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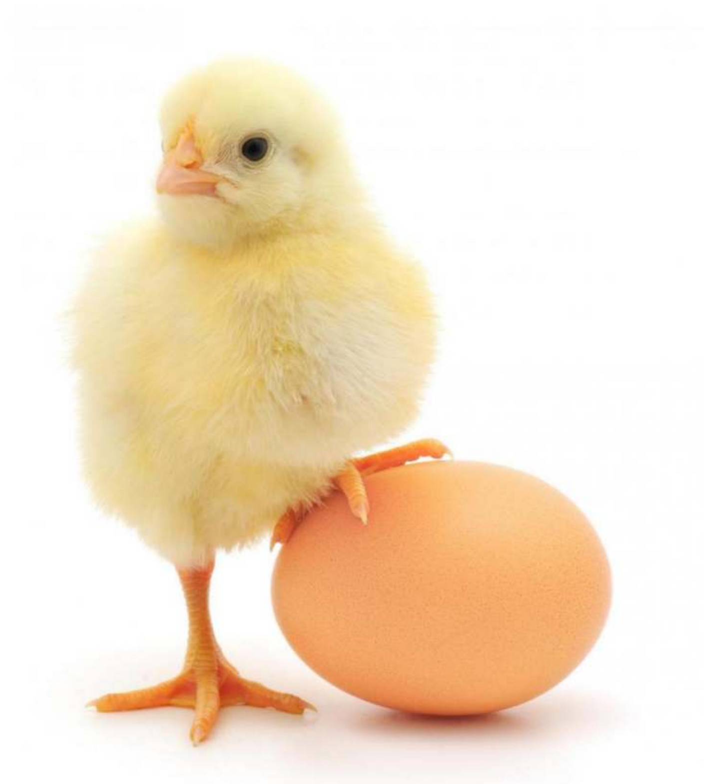

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Research Article
Effect of Magnetically Treated Water on Physiological and Biochemical Blood Parameters of Japanese Quail

Ali H. Al-Hilali

Department of Animal Production, College of Agriculture, Al-Muthana University, Al-Muthana, Iraq

Abstract
Objective: The present study examined the effect of magnetically treated water (MTW) on the physiology and biochemistry of Japanese quail to increase the production of poultry in Iraq. **Materials and Methods:** Sixty male Japanese quail were divided into three groups and given tap water treated with a 0 (control), 500 (T1), or 1000 (T2) Gauss magnetic field for 60 days. Changes in the chemical and physical properties of the water were examined with and without magnetization. Biochemical and physiological parameters measured in quail included red and white blood cell counts (RBCs and WBCs, respectively), packed cell volume (PCV), hemoglobin (Hb), cholesterol, lactate dehydrogenase (LDH), very low density lipoprotein (VLDL), low density lipoprotein (LDL), triglycerides, glucose, total protein levels, aspartate transferase, alanine transferase, alkaline phosphatase (AP) activity, aspartate transaminase (AST), alanine aminotransferase (ALT) and the mitotic index. **Results:** Magnetic treatment of water significantly increased RBCs, WBCs, Hb, PCV%, total protein levels, AP activity and the mitotic index but caused a significant decline in total cholesterol, LDL, VLDL, triglycerides, glutathione (GSH) and glucose levels. There were no significant differences in aspartate or alanine transferase activity between either treatment groups compared to control. **Conclusion:** The results indicate that MTW improved all biochemical and physiological properties of Japanese quail, which could help to improve poultry productivity.

Key words: Magnetic water treatment, productivity, Japanese quail, aspartate transaminase, alanine aminotransferase

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Corresponding Author: Ali H. Al-Hilali, Department of Animal Production, College of Agriculture, Al-Muthana University, Al-Muthana, Iraq

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is one of the most important components of life. Physiologically, it is needed to transport compounds via blood to maintain cellular structural integrity and regulate temperature as well as other processes[1]. Interestingly, living cells have magnetic properties and magnetic healing treatments date back as early as ancient Greece[2,3]. In recent years, several studies have suggested that magnetic fields may have possible biological effects on the health of humans and animals[4]. For example, the equilibrium of living cells has been shown to be restored with the help of magnetic field manipulation[5]. Water and water-based solutions that pass through magnetic fields acquire a finer and more homogeneous structure as various minerals are dissolved and removed. This increases fluidity and improves the biological activity of these solutions, which positively affects the performance of humans, animals and plants that consume or absorb them[6]. The effects of magnetically treated water (MTW) on poultry have been previously reported[7]. Studies have found that use of MTW increased animal yield 5-30%[8] and significantly increased lipid and protein metabolism as reflected by altered blood glucose levels and accelerated glycolysis and glycogenesis in humans[9]. Moreover, cows that drank MTW have been shown to produce more milk and were generally healthier than cows drinking untreated water[10]. In addition, sheep produce more wool and meat[11], chickens lay more eggs[12] and all farm animals examined survive longer when given MTW compared to untreated water[13]. The present study examined the effects of MTW on physiological and biochemical parameters of Japanese quail to increase poultry production in Iraq. The effects of magnetic treatment on various physical and chemical water properties was also evaluated.

MATERIALS AND METHODS

Animals and housing: Sixty male Japanese quail (*Coturnix coturnix japonica*) weighing 10-11 g each at the beginning of the experiment were maintained under normoxic conditions and housed in groups (ten birds per one cage) in stainless steel cages at 25°C with a 16 h light/8 h dark cycle. Birds were randomly divided into three equal groups (n = 20 each) and given untreated tap water (control) or tap water treated with a 500 (T1) or 1000 (T2) Gauss (Gs) magnetic field. Birds were provided a basal diet and drinking water *ad libitum* for 60 days, with fresh water provided every 8 h, following the magnetic funnel manufacturer's recommendations. Magnetized water was prepared by passing it through a 500 or 1000 Gs magnetic funnel (Magnetic Technologies, LLC, Germany, registered patent No. 1826921) at a relatively low speed to prevent overflow and collected into graduated cylinders for distribution.

Biochemical analysis of blood: At the end of the experiment, blood samples (2 mL each) were collected into vials containing (ethylenediaminetetraacetic acid) EDTA for hematological analyses or heparin for biochemical studies, blood was drawn by cardiac puncture. Blood samples were divided into two equal parts. One part was used to measure red and white blood cell (RBCs and WBCs, respectively) numbers, hemoglobin (Hb) levels and packed cell volume (PCV) and the other was used for measuring physiological and biochemical aspects. The RBCs and WBCs were measured according to a previous method using a hemocytometer[14] and Hb was measured using a cyanomethemoglobin method[15]. Blood collected in chilled, heparinized tubes was immediately centrifuged at 3000 rpm for 15 min and aliquots of plasma were stored at -20°C for lipid profile analyses. Serum lipids examined included triacylglycerol, total cholesterol, total protein and high density lipoprotein-cholesterol (HDL-C) using enzymatic kits (Linear Chemicals, Barcelona, Spain). Low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were assessed according to a previous method by Friedewald *et al.*[16] and plasma glucose was measured using a commercialized glucose oxidation method according to the manufacturer's instructions (Sigma Diagnostics Inc., Michigan, USA). Alanine and aspartate aminotransferase (ALT and AST, respectively) activity in plasma were measured using a commercially available kit according to the manufacturer's instructions. Serun blood tests were performed using a commercial kit (Biomagherb). Serum glutathione was measured according to a previous study[17]. Blood serum mitotic index (MI) was calculated using the following equation:

$$MI = \frac{\text{Number of bone marrow cells undergoing mitosis}}{\text{Total number of bone marrow cells}}$$

Physical and chemical properties of water: The following physical properties of magnetized and unmagnetized water were measured at the Water Technology Laboratories of the

Ministry of Science and Technology (Iraq): pH, electrical conductivity, dissolved oxygen content, surface tension, chloride concentration, salinity, total dissolved salts, viscosity and evaporation temperature.

Bone marrow extract: Bone marrow aspirations were obtained from 16 clinically healthy Japanese quail (8 male and 8 female). All birds were free of hematological abnormalities upon peripheral blood examination. The medial aspect of the proximal tibiotarsus bone, just below the femoral-tibiotarsal joint, was aseptically prepared and 22 gauge disposable marrow aspiration needles were used to obtain samples. The area was anaesthetized locally by subcutaneous infiltration of 1-1.5 mL of 2% lignocaine HCl (Cambrex Pharmaceutical Products Co., USA) over the periosteum. The aspiration biopsy needle was held perpendicular to the bone and advanced into the marrow space by applying light pressure and using slight rotatory motions. With the needle in the marrow space, the stylet was removed and a syringe was locked into the needle. The samples were collected into 5 mL syringes containing EDTA. At least five air-dried wedge slides of bone marrow smears were prepared from each pheasant. Slides were stained with Giemsa and were evaluated for cellularity and classification of erythroid, myeloid and thrombocytic precursors. Each sample was used for a 500-cell differential count to classify the marrow precursors in each cell series and to determine myeloid:erythroid (M/E) ratios for each pheasant. The M/E ratio was determined by dividing the total number of all nucleated cells of the granulocytic series by the total number of all nucleated cells of the erythrocytic series[18].

Statistical analysis: Data were analyzed using a completely random design. The data shown represent means±standard error. Significant differences between means were calculated using least significant differences (LSD). The data were analyzed by SPSS software version 21.0 for Windows 7 (SPSS Inc., Chicago, IL, USA)[19]. The results were analyzed using a t-test for comparison between two sexes. Statistical significance was set at $p<0.05$.

RESULTS

Physical and chemical effects of magnetic treatment of water: The effects of magnetic treatment on various physical and chemical properties of water are summarized in Table 1. The conductivity and total amount of dissolved salts in MTW significantly increased, especially with 1000 Gs, compared to untreated tap water, while the density, viscosity and surface tension decreased. No significant changes were observed in pH, salinity, dissolved oxygen content or chloride concentration.

Effect of MTW on physiological performance of quail: The effect of MTW on RBCs, WBCs, Hb and PCV are summarized in Table 2. The results showed that MTW treatment significantly increased ($p\leq0.05$) serum Hb levels, RBC and WBC numbers and PCV compared to controls. The mean number of RBCs in control birds was 3.60±0.4 (10^6 cells mm^{-3}) and the mean percentage of PCV in T1 and T2 birds was 35.09 and 35.56%, respectively (Table 2).

Table 1: Physical and chemical properties of untreated and magnetically treated water

Parameters	Untreated	500 Gs	1000 Gs	LSD value
Conductivity (ms cm^{-1})	650.00 ± 8.12^b	655.00 ± 8.6^b	710.00 ± 8.9^a	25.50
Density (mn mL^{-1})	50.10 ± 2.25^a	40.00 ± 2.01^b	40.04 ± 2.12^b	8.25
Total dissolved salts (ppm)	4.93 ± 0.21^a	4.96 ± 0.16^a	5.15 ± 0.19^a	0.12
Dissolved O_2 (mg L^{-1})	496.00 ± 23.3^b	498.00 ± 20.3^b	456.00 ± 25.56^a	150.25
pH	7.60 ± 0.07^b	7.62 ± 0.05^b	7.85 ± 0.02^a	0.10
Salinity (ppt)	0.28 ± 0.002^a	0.29 ± 0.003^a	0.30 ± 0.002^a	0.08
Surface tension (dyn cm^{-2})	60.52 ± 2.81^a	52.40 ± 2.85^b	50.42 ± 2.93^b	2.56
Chloride concentration (ppm)	55.52 ± 2.81^a	47.62 ± 2.25^b	42.62 ± 0.28^b	3.56
Viscosity (cSt)	0.79 ± 0.01^a	0.81 ± 0.008^a	0.82 ± 0.006^a	0.05
Evaporation temperature (g h^{-1})	0.76 ± 0.23^a	0.77 ± 0.22^a	0.75 ± 0.21^a	0.08

[a,b,c]Means within a row lacking a common superscript differ significantly. Gs: Gauss, LSD: Least significant difference, Dyne per square centimeter (dyn cm^{-2})

Table 2: Effect of magnetically treated water on blood cells in Japanese quail

Parameters	Control	T1	T2	LSD value
RBC (10^6 cells mm^{-3})	3.60 ± 0.40^c	4.36 ± 0.42^b	5.32 ± 0.46^a	0.54
WBC (10^6 cells mm^{-3})	6.16 ± 0.52^b	$6.52\pm0.50^{a,b}$	6.95 ± 0.45^a	0.63
Hb (g dL^{-1})	13.20 ± 1.06^b	13.46 ± 1.54^b	14.05 ± 1.35^a	0.34
PCV (%)	33.50 ± 2.01^c	35.09 ± 2.20^b	35.56 ± 2.05^a	0.88

[a,b,c]Means within a row lacking a common superscript differ significantly. Control: Quail given water treated with 0 Gauss, T1: Quail given water treated with 500 Gauss, T2: Quail given water treated with 1000 Gauss. RBC: Red blood cells, WBC: White blood cells, Hb: Hemoglobin

Table 3: Effect of magnetically treated water on biochemical parameters in serum of Japanese quail

Parameters	Control	T1	T2	LSD value
Glucose	108.02±2.54[a]	94.02±3.75[b]	76.15±4.50[c]	8.15
Total protein (g L^{-1})	3.13±0.21[b]	3.26±0.15[b]	3.42±0.11[a]	0.24
Total cholesterol (mg dL^{-1})	95.00±4.25[a]	75.30±3.75[b]	75.00±4.46[c]	12.50
LDL-C (mg dL^{-1})	92.70±9.91[a]	79.61±8.36[b]	68.36±6.52[c]	8.42
VLDL-C (mg dL^{-1})	21.79±1.34[a]	19.64±1.40[b]	17.36±1.53[c]	1.46
HDL-C (mg dL^{-1})	33.07±0.67[c]	34.94±0.94[b]	38.26±0.26[a]	3.62
Triglycerides (mg dL^{-1})	100.60±4.02[a]	92.30±3.96[a]	86.80±3.88[a]	9.16

[a,b,c]Means within a row lacking a common superscript differ significantly. Control: Quail given water treated with 0 Gauss, T1: Quail given water treated with 500 Gauss, T2: Quail given water treated with 1000 Gauss. LSD: Least significant difference, LDL-C: Low density lipoprotein-cholesterol, VLDL-C: Very low density lipoprotein-cholesterol, HDL-C: High density lipoprotein-cholesterol

Table 4: Effect of magnetically treated water on enzyme activity in serum of Japanese quail

Parameters	Control	T1	T2	LSD value
AST (U L^{-1})	70.20±1.90[a]	70.00±1.70[a]	71.00±1.80[a]	1.18
ALT (U L^{-1})	7.62±0.23[a]	7.42±0.20[a]	7.45±0.25[a]	0.96
ALKP (U L^{-1})	96.00±4.60[b]	100.04±4.16[a,b]	120.00±4.22[a]	10.54
GSH (mg dL^{-1})	2.70±0.18[c]	3.16±0.22[b]	4.90±0.26[a]	0.35

[a,b,c]Means within a row lacking a common superscript differ significantly. Control: Quail given water treated with 0 Gauss, T1: Quail given water treated with 500 Gauss, T2: Quail given water treated with 1000 Gauss. LSD: Least significant difference, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALKP: Alkaline phosphatase, GSH: Glutathione

Table 5: Effect of magnetically treated water on Mitotic index in bone marrow in Japanese quail

Parameter	Control (%)	T1 (%)	T2 (%)	LSD value
Mitotic index	5.45±0.62[c]	6.3±0.75[b]	8.5±0.77[a]	0.78

[a,b,c]Means within a row lacking a common superscript differ significantly. Control: Quail given water treated with 0 Gauss, T1: Quail given water treated with 500 Gauss, T2: Quail given water treated with 1000 Gauss, LSD: Least significant difference

Effect of MTW on serum biochemical and lipid profiles: Remarkably, consumption of MTW significantly decreased ($p \leq 0.05$) serum glucose, total cholesterol, LDL-C, VLDL-C and triacylglycerol levels compared to controls but significantly increased ($p<0.05$) total serum protein and HDL-C levels (Table 3). These trends became more apparent from T1 to T2 groups.

Effect of MTW on serum AST, ALT, alkaline phosphatase (ALKP) and reduced GSH levels: Serum AST and ALT activities did not change significantly when Japanese quail were supplied MTW compared to controls given untreated tap water (Table 4). On the other hand, ALKP activity in T2 birds significantly increased ($p<0.05$) versus both control and T1 groups, however, no significant difference was overserved between T1 and control treatment. Furthermore, the MTW was found to have a protective effect on the antioxidant status of the Japanese quail since a significant elevation ($p<0.05$) in serum GSH concentration (μmol L^{-1}) was detected in T1 and T2 groups compared to controls (Table 4).

Effect of magnetized water on the mitotic index of bone marrow: The mitotic index is an important test for determining the extent of genetic damage. The results showed a significant increase ($p \leq 0.05$) in the mitotic index of birds supplied MTW relative to controls (5.45%). In addition, this increase was enhanced from T1 (6.3%) to T2 (8.5%) treatment groups (Table 5).

DISCUSSION

The results of the present study showed that exposure to MTW caused a variety of significant metabolic and physiological changes within Japanese quail, which were related to changes in the chemical and physical properties of drinking water exposed to a magnetic field. A previous study showed that the crystal shape of water molecules changes from substrate-bound solidification to separate disc-shaped crystals and this phenomenon was used to measure the quantum magnetic field by calculating the reduction in substrate-bound levels[20]. Moreover, changes in the physical and chemical properties of MTW have been shown to be caused by changes in the bond angles of molecules from 105-103°[21].

In physics, water is known to change weight under the influence of magnetic fields. At the chemical level, more hydroxyl ions are created, which form alkaline molecules and reduce acidity. Indeed, an increase in both the conductivity and dielectric constant of water has been documented following exposure to magnetic fields[22]. Some studies have also reported that magnetic treatment of water affects properties, such as light absorbance, pH, surface tension[23] and the amount of dissolved oxygen[24]. Changes in the surface tension of water over time can be the key to tracing impurities[25]. Furthermore, normal water has a pH of approximately 7, whereas magnetized water can reach a pH of 9.2 following exposure to 7000 Gs for a long period of time[26]. Thus, an applied magnetic field may affect the formation of hydrogen bonds in water, leading to conformational changes and these changes may be the cause of the observed variations in both conductivity and dielectric constant[21]. Hence, it was hypothesized that MTW may be able to increase Hb levels in Japanese quail due to the physical and chemical effect of the magnetic field on water in the blood. Administration of MTW to animals has been shown to increase the ability of blood to supply nutrients and gases to the body, glands and cells[27]. This was thought to be caused by the decreased viscosity of MTW in the bloodstream, which increases flow through vessels, thereby increasing Hb movement[28]. Moreover, MTW has an increased dissolved oxygen content, which enhances blood oxygen levels by making more oxygen available to bind Hb molecules.

The increased PCV in birds that were provided MTW in the current study may be due to a positive correlation between PCV and Hb. The benefit of MTW on PCV and Hb may be attributed to increased production of RBCs and WBCs in the bone marrow, changes in hormonal regulation of the circulatory system[12] and/or improved immune function. The present study showed that supplementation of MTW resulted in a significant increase in RBCs, which is in agreement with previous studies[29,30]. This was attributed to their increased production in the bone marrow. This result was predicted to significantly change the metabolic status of cells when coupled with increased blood flow, amount of available oxygen and Hb (a main component of RBCs) levels[31]. The magnetic field causes an increase in the attraction of iron from the blood and this connection produces large blood quantities with a concomitant increase in the number of RBCs and Hb[27]. In this study authors detected a shift in cellular metabolism, since the glucose levels in quail provided MTW were significantly reduced compared to controls. This is in direct contrast to results of other studies that have reported increases in serum glucose levels[29,32], however, one of these studies examined the effects of subacute exposure to a static magnetic field[28], which is likely the cause of the inconsistency. On the other hand, it is possible that prolonged exposure to a magnetic field might induce an adaptive response that leads to an increase in glucogenesis to compensate for the increase in metabolic activity[33]. Furthermore, an increase in Hb levels also inherently implies an increase in iron levels, since this element is a key component of each Hb subunit. Taken together with the increased conductivity of MTW, this may also increase the conductivity of the blood[34].

The decrease in total cholesterol, triglycerides and VLDL-C levels as well as the elevation in HDL-C levels detected in the sera of MTW treated birds indicates that MTW may induce hypotriglyceridemia and the beneficial elevation of "good" cholesterol. The therapeutic effect of a single exposure to an electromagnetic force on obesity and complicated hyperlipidemia was previously shown to increase serum HDL-C levels but decrease total cholesterol in the liver of rats[35,36]. Current study showing that MTW increased total serum protein levels suggests a change in protein levels of stressed poultry, while neither AST nor ALT activity was affected by MTW. These results slightly contradict those of a previous study showing that serum protein levels as well as ALT and AST activity remained unchanged after exposure to a static magnetic field, leading to the conclusion that drinking MTW does not harmfully affect hepatocytes[12]. On the other hand, MTW (especially 1000 Gs) significantly increased ALKP activity and GSH levels in sera of treated birds compared to controls in the current study. These results suggest that exposure to MTW affects the antioxidant capacity of cells in addition to lipid peroxide levels, further supporting the likelihood of improved immune function. Finally, the mitotic index was also found to be increased from 5.4% in controls to 6.3% in T1 and 7.5% in T2 birds. The heightened mitotic index in T2 quail was due to activation of mitotic indices in the bone marrow as a result of the decreased surface potential of water, which increased cell division.

CONCLUSION

It is concluded that magnetically treated water (MTW) improved all biochemical and physiological properties of Japanese quail, which could help to improve poultry productivity.

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