

EFFECT OF DRINKING IONIZED WATER ON HISTOLOGICAL CHANGES, BACTERIA COUNT, AND SOME HEMATOLOGICAL PARAMETERS OF JAPANESE QUAIL

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ABSTRACT

This experiment was conducted to study the effect of drinking ionized water on histological changes, bacteria count, and some hematological parameters of Japanese quail. A total of 200-42-day-old female Japanese quails were divided into five treatments four replicates each treatment (40 birds/replicate). The birds of each treatment were divided into four replicates. The pH level of drinking water of the first treatment was 7, whereas the pH levels of drinking water of other treatments were 8, 9, 6, and 5 respectively. The results showed that villi height, crypt depth, and mucous layer thickness of the duodenum were increased ($P<0.05$) in T4 and T5. The normal histological structure was observed in the microscopic section of the liver of the treated group. Also, the pH9 of drinking water showed large vacuoles in the epithelial layer which support the lymphoid follicles in the section of the bursa. There were thickening and degeneration changes in the capsule with lymphocyte aggregation in birds that received pH9 of drinking water. The levels of pH of drinking water affect WBC, Hb, and PCV. Drinking acidic ionized water increased the number of *Lactobacilli* bacteria and decreased the number of *Coliform* bacteria.

Key words: ionized water, villi high, crypt depth, blood parameters, Japanese quails

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تأثير شرب الماء المتأين على التغيرات النسيجية، العد الميكروبي وبعض قياسات الدم للسمان الياباني
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المستخلص

أجريت هذه التجربة لدراسة تأثير شرب الماء المتأين على التغيرات النسيجية، العد الميكروبي ومعلمات الدم لطيور السمان الياباني. وزعت 200 اناث طيور السمان بعمر 42 يوم الى خمس معاملات وطيور كل معاملة قسمت الى أربع مكررات (40 طير/مكرر). مستوى الاس الهيدروجيني لماء الشرب لطيور المعاملة الأولى 7بينما مستويات الاس الهيدروجيني لماء الشرب للمعاملات الأخرى 8، 9، 6 و 5 على التوالي. أشارت النتائج الى ارتفاع ($P<0.05$) طول الزغابات وعمق الخبايا وسمك الطبقة المخاطية في صائم طيور المعاملة الرابعة والخامسة. لوحظ نسيج طبيعي في المقطع المجهرى لكبد الطيور المعاملة بالماء المتأين. اثرت ($p<0.05$) مستويات الاس الهيدروجيني لماء الشرب على كريات الدم البيض WBC و الهيموكلوبين Hb و حجم الخلايا المكدسة PCV. زاد الماء المتأين من ارتفاع في عدد بكتريا العصيات اللبنية *Lactobacilli* وخفض عدد بكتريا القولونية *Coliform*.

كلمات مفتاحية: الماء المتأين، طول الزغابة، عمق الخبايا، قياسات الدم، طائر السمان.

INTRODUCTION

The domesticated quail birds originated from domesticated Japanese quail *Coturnix Japonica* of Japan in the eleventh century. The bird is available in many countries such as Spain, France, Japan, China, Egypt, Saudi Arabia, and Syria (18). The originating of the largest amount of quail meat is exported by France and America, and quail layers are mostly exported by Japan, and Korea (9). Ionized water is a specific type of functional water and is produced using electrolysis or a magnetic field. The water is generated near the cathode or anode is electrolyzed water. The electrolyzed oxidizing water has sterilization activity due to hypochlorous acid, chlorine gas, and ozone (12). The raw water parameters in the filter stations usually include agglomeration, sedimentation, and filtration as well as adding chlorine. The use of neutral electrolyzed water in poultry has a positive impact on the health of the birds (20). (6,22) noted that the low intestine pH led to improvements in the productive performance of birds. (7) noted that drinking electrolyzed water significantly improved growth performance, feed consumption, and antioxidant activity in chicks exposed to a temperature of 34° C. (1) noted that using acetic acid in bird diets led to a improvement in body weight, body weight gain, feed conversion ratio, the morphology of intestine parts, and *Lactobacillus* count. Food Sterilization using electrolyzed water does not affect the smell, color, consistency, or flavor (4). (3 ,21) noted acidification with addition propionic acid to the diet has appositive effect on productive performance. (13) noted that acidic water led to an increase in calcium content, HDL, total protein, and albumin. But decrease cholesterol content of blood serum. (2) noted that using acidic water led to improvement of villi high, crypt depth, and the thickness of intestinal mucosa. The effect of ionized drinking water on the histological changes and microbial count of Japanese quail was not studied yet. That is why performing the current study.

MATERIALS AND METHODS

The experiment was conducted at a private farm. The birds were raised for eighteen weeks. Two hundred Japanese quail 42 days old were divided randomly into five treatments, each four replicates formed one treatment. Feed and water were provided ad libitum (Table 1). The diets were formulated according to (19). The birds were housed in metal battery cages. The treatments were as follows: the birds received drinking water (pH7) were the first treatment (control). The birds received levels 8, 9, 6, and 5 of pH drinking water were the other treatments respectively. Ionized water (acidic or alkaline) was produced according to the method described by a Bawell apparatus, as shown in figure 1.

Table 1. The chemical compositions of the experiment diet

Ingredients	%
Corn	30
Wheat	31.9
Soybean	25
Concentrated Protein*	5
Corn Oil	2
Limestone	5.5
D.C.P.	0.3
NaCl	0.3
Total	100
Calculated analysis	
Energy (Kg/cal)	2894
Protein (%)	19.5
Lysine (%)	1.2
Methionine (%)	0.57
Ca (%)	2.5
Available P (%)	0.49

The chemical composition of the feed was calculated according to the 20. Hold Mix type concentrated protein from Jordan was used. Each kg of the feed contained 40% raw protein, 3.5% fat, 1% raw fiber, 6% calcium, 2100 kca of assimilated energy, 3% phosphorous, 2.20% salt, 3.25% lysine, 3.50% methionine, 3.90% methionine + cysteine, 40,000 international units of vitamin D₃, 15 mg of vitamin B₁, 300 mg of vitamin B₁₂, 30 mg of vitamin K₃, 100 Mcg of biotin, 100 mg of copper, 1200 mg of manganese, 15 mg of iodine, 2 mg of selenium, and 10 mg of folic acid.



Figure 1.Bawell_SM1 Water Ionizer

Histological analysis

The samples (1cm²) of the liver, and duodenum were taken at 18 weeks and stored immediately in 10% buffer formaldehyde solution. The samples were conducted for histological analysis according to the method described by (24).

Blood tests

At 18 weeks of age, five blood samples of each treatment were collected from the brachial wing vein of birds. The collected blood samples in anti-clotting K2EDTA tubes were subjected to biochemical analysis. Hemoglobin concentration (Hb) was determined according to the method described by (28). RBC and WBC were conducted according to the method described by (10). PCV was determined using the micro hematocrit method.

Bacterial count

At the end of the experiment, four birds from each treatment were slaughtered, and the guts

were opened immediately. Count of *Lactobacilli* and *Coliform* bacteria was performed as the method described by (23)

Statistical analysis

The design of this experiment was a completely randomized design (CRD). The Duncan test (11) was used to compare among means using the SAS program (25).

RESULTS AND DISCUSSION

The significant differences in morphological values among treatments were shown in Table 2. Birds that drank drinking water pH 5 and 6 had higher villi height ($P \leq 0.05$), and mucosa thickness ($P \leq 0.05$), While birds that drank drinking water pH5 had the highest crypt depth ($P \leq 0.05$). Also, birds that drank normal drinking water with pH 7 had the highest villi width ($P \leq 0.05$). In addition, birds drank drinking water at pH 5 had the lowest ($P \leq 0.05$) length epithelium compared with other treatments.

Table 2. Effect of ionized water on duodenum morphology of Japanese quail

Treatments	Villi height	Villi width	Crypt depth	Epithelium length	Mucous layer thickness
T1	1125.00±42.57 ^b	350.00±17.68 ^a	220.00±25.50 ^b	50.00±3.45 ^a	1490.00±50.99 ^b
T2	1145.00±61.93 ^b	162.00±5.83 ^b	124.00±9.27 ^c	50.50±3.91 ^a	1465.00±26.93 ^b
T3	890.00±34.1 ^c	180.00±16.58 ^b	146.00±5.1 ^c	53.50±1.87 ^a	1080.00±18.37 ^c
T4	1420.00±26.69 ^a	200.00±17.68 ^b	118.00±9.17 ^c	37.50±3.45 ^b	1790.00±23.18 ^a
T5	1450.00±23.72 ^a	168.00±9.7 ^b	262.00±9.7 ^a	56.00±2.03 ^a	1752.00±46.77 ^a
Level of significance	*	*	*	*	*

Mean± the standard error.*Different letters in the same column indicate significant differences between treatments at P < 0.05.T1: the control treatment, T2: pH 8, T3: pH 9, T4: pH 6, and T5: pH 5.

Histological results

Control group: Liver: The tissue section of the control group showed normal histology structure Fig. (1).

Treated groups

Livers: The tissue section of the all-treated group showed normal histology structure Fig. (2 and 3).

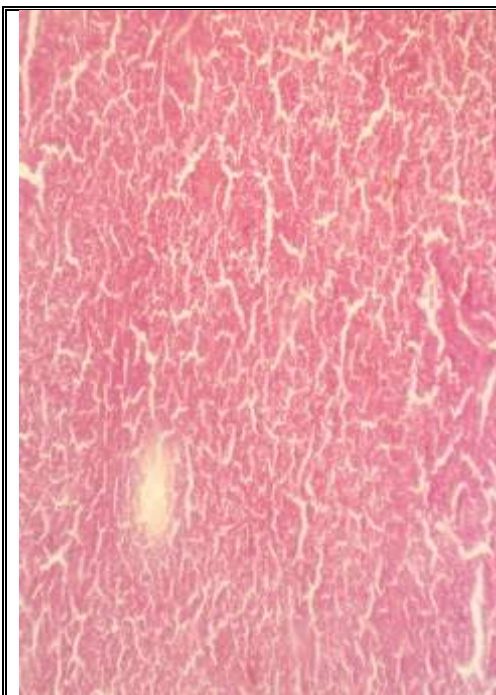


Figure1. T1, Liver of control group showing normal histological structure (H &E 400X)

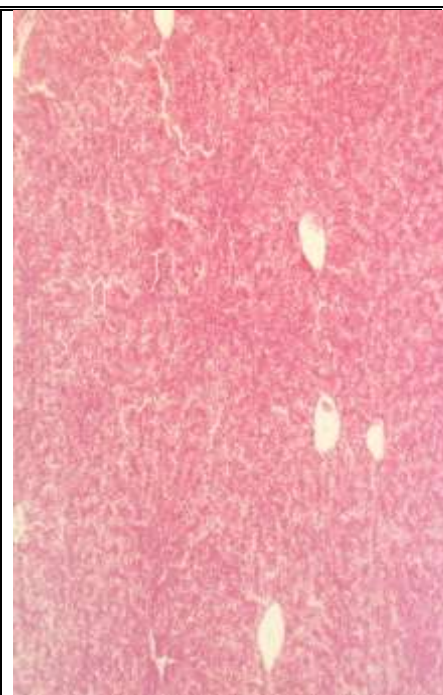


Figure 2. T3,Liver of Treated group showing normal histological structure (H &E 400 X).

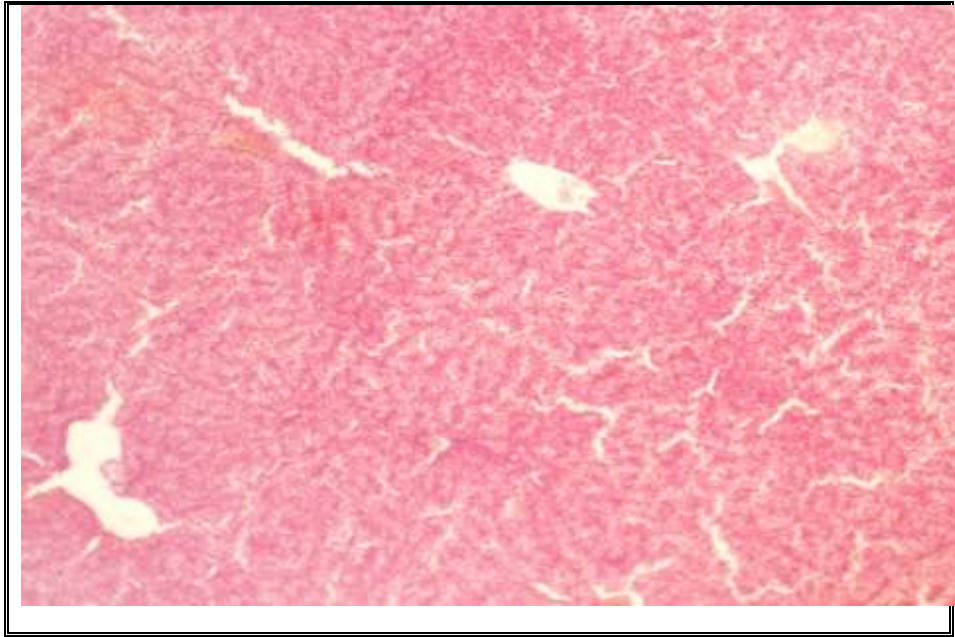


Figure 3. T2, Liver of Treated group showing normal histologic structure (H&E400X).

Blood analysis

There was no significant effect among treatments values of RBC of the birds compared with the control group ($p > 0.05$).

However, the values of WBC, Hb, and PCV were significantly increased in birds that received ionized drinking water compared to the control group (Table 3).

Table 3. Effect of ionized water on hematological parameters of Japanese quail

Treatments	RBC	WBC	Hb	PCV
T1	2.67±0.2	22.23±0.86 ^b	10.26±0.74 ^b	27.21±1.55 ^b
T2	2.50±0.13	29.44±29.44 ^a	11.57±0.71 ^{ab}	32.58±0.77 ^a
T3	2.63±0.16	24.71±0.89 ^b	12.60±12.60 ^a	32.20±0.70 ^a
T4	2.68±0.12	23.94±1.56 ^b	10.92±0.35 ^{ab}	30.80±0.59 ^a
T5	2.85±2.85	21.56±1.33 ^b	10.87±0.84 ^{ab}	25.87±1.25 ^b
Level of significance	NS	*	*	*

Mean± the standard error. *Different letters in the same column indicate significant differences between treatments at $P < 0.05$. N.S.: no significant difference. T1: the control treatment, T2: pH8, T3: pH 9, T4: pH 6, and T5: pH 5.

Bacteria count

Table 4 shows the effect of ionized water on the numbers of *Lactobacilli* and *Coliform* bacteria. The results showed that ionized water

decreased the number of *Coliform* bacteria and increased the number of *Lactobacilli* bacteria compared with the control group ($p \leq 0.05$).

Table 4. Effect of ionized water on count of *Lactobacilli* bacteria and *Coliform* bacteria of Japanese quail

Treatments	<i>Lactobacilli</i> bacteria	<i>Coliform</i> bacteria
T1	7.12±0.02 ^b	6.22±0.57 ^a
T2	6.76±0.36 ^b	6.85±0.25 ^a
T3	6.96±0.06 ^b	6.93±0.18 ^a
T4	8.55±0.15 ^a	4.70±0.05 ^b
T5	9.18±0.32 ^a	3.45±0.10 ^c
Level of significance	**	**

Mean± the standard error.**Different letters in the same column indicate significant differences between treatments at P < 0.05. T1: the control treatment, T2: pH 8, T3: pH 9, T4: pH 6, and T5: pH 5.

The high value of *Lactobacillus* in the gastrointestinal tract affected positively villi height. The bacteria provided the intestinal cells (Enterocytes) with energy for sustaining and vital functions by producing a range of fatty acids short chain, (18). Magnetic water decreased the acidity of the intestine as well as increased the absorption and secretion in the intestine (20). (16) mentioned that birds were exposed to stress led to an increase in the hormone ACTH level in the blood plasma (Adrenocortico Trophic Hormone). The hormone will remove the epithelial layer lining of the digestive tract by destroying its mucin protein. The mucin is used to provide the intestinal flora with nutrients thus mending the environment of the gut microbiology component of flora and the contents of the chyme (Chymos). Therefore, drinking magnetic water led to protect the mucous layer of the stomach and small intestine, increases digestion and absorption processes, reduces stress, and increases the oxygen of cells (17,22). (26) noted that there was a relation between the feed and the activity of enzymes in the intestines of poultry birds. The organic acids are used as feed preservatives and growth promoters. The inclusion of the organic acid in the diet decreased the pH, and the bacteria contamination in the diet thus led to prolong storage time (1, 15). These results agreed with (5, 13, 27) who indicated that drinking acidic or alkaline water did not affect significantly blood parameters (RBC), PCV volume, and hemoglobin (Hb). Acidic ionized water provides a suitable acidic environment for the growth and production the beneficial

bacteria such as *Lactobacilli*. The beneficial bacteria cover the lining of the small intestine thus expelling and reducing the presence of pathogen bacteria such as the *Coliform* by preventing them from sticking to the intestinal receptors (6,21). Drinking ionized water increased the feed intake of laying hens (14). Also, improved calcium and phosphorous contents of serum, blood characteristics, and metabolize nutrients of breeder rooster (8).

CONCLUSIONS

Drinking ionized water (pH 8, 5) affected positively the histological changes, duodenum morphology, and bacterial count as well as some blood parameters of Japanese quail.

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