Impact of using magnetized water and time-restricted feeding on hematological cellular parameters in Japanese quail (*Coturnix Japonica*)

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□ ABSTRACT □

This study aimed to evaluate the effects of magnetized water, time-restricted feeding, and their interaction on hematological traits of Japanese quail. A total of 312 chicks were randomly assigned to four treatments (78 birds each): T1 (natural water with ad libitum feeding), T2 (natural water with 5 h feed restriction), T3 (magnetized water with ad libitum feeding), and T4 (magnetized water with 5 h feed restriction).

The results showed significant increases ($P \leq 0.05$) in red blood cell count, hemoglobin concentration, and hematocrit, and a decrease in white blood cell count and H/L ratio in males and females consuming magnetized water compared with normal water. Feed restriction had no significant effect in the blood parameters of males, whereas females showed reduced red blood cells and hemoglobin without changes in hematocrit.

Interaction results showed that T3 and T4 had higher red blood cell count, hemoglobin concentration, and hematocrit, and lower white blood cell count and H/L ratio than T1 and T2. All hematological values remained within or very close to normal physiological ranges, magnetized water improved physiological status in Japanese quail.

Keywords: Magnetized water, Time-restricted feeding, Japanese quail, Hematological parameters**.**

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تأثير استخدام الماء الممغنط والتقنين الغذائي الزمني في بعض المؤشرات الدموية الخلوية لدى طيور الفري الياباني (*Coturnix japonica*)

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□ ملخّص □

يهدف البحث إلى دراسة تأثير استخدام الماء المعالج مغناطيسياً وبرنامج التقنين الغذائي الزمني والتداخل بينهما في بعض المؤشرات الدموية لدى طيور الفري الياباني. قُسم 312 فرخاً بشكل عشوائي على أربع معاملات (T1: معاملة الماء العادي والتغذية الحرة، T2: معاملة الماء العادي والتقنين الغذائي لمدة 5 ساعات يومياً، T3: معاملة الماء الممغنط والتغذية الحرة، T4: معاملة الماء الممغنط والتقنين الغذائي لمدة 5 ساعات).

أظهرت النتائج ارتفاعاً معنوياً ($P\leq0.05$) في عدد كريات الدم الحمراء وتركيز خضاب الدم ومكداس الدم، وانخفاضاً معنوياً في عدد الكريات البيضاء ونسبة H/L في ذكور وإناث المعاملة التي استهلكت الماء الممغنط بالمقارنة مع التي استهلكت الماء العادي. لم يظهر التقنين الغذائي فروق معنوية في المؤشرات الدموية لذكور الفري، بينما سجلت الإناث انخفاضاً معنوياً في كريات الدم الحمر والهيموغلوبين دون وجود فروق معنوية في تركيز مكداس الدم. كما بينت نتائج التداخل تفوق ذكور وإناث معاملات T3 و T4 معنوياً في عدد كريات الدم الحمراء وتركيز الهيموغلوبين ومكداس الدم وانخفاض في عدد الكريات البيض ونسبة H/L مقارنة بمعاملات T1 و T2، وكانت جميع القيم الدموية ضمن النطاقات الفيزيولوجية الطبيعية أو قريبة منها، وحسّن استخدام الماء الممغنط الحالة الفيزيولوجية لدى طيور الفري الياباني.

الكلمات المفتاحية: الماء الممغنط – التقنين الغذائي الزمني– الفري الياباني – المؤشرات الدموية.

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Introduction:

Water is one of the most essential requirements for poultry and represents a critical strategic resource in all forms of life, being the second most vital component for living organisms after oxygen. It is indispensable for digestion, nutrient absorption, body temperature regulation, and immune system function [1]. Water quality directly and indirectly affects the health and growth of birds, particularly when it contains harmful substances, elevated mineral levels, altered color, pH, salinity, or certain bacterial species, which can cause pollution and significantly impact physiological functions during poultry development [2,3].

With advances in technology and scientific research, water treatment has become crucial to enhance its properties and positively influence poultry growth. Studies have shown that Using magnetization can change the properties of water to become more homogeneous by reorganizing water molecules, which have random charges in ordinary water [4,5]. Magnetization process affects the bond angle between the oxygen atom and the two hydrogen atoms in the water molecule, causing the hydrogen bonds to break. This angle becomes 103 degrees instead of 105 degrees in ordinary water molecules, resulting in smaller water clusters, This is attributed to the water molecules gathering into groups of (6-7) molecules instead of (12-17-100) molecules per cluster, meaning the clusters are reduced to smaller molecules [6], This results in increased partial permeability of magnetized water and the substances it carries through cell walls, thus facilitating the penetration of magnetically treated water into cell membranes and consequently increasing the utilization of ingested feed by enhancing the absorption of nutrients and minerals in the body [7,8,9]. Additionally, the magnetic field also contributes to reducing water viscosity due to decreased surface tension, achieved by reducing the number and strength of hydrogen bonds in the water. This reduces its viscosity by up to 4%, making it a highly effective solvent, which helps it carry larger quantities of nutrients. This facilitates the transport and absorption of nutrients, minerals, and vitamins, furthermore lower surface tension helps increase diffusion and the movement of water molecules to all parts of the body compared to ordinary water [10,11,12,13], treating water with a magnetic field also raises the pH from neutral to slightly alkaline, as exposing water to a magnetic field produces hydroxyl ions (OH) and makes water molecules alkaline. [6, 13,14,15].

Poultry is a cornerstone of animal protein production, playing a vital role in meeting human protein needs. While Japanese quail farming is not a recent development, it still lacks an optimal, cost-effective feeding system to achieve maximum productivity at the lowest possible cost. Quail meat is an important food source in the poultry industry, as the bird's nutritional requirements are limited. Therefore, implementing timed feeding rationing programs helps ensure these requirements are met and reduces waste [16]. These programs aim to reduce carcass fat deposition [17,18], as excess fat increases feed consumption and production costs, representing an economic loss for producers [19].

Moreover, the use of time-restricted feeding with a limited number of hours contributes to stimulating compensatory growth, which enables birds to reach a suitable body weight at marketing age, similar to the weight of free-feeding birds, while reducing feed waste [20,21,22, 23].

Significance and Objectives of the Study:

The importance of this research stems from its focus on the impact of using magnetized water as a modern trend in improving physiological performance, alongside time-restricted feeding, a widely used dietary practice that has garnered increasing attention in recent studies. Given that blood is the primary biomarker reflecting the health and physiological state of an organism, studying the changes occurring in its cellular components as a result of applying these two factors, either together or separately, represents a crucial step in understanding physiological responses. This contributes to exploring the extent to which this integration enhances overall health and supports productive efficiency in the animal sector. Therefore, the research objectives focused on:

1. Determining the effects of magnetized water and time-restricted feeding on certain blood cellular traits.
2. Evaluating the interaction between magnetized water and time-restricted feeding to identify the optimal program for improving physiological performance in birds.

Materials and methods:

Location where the experiment was conducted:

The study was conducted in a private poultry farm located in the Maarashhour area of Hama Governorate, Syria, during the period from April 4 to November 20, 2024.

Experimental design:

A total of 312 unsexed Japanese quail chicks, one day old and approximately uniform in body weight, were used in the experiment. The chicks were randomly divided into two treatments during the first and second weeks of rearing, with each treatment comprising 156 chicks. The treatments were as follows: (T1) natural water treatment, (T2) magnetized water treatment, each treatment included six replicates, with 26 chicks per replicate. Magnetized water was provided to the T2 group from the start of the rearing period at one day of age until the end of the experiment. Feed was offered ad libitum during these two weeks. Table (1) shows the number of treatments chicks and the intensity of magnetization applied during the first two weeks of the rearing period.

Table 1. illustrates the number of treatments, chicks, and the magnetization intensity applied during the first two weeks of the rearing period.

Treatments	Type of water	Magnetization intensity (Gauss)	Time required for water to circulate from the tank to the magnetizer (in minutes)	Number of birds per replicate	Number of birds
T1	Natural water	-	-	26	156
T2	Magnetized water	3323	60	26	156

Starting from the third week, a timed feeding program was introduced for 5 hours daily, from 12 pm to 5 pm, and continued until the end of the trial period in three replicates for both birds consuming natural water and birds consuming magnetized water, while maintaining free feeding in the other three replicates, where each of the three replicates was considered a treatment. Consequently, the chicks were divided into four treatments starting from the third week, with each treatment comprising 78 chicks:

T1: Natural water with ad libitum feeding.
T2: Natural water with time-restricted feeding for 5 hours per day.
T3: Magnetized water with ad libitum feeding.
T4: Magnetized water with time-restricted feeding for 5 hours per day.

Each replicate contained 26 chicks (Table 2). Prior to the implementation of this program, some technical modifications were made within the poultry house: a section of 25 m² was divided into four compartments (treatments) using partitions. The chicks were reared on wood shavings litter, and plastic drinkers with a capacity of 1.5 liters were used. The time-restricted feeding program continued until the end of the experiment.

Source of Magnetized Water:

The magnetization of water was performed using a water magnetization device with an intensity of 3,323 Gauss, equivalent to 332.3 milliTesla (Figure 1). The magnetic field intensity was measured using a (Sensor Mobile-CASSY 524 009) device from (LD Didactic GmbH, Germany), in the laboratories of the Faculty of Science (Physics Department) at the University of Latakia (Figure 2). A water treatment device was installed on the farm's main tank, and the water flow rate through the magnetization device was (0.058 L/S). A plastic tank was used to collect the water after magnetization.

The water was recycled from the tank back to the magnetizer using a pump for 60 minutes to ensure the quality of the magnetization process. (Table 1, 2).

Table 2. Experimental design and treatments from the third week onwards.

Treatments	Type of water	Magnetization intensity (Gauss)	Time required for water to circulate from the tank to the magnetizer (in minutes)	Number of birds	Number of birds per replicate
T1	Natural water	-	-	78	26
T2	Natural water	-	-	78	26
T3	Magnetized water	3323		78	26
T4	Magnetized water	3323		78	26

Figure 1. Measuring the magnetic field strength of a magnetization device.

Figure 2. Measuring the magnetic field strength of a magnetization device.

Bird Watering:

Regular and magnetized water were continuously provided to the experimental treatments using 1.5-liter drinkers. The drinkers were emptied and refilled every 6 hours periodically to maintain the properties of the magnetically treated water. natural water was supplied to the control group (natural water treatment) before magnetic treatment.

Feeding:

The birds were fed three pelleted diet formulations. Table (3) presents the feeding program applied during the rearing period, including the composition of the feed mixtures and their energy and protein contents, formulated according to the nutritional requirements of the birds at different growth stages.

Table 3. Percentage composition and energy and protein contents of the experimental diets for quail.

Ingredient %	Starter diet (1–4 weeks)	Grower diet (4–6 weeks)	Layer/Production diet (6 weeks until the end of the experiment)
Yellow corn	31.8	48	43
Wheat	25	9	20
Soybean meal	32	34	22
Protein concentrate	9	5	6
Fat	0.7	2	2
Limestone	1.25	1.7	6.75
Salt	0.25	0.3	0.25
Diet composition during the rearing period			
Crude protein %	24.66	21.7	20.04
Metabolizable energy (kcal/kg)	2999.2	2900	3106.7

All experimental birds were treated under the same management, care and feeding conditions throughout the experiment. All birds in the different treatments were exposed to continuous lighting (day and night) during the first two weeks of the birds' lives. Then the number of hours of lighting was reduced to 22 hours during the third and fourth weeks, to 21 hours during the fifth week, then to 20 hours during the sixth week, and then reduced to 17 hours until the end of the experiment.

Blood Sampling:

Collection of blood samples: Blood samples were taken from 96 birds aged 44 days, at a rate of 2 ml of blood. Some samples were taken at the time of slaughter of (3 males, 3 females) from each replicate, with (9 males, 9 females) from each treatment. To increase accuracy, additional blood samples were taken from the wing vein of the birds, with (1 male, 1 female) from each replicate, i.e. (3 males, 3 females) from each treatment, Therefore, the total number of blood samples taken from the birds became 24 samples (12 males and 12 females) from each treatment, and placed in tubes containing the anticoagulant heparin to estimate some cellular characteristics.

Hematological Parameters:

Red Blood Cell Count, Hemoglobin Concentration, and Hematocrit: I use a Mindray analyzer to analyze Japanese quail blood samples to measure some cellular blood indicators, including red blood cell count (RBC), hemoglobin concentration (Hb), and hematocrit (HCT).

White Blood Cell Count:

The direct method for white blood cell counting, involving the use of Natt and Herick's solution, was employed with a hemocytometer. Blood was drawn to the 0.5 mark in the dilution pipette, and the diluent was drawn to the 101 mark to achieve a dilution ratio of 200:1. After thoroughly mixing the blood and diluent, a drop of the diluted blood (after discarding the first two drops) was placed over the hemocytometer's counting chamber. The white blood cells were then counted in nine large squares within the ruler of the hemocytometer chamber. The white blood cells appear dark blue and may be granular in shape. The white blood cell count was calculated using the following equation:

White blood cell count ($\times 10^3$ cells/mm^3) = Total number of cells in nine squares + (10% of the total white blood cell count) × 200.

Heterophil/Lymphocyte Ratio (H/L Ratio):

The H/L ratio was calculated using the following formula [24]:

$$H/L = \frac{\text{Number of heterophil cells per 100 cells(H)}}{\text{Number of lymphocyte cells per 100 cells(L)}}$$

Statistical Analysis:

The data of this study were analyzed using a Completely Randomized Design (CRD) based on a factorial experiment with two factors: the first being water type (normal water and magnetized water), and the second being feeding regime (ad libitum feeding and feed restriction for 5 hours per day), including their interaction effects.

Data analysis was performed using the statistical software SPSS version 24, and the significance of differences among the studied means was tested using the Least Significant Difference (LSD) test at a 5% level of significance ($P \leq 0.05$). In addition, the GenStat software was used to determine the LSD values for the interaction effects.

The mathematical model used for data analysis was as follows:

$Y_{ijk} = \mu + D_i + E_j + (DE)_{ij} + e_{ijk}$

Where:

Y_{ijk} = the observation value of the k-th replicate under the i-th water type and the j-th feeding regime.

μ = overall mean.

D_i = effect of the water type (i = magnetized or normal).

E_j = effect of the feeding regime (j = ad libitum or feed restriction for 5 hours per day).

$(DE)_{ij}$ = interaction effect between water type and feeding regime.

e_{ijk} = experimental error.

Results and Discussion:

Red Blood Cells (RBCs):

Table (4) illustrates the effect of water quality and time-restricted feeding on red blood cell counts in male and female Japanese quail at 44 days of age. A significant effect ($P \leq 0.05$) of water quality was observed on RBC counts in both sexes. Birds that consumed magnetized water exhibited higher RBC counts, reaching 4.03 and 3.37 million cells/mm³ in males and females, respectively, compared with male and female birds that consumed ordinary water, the cell count was (3.66, 3.11) million cells/mm3 respectively. It is noteworthy that despite the significant differences observed among treatments, all recorded RBC values remained within the normal physiological range reported for Japanese quail (approximately $2.90 – 4.00 \times 10^6$ cells/mm³) at 42 days of age [25]. Therefore, the observed increases and decreases reflected normal physiological responses to the experimental treatments. This increase may be attributed to increased red blood cell production in the bone marrow [10]. Magnetized water is characterized by its low viscosity, which facilitates nutrient absorption, improves blood circulation in tissues, and enhances the absorption of essential hematopoietic minerals, particularly iron and compounds involved in hemoglobin synthesis, thus increasing hemoglobin and red blood cell levels in the body [7]. In addition, magnetized water may exert an antioxidant effect by reducing free radical formation and maintaining the integrity of blood cells, thereby reducing red blood cell destruction and positively affecting blood parameters and the overall physiological state of birds [10, 26]. On the other hand, the increased red blood cell count in the magnetized water treatment can be attributed to the rise in live body weight, there is a direct relationship between live weight and red blood cell count [27]. At 44 days of age, the live body weight in males and females treated with magnetized water was 192.97 and 265.83 g, respectively, while in males and females treated with natural water it was 183.56 and 246.16 g, respectively. These results are consistent with the findings of [10, 28, 29] who all reported a significant increase ($P \leq 0.05$) in RBC counts in quail consuming magnetized water compared to those consuming natural water. However, they contrast with the findings of [30, 31, 32], who did not observe a significant effect of magnetized water administration on red blood cell count in broiler chickens compared to birds that consumed natural water.

Regarding the effect of feeding regime, a non-significant increase ($P > 0.05$) in RBC counts was observed in male quail under ad libitum feeding compared to those subjected to 5-hour feed restriction. This finding agrees with [33], who reported no significant effect on RBC counts in ducks subjected to feed withdrawal or light restriction for 5 hours/day compared to ad libitum feeding. In contrast, a significant increase ($P \leq 0.05$) in RBC counts was observed in females under ad libitum feeding, which reached 3.29 million cells/mm³, compared with 3.18 million cells/mm³ in restricted-fed females, corresponding to a 3.23% increase. This result is consistent with [34], who showed that following a feed-withholding regimen for (3, 4,5, 6) non-consecutive days from 8 to 18 days of age had a negative effect and led to a significant decrease ($P \leq 0.05$) in the red blood cell count for the 6-day feed-withholding treatment compared to the other restriction treatments. This deficiency may be attributed to the lack of continuity in the internal relative stability of blood components due to stress and its hormones affecting the metabolism (breakdown) of some cell components in general, including red blood cells, when there is an increased need to form glucose from non-carbohydrate sources [35, 36]. The significant increase ($P \leq 0.05$) in RBC counts in females under ad libitum feeding may therefore be linked to higher live body weights, It was found that the average live weight of free-feeding females at 44 days of age was (258.85)g, which was significantly higher ($P \leq 0.05$) compared to the females in the restrictive diet treatment, which was (253.15)g, in agreement with the positive correlation between body weight and hematological traits such as RBC count, hemoglobin concentration, and hematocrit [27]. These findings also align with [37], who observed significantly ($P \leq 0.05$) higher RBC counts in heavier quail compared with lighter birds. However, they do not agree with [38], who noted a significant increase in the number of red blood cells at 42 days of age for the rationing treatments compared to the control treatment (ad libitum).

As for the interaction between water type and feeding regime, analysis of variance revealed a significant effect ($P \leq 0.05$) on RBC counts. Male and female quail in the magnetized water treatments under both ad libitum feeding (T3) and feed restriction (T4) showed higher RBC counts of 4.06, 4.00, 3.40, and 3.34 million cells/mm^3, respectively, compared with 3.72, 3.60, 3.19, and 3.03 million cells/mm^3 in the natural water treatments under ad libitum feeding (T1) and feed restriction (T2). This effect may be attributed to the role of magnetized water in alleviating stress and stress- and its hormones that affect the metabolism (breakdown) of certain cellular components [22], in addition to its role in increasing live body weight, enhancing nutrient absorption, and blood flow through the blood vessels from the cells [13]. A significant decrease in the number of red blood cells in the blood of female birds treated with T2 was also observed, reaching (3.03) million cells/mm^3 compared to treatment T1 (3.19) million cells/mm^3, this may be due to the effect of stress resulting from dietary restriction [35, 36].

Table 4. Red blood cell count in the blood of Japanese quail under the studied treatments during the experimental period (mean ±SD).

Red blood cell count (10^6 cells/mm^3)			
Sex		Males	Females
Effect of water			
Natural water		3.66 ± 0.115^b	3.11 ± 0.108^b
Magnetized water		4.03 ± 0.089^a	3.37 ± 0.082^a
Effect of feed restriction			
Ad libitum feeding		3.89 ± 0.203^a	3.29 ± 0.139^a
Feed restriction (5 hours)		3.80 ± 0.236^a	3.18 ± 0.182^b
Effect of interaction			
Natural water	Ad libitum feeding	3.72 ± 0.098^b	3.19 ± 0.088^b
	Feed restriction (5 hours)	3.60 ± 0.116^b	3.03 ± 0.050^c
Magnetized water	Ad libitum feeding	4.06 ± 0.095^a	3.40 ± 0.083^a
	Feed restriction (5 hours)	4.00 ± 0.092^a	3.34 ± 0.085^a
L.S.D for interaction		0.1909	0.0640

Different letters beside the means within each column indicate significant differences ($P \leq 0.05$).

Hemoglobin concentration:

Table (5) shows a significant effect ($P \leq 0.05$) of water type on hemoglobin concentration in the blood of male and female Japanese quail. The treatment using magnetized water resulted in higher hemoglobin concentrations, reaching 18.41 g/100 ml in males and 15.15 g/100 ml in females, compared to the treatment using natural water, where hemoglobin concentrations in the blood of males and females in this treatment were 16.56 and 13.95 g/100 ml, respectively, that consumed normal water. It is noteworthy that despite the significant differences observed among treatments, the hemoglobin values recorded in the present study remained within or very close to the physiological range reported for Japanese quail of a similar age, which is (12–17 g/100 ml) ml at 42 days old [39]. Therefore, the observed increases and decreases in hemoglobin concentration can be considered normal physiological responses to the experimental treatments. This increase may be attributed to the role of magnetized water in improving the bioavailability of minerals and nutrients essential for hemoglobin synthesis, particularly iron, thereby enhancing heme synthesis processes within hematopoietic tissues [7, 10]. Furthermore, the antioxidant effect of magnetized water may help maintain erythrocyte integrity and hemoglobin stability within red blood cells, while reducing their rate of destruction, which is reflected in increased hemoglobin concentration in the blood [10, 26]. The results may also to an increased number of red blood cells in birds that consumed magnetized water, since hemoglobin concentration depends on RBC numbers, given that hemoglobin is a protein carried on these cells, a positive correlation between hemoglobin values and RBC counts has been reported [40]. These findings are consistent with [7, 10, 32], who reported a significant (P≤0.05) increase in blood hemoglobin in Japanese quail and broiler chickens consuming magnetized water compared to normal water. In contrast, [31] did not observe a significant effect (P≤0.05) in the hemoglobin ratio of birds that consumed magnetized water from day one to 32 days of age compared to birds that consumed natural water.

As shown in Table (5) shows that there was no significant effect (P≤0.05) of using dietary restriction on hemoglobin concentration in the blood of Japanese quail males between the treatment that consumed ad libitum and the treatment that was subjected to dietary restriction, although a slight numerical increase was observed in the ad libitum treatment. This is consistent with [33], who did not observe any significant differences (P>0.05) in hemoglobin levels between ducks that were rationed and those that were ad libitum. In contrast, a significant reduction (P≤0.05) in hemoglobin concentration was observed in female quail exposed to feed restriction (13.81 g/100 ml) compared to females fed ad libitum (14.68 g/100 ml). This reduction may be attributed to the lower red blood cell count [40]. Similar findings were reported by [34], who observed a significant decrease (P≤0.05) in blood hemoglobin concentration in broilers subjected to feed restriction. The higher hemoglobin concentration in males compared to females may be attributed to the physiological effects of male hormones, particularly testosterone, which stimulates red blood cell production and increases hemoglobin synthesis. Conversely, the relatively lower values in females may be due to the use of some iron and nutrients in reproductive processes such as egg formation [41].

Regarding the interaction, Table (5) indicates a significant increase (P≤0.05) in hemoglobin concentration in both male and female quail of treatments T3 and T4, reaching 18.49, 18.34, 15.25, and 15.05 g/100 ml, respectively, compared to T1 and T2, which recorded 16.70, 16.42, 14.10, and 13.81 g/100 ml, respectively. This increase may be attributed to the role of magnetized water in enhancing red blood cell counts [7,8,9, 10].

Table 5. Hemoglobin concentration in the blood of Japanese quail under the studied treatments during the experimental period (mean ±SD).

Hemoglobin concentration (g\100 ml)			
Sex		**Males**	**Females**
Effect of water			
Natural water		**16.56±0.363^{b}**	**13.95±0.210^{b}**
Magnetized water		**18.41±0.244^{a}**	**15.15±0.211^{a}**
Effect of feed restriction			
Ad libitum feeding		**17.59±1.045^{a}**	**14.68±0.661^{a}**
Feed restriction (5 hours)		**17.38±1.066^{a}**	**14.43±0.684^{b}**
Effect of interaction			
Natural water	**Ad libitum feeding**	**16.70±0.461^{b}**	**14.10±0.201^{b}**
	Feed restriction (5 hours)	**16.42±0.240^{b}**	**13.81±0.065^{b}**
Magnetized water	**Ad libitum feeding**	**18.49±0.338^{a}**	**15.25±0.261^{a}**
	Feed restriction (5 hours)	**18.34±0.136^{a}**	**15.05±0.111^{a}**
L.S.D for interaction		**0.5985**	**0.3332**

Different letters beside the means within each column indicate significant differences (P ≤0.05).

Hematocrit concentration:

Table (6) shows a significant effect (P≤0.05) of water type on hematocrit percentage. Male and female Japanese quail that consumed magnetized water showed a significant increase (P≤0.05) in hematocrit levels of 5.40% and 9.83%, respectively, compared to the treatment with natural water. It is noteworthy that all hematocrit values recorded in the present study were generally within the normal physiological range reported for Japanese quail (approximately 35 – 46%) at 42 days of age [42], with only slight elevation observed in the upper values, indicating that the changes remained within acceptable biological limits and reflected physiological. This improvement is attributed to the positive relationship between red blood cell counts and hematocrit percentage [10, 27, 43], supporting the physiological link between these hematological parameters. These results are consistent with [10, 32], who observed a significant increase (P≤0.05) in hematocrit levels in Japanese quail and broiler chickens consuming magnetized water compared to those consuming normal water, while [31] found a non-significant decrease in the hematocrit ratio in the blood serum of broiler chickens that consumed magnetized water compared to birds that consumed natural water.

On the other hand, Table (6) indicates no significant differences (P>0.05) in hematocrit concentration in the blood of between male and female Japanese quail fed ad libitum compared to those under feed restriction, a slight increase was observed in favor of ad libitum feeding, which may be attributed to the positive relationship between hematocrit percentage and red blood cell counts [43]. This aligns with [44], who observed no significant differences in hematocrit concentration when using feed withholding feed in broiler chickens compared to free-feeding birds, whereas [45] noted a significant decrease (P≤0.05) in hematocrit due to feed restriction, which progressively worsened with increasing restriction levels.

The table also shows a significant interaction (P≤0.05) between water type and feeding regime on hematocrit percentage. A significant increase (P≤0.05) was observed in males and females receiving magnetized water under ad libitum feeding (T3) and feed restriction (T4), with hematocrit levels of 47.09, 46.60, 43.64, and 42.42%, respectively. In contrast, males and females consuming normal water under ad libitum feeding (T1) and feed restriction (T2) recorded 44.86, 44.02, 39.81, and 38.56%, respectively. This effect is likely due to the role of magnetized water in increasing red blood cell counts and body weight [7,8,9,10,11,12, 13].

Table 6. Hematocrit concentration (%) in the blood of Japanese quail under the studied treatments during the experimental period (mean ±SD).

Hematocrit concentration (%)			
Sex		Males	Females
Effect of water			
Natural water		44.44±0.980[b]	39.18±1.145[b]
Magnetized water		46.84±0.814[a]	43.03±1.106[a]
Effect of feed restriction			
Ad libitum feeding		45.97±1.594[a]	41.72±2.272[a]
Feed restriction (5 hours)		45.31±1.510[a]	40.49±2.311[a]
Effect of interaction			
Natural water	Ad libitum feeding	44.86±1.215[b]	39.81±1.045[b]
	Feed restriction (5 hours)	44.02±0.635[b]	38.56±1.008[b]
Magnetized water	Ad libitum feeding	47.09±1.076[a]	43.64±0.890[a]
	Feed restriction (5 hours)	46.60±0.565[a]	42.42±1.072[a]
L.S.D for interaction		1.726	1.895

Different letters beside the means within each column indicate significant differences (P ≤0.05).

White Blood Cell Count:

Table (7) shows a significant reduction (P ≤0.05) in white blood cell (WBC) counts in both male and female birds consuming magnetized water, reaching 26.60 and 22.05 $\times 10^3$ cells/ mm^3, respectively, compared to 28.54 and 23.86 $\times 10^3$ cells/ mm^3 in birds consuming Natural water. It is noteworthy that all WBC values recorded in the present study were generally consistent with the normal physiological limits reported for Japanese quail (approximately 20–27 $\times 10^3$ cells/mm^3) at 42 days of age [46], suggesting that these variations fall within the expected biological variability and do not indicate pathological leukocytic alterations Values close to the upper limit may indicate a minor physiological response rather. This may be attributed to the role of magnetized water in reducing stress, as a high stress level may increase the number of white blood cells [30, 47].

The absence of a significant effect of feed restriction on WBC counts in the current study indicates that the applied time-restricted feeding program did not impose sufficient physiological stress to alter leukocyte profiles. This finding is consistent with [34], who indicated no significant differences in white blood cell count at 56 days of age between treatments of removing feed from broiler chickens for (3,4,5,6) non-consecutive days from 8-18 days of age compared to the free-feeding treatment. In contrast, [38] reported a significant increase in WBC counts in Japanese quail subjected to feed deprivation compared to freely fed birds.

As observed in Table (7), there was a significant decrease (P≤0.05) in the white blood cell count in males and females treated with magnetized water and free nutrition (T3) and in those treated with magnetized water and dietary restriction (T4). The counts were (26.31, 21.75, 26.88, 22.36) thousand cells/mm3 respectively, compared to males and females treated with plain water and dietary restriction (T2) (28.90, 24.28) thousand cells/mm^3. This may be attributed to the role of magnetized water in reducing stress even with the use of dietary restriction programs, and thus led to a decrease in the number of white blood cells, which is associated with a decrease in the H/L ratio in the blood as an indicator of reduced [28, 47].

Table 7. White blood cell counts in quail blood across the studied treatments during the experimental period (mean ±SD).

White Blood Cell Count (10^3 Cells \mm^3)			
Sex		**Males**	**Females**
Effect of water			
Natural water		**28.54±0.615^a**	**23.86±1.087^a**
Magnetized water		**26.60±1.276^b**	**22.05±0.879^b**
Effect of feed restriction			
Ad libitum feeding		**27.24±1.459^a**	**22.59±1.237^a**
Feed restriction (5 hours)		**27.89±1.375^a**	**23.32±1.436^a**
Effect of interaction			
Natural water	**Ad libitum feeding**	**28.18±0.482^{ab}**	**23.44±1.133^{ab}**
	Feed restriction (5 hours)	**28.90±0.567^a**	**24.28±1.064^a**
Magnetized water	**Ad libitum feeding**	**26.31±1.576^b**	**21.75±0.645^b**
	Feed restriction (5 hours)	**26.88±1.160^b**	**22.36±1.115^b**
L.S.D for interaction		**1.972**	**1.901**

Different letters beside the means within each column indicate significant differences (P ≤0.05).

Heterophil-to-Lymphocyte (H/L) Ratio:

Table 8 shows a significant decrease (P≤0.05) in the heterophil-to-lymphocyte (H/L) ratio in both male and female quail consuming magnetized water, reaching 0.325 and 0.324, respectively. The H/L values obtained in the present study (0.322–0.367) correspond to the low-stress category described for avian species, where H/L ratios of approximately 0.2, 0.5, and 0.8 are indicative of low, moderate, and high stress levels, respectively. This suggests that the experimental treatments did not induce marked physiological stress and that the birds maintained a satisfactory physiological status throughout the experimental period [48]. This reduction may be attributed to the stress-reducing effect of magnetized water [47], as elevated WBC counts and H/L ratios are recognized physiological indicators of stress in birds [49]. The H/L ratio is widely used as a reliable indicator of stress in birds, as it is closely associated with activation of the hypothalamic–pituitary–adrenal axis and increased corticosterone secretion under stressful conditions [50, 51] these findings are consistent with [28], who reported a significant decrease in heterophil percentages in Japanese quail consuming magnetized water at 1000 and 1500 Gauss compared to birds consuming natural water at six weeks of age. Conversely, lymphocyte percentages increased significantly in the magnetized water treatments, resulting in a reduced H/L ratio. In contrast, [31] observed a slight, non-significant reduction in H/L ratios at 36 days of age in broilers consuming magnetized water compared to those receiving natural water.

Regarding feed restriction, Table 8 indicates no significant effect (P>0.05) on the H/L ratio in male and female quail, the absence of a significant effect of feed restriction on the H/L ratio in the present experiment indicates that the applied five-hour daily feed restriction was insufficient to induce a pronounced stress response in Japanese quail. Although a numerical increase in the H/L ratio was observed in restricted-fed birds, this increase did not reach statistical significance, implying that short-term feed deprivation can be tolerated without major alterations in leukocyte distribution. This is consistent with [52], who indicated that there were no significant differences in the H/L ratio at 42 days of age between the feed restriction treatments (withholding feed for 3 and 4 hours daily at 7, 9, 11, 13, 15, 17, 19, and 21 days) and the free-feeding treatment. However, [53] observed a significant increase in H/L ratios in broiler breeders aged 12 weeks exposed to intermittent feed restriction programs (2 non-consecutive days of deprivation followed by 5 days of ad libitum feeding from 6 to 9 weeks) compared to ad libitum-fed controls.

Table 8. Heterophil-to-lymphocyte (H/L) ratio in quail blood across the studied treatments during the experimental period (mean ±SD).

Heterophil-to-Lymphocyte (H/L) Ratio			
Sex		Males	Females
Effect of water			
Natural water		0.354 ± 0.021^{a}	0.353 ± 0.024^{a}
Magnetized water		0.325 ± 0.017^{b}	0.324 ± 0.019^{b}
Effect of feed restriction			
Ad libitum feeding		0.333 ± 0.020^{a}	0.331 ± 0.018^{a}
Feed restriction (5 hours)		0.344 ± 0.028^{a}	0.346 ± 0.031^{a}
Effect of interaction			
Natural water	Ad libitum feeding	0.342 ± 0.019^{ab}	0.339 ± 0.020^{ab}
	Feed restriction (5 hours)	0.363 ± 0.021^{a}	0.367 ± 0.022^{a}
Magnetized water	Ad libitum feeding	0.323 ± 0.019^{b}	0.322 ± 0.013^{b}
	Feed restriction (5 hours)	0.324 ± 0.020^{b}	0.325 ± 0.027^{b}
L.S.D for interaction		0.03833	0.03833

Different letters beside the means within each column indicate significant differences (P ≤0.05).

Analysis of variance indicates a significant interaction (P≤0.05) between the effect of magnetized water and dietary restriction on the H/L ratio. A significant decrease (P≤0.05) was observed in males and females of treatments T3 and T4, reaching (0.323, 0.324, 0.322, 0.325) respectively, compared to males and females of treatment T2 (0.363, 0.367) respectively. This may be attributed to the role of magnetized water in reducing stress [47]. No significant difference in the H/L ratio was observed between treatments T3, T4, and treatment T1.

Conclusions and Recommendations:

Conclusions:

The use of magnetized water has had a positive effect on the efficiency of blood functions, as reflected by significant increases in red blood cell (RBC) counts, hemoglobin concentration, and hematocrit values, indicating improved oxygen-carrying capacity of the blood. Conversely, significant reductions in white blood cell (WBC) counts and the heterophil-to-lymphocyte (H/L) ratio suggest a potential role of magnetized water in reducing stress response and improving the physiological status of birds.

Time-restricted feeding for five hours had no significant effect on most hematological parameters. However, female Japanese quail exhibited significant reductions in RBC counts and hemoglobin concentration, suggesting that females were more sensitive to feed restriction than males. No significant differences were observed in hematocrit values, WBC counts, or H/L ratios between ad libitum-fed and feed-restricted birds.

The positive role of the intervention when using magnetized water, whether with free feeding or time-restricted feeding, contributed to improving some blood indicators by increasing the number of red blood cells and hemoglobin concentration, with a significant reduction in the number of white blood cells and the H/L ratio Compared to the treatment of ordinary water and feed rationing.

Recommendations:

The use of magnetized water in the care of Japanese quail has shown positive effects in improving some productive and blood indicators, which may contribute to raising productive efficiency and improving the physiological condition of the birds.

Investigate sex differences when implementing time-restricted feeding programs, especially since females have shown greater sensitivity in hematological parameters compared to males, necessitating the design of sex-specific experiments.

Study different time-restricted feeding periods to determine the optimal duration that minimizes negative effects on females while maintaining physiological balance.

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