen/Final body weight	0.09 ± 0.007	0.09 ± 0.006	0.10 ± 0.008
Abdominal fat/Final body weight	1.45 ± 0.12[a]	1.25 ± 0.11[a,b]	1.10 ± 0.08[b]

[a,b]Mean values within a row followed by the same letter are not significantly different ($P < 0.05$).

[1]Each data entry represents the mean ± standard error.

[2]Treatments: CON = tap water group; HNW = hydrogenated water group; ONW = oxygenated water group.

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The serum levels of TAG, TCL, and LDL-cholesterol was decreased in broiler chicks supplied with either HNW or ONW for five weeks compared to CON broiler chicks ($P < 0.05$, Table 2); there was no significance between the HNW and ONW groups ($P > 0.05$). The total protein and albumin contents were not significantly altered between groups ($P > 0.05$), whereas the amount of globulin available in broiler chick serum was influenced by HNW supplementation. Specifically, broiler chicks in the HNW group had a globulin level of 2.30 ± 0.04 g/dL, which was 0.28 g/dL higher than that of broiler chicks in the CON group ($P < 0.05$). While the highest globulin level in this study was observed for the HNW group, generation of IgG and IgM, the levels of which provide an overall picture of immune function, was higher in broiler chicks furnished with ONW, which increased up to 10.58% and 32.97%, respectively, as compared to those of CON broiler chicks ($P < 0.05$). The serum triglyceride, total cholesterol, and low-density lipoprotein-cholesterol in ONW were significantly increased as compared to the other groups. This data was consistent with the long-term study by Nugrahani (2013), who reported that consumption of oxygenated water consumption lowered total cholesterol and LDL-cholesterol levels in the blood plasma of 12 male students who drank oxygenated water and then exercised. Based on the slightly negative and positive charges of oxygen and hydrogen in water, addition molecular oxygen and hydrogen may bind to hydrogen and oxygen of water through ionic bonds. Addition to additional oxygen and hydrogen delivered through oxygenated and hydrogenated water seems to increase oxygen saturation and free hydrogen ion levels in the blood stream of broiler chicks as a result of diffusion (Ignacio et al., 2013).

Table 2.

Effects of hydrogenated and oxygenated water supplementation on broiler chicken blood and immune parameters.[1]

	Treatment[2]		
Items	**CON**	**HNW**	**ONW**
Glucose (mg/dL)	214.4 ± 3.85	208.3 ± 3.13	208.8 ± 4.07
Triacylglyceride (mg/dL)	51.53 ± 3.68[a]	41.49 ± 2.36[b]	42.86 ± 1.96[b]
Total cholesterol (mg/dL)	150.0 ± 9.06[a]	122.2 ± 3.20[b]	113.4 ± 4.08[b]
HDL-cholesterol (mg/dL)	66.38 ± 4.28	65.88 ± 4.14	63.94 ± 3.60
LDL-cholesterol (mg/dL)	206.1 ± 7.97[a]	180.7 ± 5.73[b]	168.8 ± 5.76[b]
Total protein (g/dL)	3.09 ± 0.10	3.18 ± 0.14	3.08 ± 0.08
Albumin (g/dL)	1.09 ± 0.04	0.99 ± 0.04	1.08 ± 0.03
Globulin (g/dL)	2.02 ± 0.07[b]	2.30 ± 0.04[a]	2.10 ± 0.07[a,b]
IgG (mg/mL)	18.62 ± 0.51[b]	19.67 ± 0.58[a,b]	20.59 ± 0.42[a]
IgM (ug/mL)	138.9 ± 13.7[b]	153.7 ± 10.3[a,b]	184.7 ± 13.2[a]

[a,b]Mean values within a row followed by the same letter are not significantly different ($P < 0.05$).

[1]Each data entry represents the mean ± standard error.

[2]Treatments: CON = tap water group; HNW = hydrogenated water group; ONW = oxygenated water group.

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The levels of IgG and IgM were significantly increased in HNW and ONW as compared to the control group. But the trend of globulin levels was not directly correlated with that of IgG and IgM in HNW, which may suggest that hydrogen supplementation did not influence globulin members other than gamma-globulins. Globulins are water-insoluble proteins (Singh et al., 2001), and among the four different types of globulins, gamma-globulin is one of the main globulins and also a component of immunoglobulin (Hodek and Stiborová,

2003). Therefore, an elevated amount of globulin may represent an increase in the ability to produce additional IgG and IgM. The increase in oxidative stress causes decreased immunoglobulin levels and antioxidant enzymes (Ercal et al., 2000) and consumption of fat that contributes the alteration of immunoglobulin levels (IgG and IgM). Our results show administration of HNW and ONW enhanced the IgG, IgM, SOD, and decreased the fat levels. There may be some interconnection among the SOD immunoglobulin and fat. Therefore, we suggest that increased SOD and decreased fat level may influence immunoglobulin such as IgG and IgM. In addition, amelioration of both IgG and IgM may be induced due to the release of interleukin-10 (IL-10) as described by Okada et al. (2003), and excess oxygen levels leading to ROS formation is associated with increased IL-10 production (Kelly et al., 2010). However, as mentioned above, ROS formed during basal metabolism appeared to be scavenged or quenched due to oxygen and hydrogen supplementation, leading to increased levels of both IgG and IgM, which was thought to be due to another interleukin or chemokine such as IL-10, although we did not specifically measure levels of IL-10 in broiler chickens.

Both HNW and ONW supplementation enhanced the antioxidant capacity of broiler chickens to 4.69 mM and 4.36 mM, respectively ($P < 0.05$, Table 3). The catalase was not significantly different between treatments. Along with the improvement of antioxidant effects, an enrichment in SOD activity from 49 to 52% and 20 to 37% in both serum and tissue was observed ($P < 0.05$), respectively. And GSH-Px activities in the serum was not significantly different as compared to Con but HNW and ONW in the breasts was significantly decreased than Con ($P < 0.05$). Both HNW and ONW supplementation significantly enhanced the antioxidant and SOD of broiler chickens ($P < 0.05$). The antioxidant enzyme activity is the first line of defense against ROS and decomposes H_2O_2 to H_2O and O_2 in peroxisomes (Ray and Husain, 2002; Yang and Poovaiah, 2002). This finding was consistent with earlier reports Öztürk-Ürek et al. (2001), who did not observe any improvement in catalase activity but instead in SOD activity following the addition of copper to a chicken's diet. Specifically, as SOD levels increase, more ROS can be converted into H_2O_2; therefore, the activities of both catalase and GSH-Px should increase. However, in this study, catalase was not affected and GSH-Px was significantly decreased ($P < 0.05$). Two different scenarios can be considered in attempting to explain this observation. First, the hydrogen and/or oxygen provided via drinking water to broiler chicks may have reacted with and stabilized ROS levels, while some of the hydrogen and/or oxygen may have been used to form H_2O_2 due to reaction with SOD. Another defense against H_2O_2 is non-enzymatic antioxidant molecules (Finkel and Holbrook, 2000). Although non-enzymatic antioxidants transform H_2O_2 to H_2O, they are not very rapid or reactive substances, but their activity may be accelerated in the presence of increased hydrogen and/or oxygen

levels (Hazra et al., 2008). Furthermore, high concentrations of H_2O_2 may lead to preferential activation of catalase over GSH-Px to convert H_2O_2 to H_2O (Öztürk-Ürek et al., 2001), which may be because GSH-Px is complex and requires additional co-factors and proteins for activation (Weydert and Cullen, 2010). Together, these effects may explain how catalase exhibited sustained activity without any reduction in its levels in the serum or tissue ($P > 0.05$).

Table 3.

Effect of hydrogenated and oxygenated water supplementation on antioxidant enzymes activity of broiler chicken serum and breast muscle.[1]

	Treatment[2]		
Items	**CON**	**HNW**	**ONW**
Antioxidant (mM)	2.50 ± 0.67[b]	4.69 ± 0.30[a]	4.36 ± 0.62[a]
Catalase			
Serum (nmol/min/mL of serum)	18.23 ± 2.07	12.56 ± 1.48	14.22 ± 2.19
Tissue (nmol/min/mg of protein)	477.00 ± 68.60	435.36 ± 22.72	484.65 ± 43.65
Superoxide dismutase			
Serum (U/mL of serum)	4.77 ± 1.04[b]	9.45 ± 1.27[a]	9.88 ± 0.94[a]
Tissue (U/mg of protein)	8.73 ± 0.55[c]	13.86 ± 0.43[a]	11.02 ± 1.08[b]
Glutathione peroxidase			
Serum (nmol/min/mL of serum)	232.4 ± 43.1	258.3 ± 56.8	333.4 ± 58.4
Tissue (nmol/min/mg of protein)	6.86 ± 1.65[a]	3.13 ± 0.60[b]	2.53 ± 0.69[b]

[a–c]Mean values within a row followed by the same letter are not significantly different ($P < 0.05$).

[1]Each data entry represents the mean ± standard error.

[2]Treatments: CON = tap water group; HNW = hydrogenated water group; ONW = oxygenated water group.

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In conclusion, the results of the present study showed that supplying either a hydrogenated or oxygenated water supply to broiler chicks for five weeks led to reduced TAG, TCL, and LDL-cholesterol levels and increased immunoglobulin levels. Additionally, enzymatic antioxidant effects were significantly improved by providing either hydrogenated or oxygenated water to broiler chicks. Along with increased TAG and cholesterol levels and antioxidant defense capacity, oxygenated water supply appears to improve the feed conversion ratio, which led to a higher final body weight. Hence, application of ONW appears to be a possible new therapeutic approach for increasing antioxidant enzymes, immune system, and lipid lowering activity in chicken and it could be responsible for the positive effects to health and animal welfare.

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