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PERFORMANCE, IMMUNITY RESPONSE, BLOOD BIOCHEMICAL AND HEMATOLOGICAL TRAITS OF GROWING MALE RABBITS AFFECTED BY TYPE WATER WITH ZINC

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Seventy two weaning V-line male rabbits with an average live body weight of 630±12.23 g weaned at 33 days of age were used and randomly distributed into two equal groups according to the type of water (tap water "TW" and water exposed to magnetic field ≈4000 gauss "MW"), rabbits in each group were assigned into three sub groups which received 0, 75 and 100 mg zinc/ liter of drinking water (as zinc sulphate) in factorial design (2 type of water x 3 zinc level).

Results showed that:

1) *Weaning rabbits exposed to MW had significant higher body weight (BW), body weight gain (BWG), best feed conversion ratio (FCR) and the lowest feed intake (FI), those data were recorded for the groups consumed MW, regardless the effect of zinc (Zn) level.*
2) *Supplementation with 100 mg zinc/ liter resulted in the heaviest final BW, while BWG was statistically equal among the groups supplied with both level of zinc. The significant lowest FI and best FCR were recorded for the growing male rabbits supplied with water containing 100 mg Zn/ liter (Z1), regardless of the effect of water type.*
3) *Rabbits consumed MW and Zn recorded a significant increase in red blood cells counts (RBCs), hemoglobin concentration (Hb), packed cells volume (PCV) and white blood cells counts (WBCs).*
4) *Consumption of MW supplied with both levels of Zn (0.75 and 100 mg/l) recorded significantly the highest lymphocyte and monocyte percentages. While, neutrophil, eosinophils and basophils percentages were decreased significantly for the groups which consumed TW without zinc.*
5) *Magnetic water and increasing zinc levels display a significant increase in plasma total protein and globulin concentrations. However, plasma albumin concentration was not significantly affected by the consumed water type.*

6) *Plasma total lipid and cholesterol concentrations decreased significantly for the groups which consumed MW; unsupplied or supplied with both levels of zinc.*
7) *Plasma creatinine concentration, AST and ALT activity decreased significantly for the groups which consumed MW; unsupplied or supplied with both levels of zinc.*
8) *The consumption of MW; unsupplied or supplied with both levels of zinc, significantly exhibited a higher immune status: IgG, IgM and IgA.*

Conclusively, *it could be concluded that the performance, hematological traits, biochemical blood, and immune response for weaning male rabbits improved due to the consumption of the magnetized water supplied with a low level of zinc (75 mg/liter).*

Key words: Magnetic Water, Zinc Sulphate, Hematology, Biochemical Blood, Immunity Index

Water quality is necessary for animal production as water is the fuel of life; it transports fluids and nutrients through the blood, maintains the integrity of cell structure and regulates body temperature (Attia *et al*., 2013).

Magnetized water is type of water from passing it through a permanent magnetic field which causes the separation of the positive and negative particles and puts them in a new structure (Coey and Cass, 2000). Many researchers have illustrated a lot of microscopic and macroscopic differences between magnetized water and tape water; which include the surface tension, contact angel, viscosity, electrical conductivity, pH and effect on the size of water molecule containing smaller chemical group, the more bioavailable the water is the easier its ability to pass through cell walls for transporting nutrients and removing waste, also the more it facilitates all of the communication systems in the body (Sueda *et al.*, 2007). Al-Mufarrej *et al.*, (2005) mentioned that a water solution which passes through a magnetic field acquires finer and more homogeneous structures which in turn increases fluidity, dissolving capability for various constituents like minerals and vitamins and consequently improving the biological activity of the solution, exerting a positive effect on the performance of animals (Khudiar and Ali, 2012).

Ma *et al*., (1992) showed that magnetized water increased glutamate decarboxylase activity which may have a role in mechanisms of the physiological and biological response of the body to magnetic water Hussen, (2002) reported that magnetic water leads to increasing blood flow and providing for oxygen and nutrients to the cells. In addition, increasing the number of crystallization centers in the fluid which leads to stronger magnetic field, that results in a weak electric media that could increase the amount of ions and blood ionized useful for body. The effect of a magnetic field on the intracellular fluid and substances; results in enzyme activation and increased biochemical reactions in the cell (Hore, 2012).

Trace elements are essential for the function of various enzymes and other proteins. The effects of trace elements' biochemistry and physiology on fertility are present for zinc (Leonhard–Marek, 2000). Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (IM, FNB, 2001), hormones production (testosterone and corticosteroids). Monsalve *et al.*, (2004), plays a role in immune function (Bao *et al.,* 2010), protein synthesis, DNA synthesis and cell division (Prasad, 1995). Zinc also supports normal growth, development, and bone development (Bao *et al*., 2010). Zinc has been shown to promote the binding of growth hormone with the cell surface prolactin receptor (Cunningham *et al*. 1990). Therefore, Zn ions act as a cellular antioxidant, in terms of membrane stabilization, as well as the protective role of zinc methionine (Zn-mt). Zinc intake has been reported to exert an antiatherogenic effect as it may have a protective effect on lipid metabolism resulting from its ability to prevent hyperlipidemia, especially hypercholesterolemia and the protection from lipid peroxidation, (Joanna Rogalska *et al.* 2009).

Therefore, the aim of this study was to evaluate the effect of two types of water with zinc sulphate on the performance, some blood traits and immunity response for weaning growing male rabbits.

MATERIALS AND METHODS

The present study was carried out at El-Sabahiea Poultry Research Station, Alexandria Governorate, belongs to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture.

Magnetic field, water and chemical used in the experiment:

1- Magnetic field strength as high as ≈ 4000 Gauss.
2- Tap water used was the normal water in El-Sabahia Poultry Research Station, Alexandria.
3- Zinc sulphate, anhydrous **(**El-Gomhoria Company for Chemicals).

Preparation of Magnetically treated Water (MTW)

Two types of permanent magnets were used for conditioning the water by using an Aqua Correct unit (Magnetic water softeners ≈ 4000 Gauss magnet, which was used for pipe water conditioning. The strength of the magnet was measured by a gauss meter before the initiation and after the termination of the experiment at Application Laboratory, City for Scientific Research and Biotechnology, Japanese University, Egypt.

Water quality

Physiological properties of ordinary and magnetically treated water were determined according to HMSO (1981). Quantitative determination of macro elements of minerals in water were measured using Atomic Absorption Spectrometer (Perkin Elemer, model 10LOB) according to Heghedűş-Mîndru et al. (2013). The analysis of water is shown in Table 1.

Experimental Design

Seventy two weaning V-line male rabbits with an average live body weight of 630±12.23 g, weaned at 33 days of age and were randomly distributed into two equal groups according to the type of water, rabbits in each group were assigned into three sub-groups which received 0, 75 and 100 mg/ liter in drinking water in factorial design (2 type of water × 3 Zinc level).
The experiment was conducted until 84 days of age as the following:
Group 1: Consumed tap water **(TWZ0), Group 2:** Consumed tap water containing 75 mg zinc/liter water **(TWZ1), Group 3:** Consumed tap water containing 100 mg zinc//liter water **(TWZ2)**, **Group 4:** Consumed magnetic water **(MWZ0), Group 5:** Consumed magnetic water containing 75 mg zinc/liter water **(MWZ1), Group 6:** Consumed magnetic water containing 100 mg zinc/liter water **(MWZ2).**

Notes:

Rabbits were fed ad libitum basal diet containing 50 mg zinc/ kg diet/ premix source + 50 mg/ kg diet/ ingredient diet. Zinc sulphate solving in drinking water.

Housing and management

The rabbits were housed in a naturally ventilated building and kept in individual wire galvanized cages (60×55×40 cm). Batteries were accommodated with feeders for pelleted rations and automatic drinkers. Animals were kept under similar management and hygienic conditions. The lighting program provided was 16 hr of photo period light per day.

Table 1: Chemical analysis of water types used in the experiment

Parameters	Unit	Tap water	
		without magnetic exposure	with magnetic exposure
pH		6.83	7.10
Conductivity	Ms/Cm	693	736
Dissolved Oxygen	ppm	6.5	7.3
Total hardness	ppm	436	452
Sodium (Na^+)	ppm	6.3	7.1
Potassium (K^+)	ppm	1.4	1.8
Calcium (Ca^{2+})	ppm	114.4	120.8
Magnesium (Mg^{2+})	ppm	114.3	116.7
Chloride (Cl^-)	ppm	2.9	3.1
Carbonate (CO_3^-)	ppm	3.8	4.1
Bicarbonate (HCO_3^-)	ppm	24.1	25.6
Organic matter	ppm	50	41

Water analyzed at laboratory of Egypt- Japan University of Science and Technology.

Diet nutrient profiles

The composition of diet is present in Table 2. Chemical analysis of the diet was done according to AOAC (2007), whereas neutral detergent fiber (NDF) and acid detergent fiber (ADF) were prepared according to Van Soest and Wine (1967).

Growth performance

The rabbits were weighed at the start and end of experiment; the weight gain was calculated by subtracting body weight at the initial weight from the body weight at the end of the experiment. Feed intake was calculated as the difference between the weight of the feed offered and the weight of the remained feed on same day of weighing the animals. Feed conversion ratio was computed as the ratio between feed intake and weight gain per period.

Blood samples collection

At 84 days of age, blood samples were withdrawn from the ears' veins of rabbits which were taken randomly from each treatment group within each water type at 9.00 am before access to feed and water, heparin was used as anticoagulant for counting white blood cells (WBC_S) and its fraction (lymphocyte, neutrophils, monocytes, eosinophils and basophils percentages) according to Jain (1986). Red blood cells counting wsa done using hemocytometer according to Helper (1966), hemoglobin concentration (Hb) was decremented using commercial kits, hematocrite values was also measured using a graduated scale. Packed cells volume (PCV) % was recorded directly according

Table (2): Composition and determined analysis (on a dry-matter basis) of the experimental commercial diet used

Ingredient	g/kg
Maize	62.2
Soybean meal, 44%	223.3
Wheat bran	233.3
Barley	150.0
Alfalfa hay	301.2
Ground limestone	10.0
Dicalcium Phosphate	12.0
Common salt	5.0
Vit. + Min. Premix*	3.0
Total	**1000**
Calculated analysis**	
Crude protein, %	18.8
Crude fiber, %	13.0
Ether extract, %	3.0
Digestible energy (kcal/kg diet)	2680
Determined analysis (g/kg)	
Dry matter	902.4
Organic matter	912.1
Crude protein	172.4
Crude fiber	138.5
Ether extract	26.2
Nitrogen-free extract	575.0
Ash	87.9
Cell wall constituents (g/kg)	
Neutral detergent fiber (NDF)	331.1
Acid detergent fiber (ADF)	160.9
Hemicellulose	170.2

Each 3kg of premix contains: Vit. A: 12,000,000 IU; Vit. D3: 3,000,000 IU; Vit. E:10.0 mg; Vit. K3: 3.0 mg; Vit. B1: 200 mg: Vit. B2: 5.0 mg Vit. B6: 3.0 mg: Vit. B12: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg: Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 70 g; Fe: 35 g; I: 1 g; Co: 0.15g and Se: 0.3g.

to Wintrobe (1965). Plasma was obtained by centrifuging the blood at 3000 rpm for 20 minutes and the obtained plasma was kept at -20°C until analysis.

Plasma biochemical constituents

Plasma total protein (g/dl) was determined using the method of Doumas *et al.* (1981), plasma albumin (g/dl) was measured using the method of Reinhold (1953), while, globulin (g/dl) was calculated by the difference between total protein and albumin according to (Coles, 1974), plasma total lipids (g/dl) were measured according to Chabrol and

Charonnat (1973). Plasma total cholesterol was measured using the method of Stein (1986). Creatinine and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by colorimetric enzymatic methods using commercial kits purchased from Diamond Diagnostic Company, Cairo, Egypt.

Immunoglobulin

Different types of immunoglobulins in blood serum (IgG, IgM and IgA; n= 5 samples per treatment) were determined using commercial ELISA kits (Kamiya Biomedical Company, USA).

Statistical analysis

The differences between means relative obtained from the experiment were tested by using factorial experimental of 3 × 2 according to the following model:

$$Y_{ijk} = \mu + R_i + W_j + Z_k + (WZ)_{jk} + e_{ijk}$$

Where: Y_{ijk} is the observation on the ijk individual , μ is the overall mean, R_i is the effect of i^{th} replicates (i=1,2,3), W_j is the effect of j^{th} water types (j=1,2), Z_k is the effect of k^{th} zinc levels (k=1,2,3), $(WZ)_{jk}$ is the interaction effect of j^{th} water type by k^{th} zinc levels, e_{ijk} is the random error.

The statistical analysis was conducted using SAS®, 2002 and all the values are expressed as statistical analysis mean ± SE. A value with $P \leq 0.05$ was considered as significant. The differences among groups' means were detected using Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Performance of weaning rabbits

Results of Table (3) illustrated the effect of drinking magnetized treated water and/or Zn supplementation and their interaction on final BW, BWG, FI and FCR

Main effect of water type on BW, BWG, FI and FCR:

The growth performance for the growing male rabbits was significantly (p= 0.0001) affected by the consumption of MW. However, the significantly highest BW (p=0.0001) and BWG (p=0.0027) were recorded for growing male rabbits that consumed MW by 6.93 and 4.85%, respectively compared with the groups consumed TW regardless of the effect of Zn level (Table 3).

The significantly (p= 0.0001) lowest FI (5364 g/ rabbit) and the significantly (p= 0.0001) best FCR (3.35) were recorded for the groups

Table (3) Effect of water treatment, zinc supplementation and their interaction on growth performance of male V-line rabbits during growing period at 84 days of age

Treatment	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Feed intake all (g)	Feed conversion (g. feed / g. gain)
Effect of water type					
TW	607.54	2120^{b}	1530^{b}	5926^{a}	3.89^{a}
MW	609.79	2267^{a}	1605^{a}	5364^{b}	3.35^{b}
SEM	5.153	17.699	19.189	67.673	0.0512
Effect of zinc level					
Z0	609.50	2108^{b}	1475^{b}	5799^{a}	3.95^{a}
Z1	609.71	2183^{b}	1581^{a}	5774^{a}	3.65^{b}
Z2	607.26	2289^{a}	1647^{a}	5360^{b}	3.26^{c}
SEM	6.326	18.372	21.888	84.326	0.077
Effect of interaction between water type and zinc level					
TWZ0	609.71	2008^{d}	1409^{c}	6129^{a}	4.35^{a}
TWZ1	609.13	2150^{c}	1582^{b}	6062^{a}	3.83^{b}
TWZ2	604.44	2202^{bc}	1600^{b}	5586^{b}	3.49^{c}
MWZ0	609.33	2209^{bc}	1540^{b}	5469^{b}	3.55^{c}
MWZ1	610.22	2216^{b}	1579^{b}	5486^{b}	3.47^{c}
MWZ2	609.80	2376^{a}	1694^{a}	5135^{c}	3.03^{d}
SEM	8.782	21.538	28.039	103.474	0.081
P value					
Water type	0.7939	0.0001	0.0027	0.0001	0.0001
Zinc level	0.9529	0.0001	0.0500	0.0001	0.0533
Interaction	0.9501	0.0009	0.0001	0.05	0.0001

The letter: within Colum, different superscripts indicate that means differed significant ($P \leq 0.0001$), TW = Tape water, MW = magnetic water, Z0 = Zinc level zero, Z1 = zinc level 75 mg/liter water, Z2 = zinc level 100 mg/liter water.

consumed MW compared with the groups consumed TW regardless of the effect of Zn level (Table 3).

Main effect of zinc levels on BW, BWG, FI and FCR:

The significantly highest final BW (p= 0.0001) was recorded for growing male rabbits that consumed water supplied with 100 mg Zn/ liter (Z1) by 8.59% compared with those consumed the lowest level of Zn. However, there were no significant differences in final BW among the groups supplied with the lowest level of Zn and control groups. Results indicated that the groups supplied with both levels of Zn showed a statistically higher BWG compared with the control group.

The significantly lowest FI (p= 0.0001) and the significantly best FCR (p= 0.0533) were recorded for the growing male rabbits that consumed water supplied with 100 mg Zn/ liter (Z1). Even though, there were no significant differences in FI among the groups supplied with the lowest level of Zn and the control group.

Interaction between water type and zinc level:

The interaction results indicated that the significantly highest BW and BWG were recorded for the growing male rabbits that consumed magnetic water supplied with 100 mg Zn/ liter of water (MWZ2) compared with the other experimental groups.

The interaction results indicated that the significantly lowest FI (p= 0.0500) and the significantly improved FCR (p= 0.0001) were for the growing male rabbits that consumed MW supplied with 100 mg Zn/ liter of water (MWZ2) compared with the other experimental groups (table, 3).

These results are in agreement with Lin (1995) who showed that magnetized TW improved the performance of farm animals compared with unexposed TW. The improved animal performance could be attributed to the influence of consuming MW on growth, renewal and recovery of cells (Laycock, 2001). In addition, Rona (2004) and Gholizadeh *et al.* (2008) reported that using magnetic drinking water for chickens resulted in shortening of the fattening period of broiler chickens and increase in growth rate by 5-7%. Al-hassani and Amin (2012) showed that Cobb-chicks drank magnetized water recorded a significant greater BWG at 4, 5 and 6^{th} weeks of age than chicks drank natural water. Mustafa (2012) found that male Cobb-500 chicks drank water exposed to magnetic field showed an increased BW compared to chicks that consumed natural water. Environmental Quality Control (EQC, 2013) reported that application of magnetizer watering system can increase weight gain in calves up to 35%, in piglets up to 48%, in lamb by up to 12%, in rabbits up to 10% and improve meat taste quality. Also, in chicks, MW can increase the process of bone formation, growth and development and improve the meat quality.

Sag Baug (2003) reported that poultry consumed MW showed a significant decrease in FI and an improvement in FCR. Rodriguez *et al.* (2003) showed a positive impact of magnetic exposure on weight gain and feed utilization of rabbit bucks. Al-Mufarrej *et al.* (2005) showed that the magnetized water significantly reduced FI of males rabbit by 7.86% while insignificantly increased FI of females by 4.76% when compared with their counterparts on the TW. Moreover, Attia *et al.* (2015) illustrated that bucks that consumed MW showed a significant increase in FI and BW.

Results obtain by Nakagawa *et al.* (1999) who showed that the dissolution of oxygen into water is significantly accelerated in the presence of a magnetic field. MW is probably effective in cell growth by increasing oxygen concentration, mineral solubility and accelerating the transfer of water and nutrients in all compartments of body via improving the permeability of cell wall as a consequence of decreased surface tension and electric conductivity. Also, Hussen (2002) indicated that the improvement in chickens and rabbit performance may be due to MW enhancing the digestion absorption of growth functions cell and circulating system and increasing the feed conversion efficiency by reducing the surface tension and increasing the permeability of the cells; this allows the expansion of the gut to take advantage of food, therefore an improvement in the body. Also, Tai *et al.* (2008) reported that subjecting water to a magnetic field modified its properties, as it became more energetic and more able to flow, thus led to raising the percentage of nutritious elements like phosphorus, potassium and zinc.

Contrary with our result, Al-Mufarrej *et al.* (2005) found that the MW significantly reduced BWG of broiler males by 6.78% while insignificantly increased BWG of females by 2.81% when compared with TW. On the other hand, Al-hassani and Amin (2012) revealed that BW, BWG, FI, FCR, mortality, viability and production index exhibited no significant differences between MW treated groups and not-treatment groups.

Regarding to Zn supplementation, Abd El-Rahim *et al.* (1995) found that dietary supplementation with zinc in rabbit diets improved live BWG, FCR. Harmony with the result found by numerous researchers on the role the zinc plays on growth and FI, Ayyat and Marai (2000) reported that supplementing rabbits with 100, 200 or 300 Zn mg. kg-1 significantly increased live BWG. El Hendy *et al.* (2001) recorded that zinc deficiency has a depressing effect on body growth traits. Selim *et al.* (2012) reported that it is quite clear that the growing rabbit responded positively to 100 mg supplemental Zn kg-1 diet, in terms of significant improvement in live BWG and FCR. Also, Meshreky *et al.* (2015) found that zinc supplementation, in organic or inorganic forms to the rabbit diet was efficient to promote body weight and feed conversion efficiency. Similar with trend results, Chrastinov *et al.* (2015) showed that supplemental Zn in the rate of 100 mg. kg-1 diet leads to improving live BWG and significantly improves FCR of the rabbit. Mahmood and Sarmad (2016) indicated that the feed diet supplemented with zinc achieved the best significant ($P< 0.05$) results in terms of increasing the BWG.

Contrary Al-Khalifa (2006) reported that supplemental dietary Zn by levels of 50, 100, or 200 ppm had no significant effect on rabbits live BWG and FCR.

Hematological traits

Data for the impact of water type with zinc sulphate on blood hematological traits are presented in Table 4.

Main effect of water type: results showed that red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and white blood cells count (WBCs) were significantly (P=0.0001) increased for rabbits that consumed MW compared to that consumed TW.

Main effect of zinc level: results revealed that the RBCs, Hb, PCV and WBCs were significantly ($P \leq 0.0001$) increased for the groups supplied with 75 and 100 mg/ liter water compared to the unsupplied group (Z0).

Interaction between water type and zinc level: results showed a higher values of RBCs (p=0.0062), Hb (p=0.0003), PCV (p=0.0001) and WBCs (p=0.0001) for growing male rabbits that consumed MW supplied or unsupplied with zinc (MWZ0, MWZ1, MWZ2) as shown in Table 3.

Hematological parameters are good to indicate productive and reproductive parameters of the physiological status of animals (Khan and Zafar, 2005). In addition, Nse-Abasi *et al.* (2014) reported that the changes in hematological parameters are often used to determine various status of the body and to determine stresses due to the environmental factor. Harmony with herein results, Shamsaldin and Al-Rawee (2012) demonstrated that consuming magnetic water could result in increasing production, transferring large amounts of RBCs (Laycock, 2007) also, the consumption of MW could elaborate blood vessel and increase the movement of RBC to enhance nutrition and the transfer of oxygen to cells (Kulish, 2004). Also, Rokicki (2006) indicated that magnetic field may causes an increase in the attraction of iron from the blood and as a result for those the number of RBC and Hb increase.

Al-Nuemi *et al.* (2015) showed significant differences in RBCs, PCV and Hb for bulls that consumed magnetic water. Another theory about the effect of MW on blood and the benefit of MW for PCV and Hb that those effects may be attributed to the increased production of those cells from bone marrow, the circulatory system under the effect of hormonal factors (Mbasa and Poulsen, 1991) or MW may improve the immune system of animal (Salem *et al.*, 2006) or may be the MW decreases viscosity of blood and increases the movement of blood in the vessels which causes a high movement of Hb (Milewski, 2004). In contrast to our results, Battocletti *et al.* (2001) mentioned that drinking MW for 32 days has no significant effect on total WBCs in monkeys. On the other hand, El Hendy *et al.* (2001) recorded

Table (4): Effect of water type, zinc supplementation and their interaction on some blood hematological of male V-line rabbits during growing period at 84 days of age.

Treatment	RBCs ($10^6/mm^3$)	Hb (g./dl)	PCV (%)	WBCs ($10^3/mm^3$)
Effect of water type				
TW	6.42^b	11.17^b	37.39^b	7.08^b
MW	6.74^a	12.21^a	38.86^a	7.74^a
SEM	0.0364	0.1109	0.2176	0.0545
Effect of zinc level				
Z0	6.48^b	10.96^b	37.12^b	7.29^b
Z1	6.70^a	12.09^a	38.76^a	7.51^a
Z2	6.61^a	12.05^a	38.50^a	7.44^a
SEM	0.0534	0.0895	0.2111	0.0741
Effect of interaction between water type and zinc level				
TWZ0	6.22^c	10.58^c	35.44^d	7.00^b
TWZ1	6.52^b	11.34^b	37.82^c	7.14^b
TWZ2	6.52^b	11.60^b	38.22^{bc}	7.10^b
MWZ0	6.73^a	12.02^a	38.80^a	7.58^a
MWZ1	6.78^a	12.10^a	39.02^a	7.88^a
MWZ2	6.70^a	12.58^a	38.77^a	7.77^a
SEM	0.0467	0.1317	0.3432	0.0654
P value				
Water type	0.0001	0.0001	0.0001	0.0001
Zinc level	0.0001	0.0001	0.0001	0.0001
Interaction	0.0062	0.0003	0.0001	0.0001

The letter: within Colum, different superscripts indicate that means differed significant ($P \leq 0.0001$), TW = Tape water, MW = magnetic water, Z0 = zero Zinc level, Z1 = zinc level 75 mg/liter water, Z2 = zinc level 100 mg/liter water.

that the hematological parameters were significantly affected by zinc insufficiency included hemoglobin, PCV and total erythrocytes' count.

Leukocyte and different types of leukocytes

Data for the impact of water type supplied and not supplied zinc on WBCs differentiation traits are presented in Table 5.

Main effect of water type: lymphocytes and monocytes percentages for the growing male rabbits were significantly (p=0.0001) increased by 7.81 and 8.54 %, respectively in the groups consumed MW compared with those

Table (5): Effect of water type, zinc supplementation and their interaction on fraction of white blood cells of male V-line rabbits during growing period at 84 days of age.

Treatment	Lymphocyte (%)	Neutrophils (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
Effect of water type					
TW	47.13^{b}	45.61^{a}	3.49^{b}	2.72^{a}	1.04^{a}
MW	48.60^{a}	44.93^{b}	3.79^{a}	2.27^{b}	0.41^{b}
SEM	0.3136	0.2676	0.0873	0.0492	0.0565
Effect of zinc level					
Z0	46.28^{b}	46.89^{a}	3.36^{c}	2.60^{a}	0.87^{a}
Z1	48.53^{a}	44.47^{b}	3.90^{a}	2.45^{b}	0.66^{b}
Z2	48.79^{a}	44.45^{b}	3.67^{b}	2.45^{b}	0.65^{b}
SEM	0.3062	0.2542	0.0881	0.0533	0.0847
Effect of interaction between water type and zinc level					
TWZ0	44.50^{c}	48.40^{a}	2.82^{b}	2.93^{a}	1.34^{a}
TWZ1	48.40^{b}	44.28^{b}	3.84^{a}	2.60^{b}	0.88^{b}
TWZ2	48.48^{b}	44.16^{b}	3.82^{a}	2.63^{b}	0.91^{b}
MWZ0	48.06^{b}	45.38^{b}	3.90^{a}	2.26^{c}	0.40^{c}
MWZ1	48.66^{b}	44.66^{b}	3.95^{a}	2.30^{c}	0.43^{c}
MWZ2	49.09^{a}	44.74^{b}	3.52^{a}	2.26^{c}	0.39^{c}
SEM	0.2258	0. 1866	0.0674	0.0554	0.2784
P value					
Water type	0.0001	0.0001	0.0001	0.0001	0.0001
Zinc level	0.0001	0.0001	0.0001	0.0001	0.0024
Interaction	0.0001	0.0001	0.0001	0.0001	0.0001

The letters within Colum, different superscripts indicate that means differed significant ($P \leq 0.0001$), TW = Tape water, MW = magnetic water, Z0 = zero Zinc level, Z1 = zinc level 75 mg/liter water, Z2 = zinc level 100 mg/liter water.

consumed TW. On the other hand, neutrophils, eosinophils and basophils percentages were significantly (p=0.0001) decreased by 6.77, 16.54 and 60.58%, respectively for the groups consumed TW compared with those consumed MW.

Main effect of zinc level: lymphocyte and monocyte percentages for the growing male rabbits were significantly increased for the groups consumed water supplied with the both levels of Zn compared with the control group. On the other hand, neutrophils, eosinophils and basophils percentages were significantly decreased for the groups supplied with both levels of Zn.

The interaction between water type and zinc level: results indicated that the significantly (p=0.0001) highest lymphocytes percentage was recorded for growing male rabbits consumed MW supplied with the highest level of Zn (MWZ2) compared with the other treated groups. However, the significantly (p=0.0001) lowest monocytes percentage was recorded for growing male rabbits consumed TWZ0 compared to the other groups, while the highest monocytes were found with the high level of Zn with magnetic water (MWZ2) compared with the other experimental groups, while there were no significant differences among the other experimental groups. On the other hand, neutrophils, eosinophils and basophils percentages were significantly (p=0.0001) decreased for the groups consumed TW supplied with both levels of Zn (TWZ1 and TWZ2) or consumed MW supplied or unsupplied with Zn (MWZ0, MWZ1 and MWZ2) compared with those consumed TW (TWZ0).

Drinking MW for 32 days had no significant effect on total WBCs, lymphocytes, monocytes and granulocytes (Battocletti *et al.*, 2001). Also, Donohue (2003) showed an increase in body immunity through the increased proportion of lymphocyte cells. Rise of lymphocyte cells percentage may be due to that MW increases the content of immune globulin in the blood and increases the number of defensive WBCs. In agreement with this study was done by Battocletti *et al.* (2001) who referred that differential and absolute WBC counts of segmented neutrophils and lymphocytes in rhesus monkeys were considerably changed during the drinking magnetized water. Herein results are contrary with that obtained by Fouad *et al.* (2016) who mentioned that rabbits drinking MW for 32 days showed no significant effect on total WBCs, lymphocytes, monocytes, granulocytes and hemoglobin concentration.

Previous study mentioned that zinc-deficient diets reduced numbers of T and B lymphocytes in peripheral blood and spleen tissues (Someya *et al.*, 2009). Peripheral blood lymphocyte and macrophage concentrations were eventually reduced by more than 50% (Fraker *et al.*, 1986). Importantly, even marginal zinc deficiency substantially suppressed peripheral blood lymphoid cell concentrations in mice and humans; not only decreased lymphocyte concentrations, but also depressed T and B lymphocyte function (Moulder *et al.*, 1989). Yui Someya *et al.* (2007) mentioned that dietary zinc deficiency increased the number of basophils, eosinophils and neutrophils and decreased the number of lymphocytes, suggesting the change in WBC distribution. Same result was obtained by Someya *et al.* (2009) who documented that zinc is known to play an important role in immune-functions. However, the effects of zinc-deficiency on the immune response system from the point of view of the distribution, changes the number of total WBCs. Therefore, the zinc-deficiency affects the number of total WBCs, neutrophil, eosinophil, basophil, monocyte and lymphocytes (T lymphocyte, B lymphocyte and Natural Killer cell) in rats. Also, Younas *et al.* (2015) showed that zinc fortified apricots significantly improved the T-lymphocytes and hemoglobin in rabbits.

Total protein, Albumin, Globulin and Immunoglobulin immunity of blood plasma:

Data for the effect of water type with zinc sulphate on total protein, Albumin, Globulin and Immunoglobulin immunity of blood plasma are presented in Table 6.

Maine effect of water type: results indicated that the plasma total protein, globulin IgG, IgM and IgA (immune index) concentrations for the growing male rabbits were significantly ($P \leq 0.0001$) higher for the groups received MW compared to that received TW. On the other hand, differences in plasma albumin between the groups either received magnetized water or TW were not significant.

Main effect of zinc level: results showed that the plasma total protein and globulin concentrations were significantly ($P \leq 0.0001$) increased for the groups supplied with 75 and 100 mg/ liter of Zn in the drinking water compared to the zero zinc. On the other hand, plasma albumin concentration was not significantly affected by Zn supplementation. The IgG, IgM and IgA immune indices had the lowest significant ($P \leq 0.0001$) effect by level Z0 for growing male rabbits, neglecting of water treatment. But, IgM ($p=0.0006$) and IgA ($p=0.0005$**)** immunity indices were significantly improved for the groups supplied with both levels of Zn in the drinking water compared with groups unsupplied with Zn.

Interaction between the water type and zinc level: results indicated that the group consumed magnetized water and supplied with 100 mg/ liter zinc (MWZ2) recorded significant higher values for total protein and globulin compared to the other treatment groups, while there was no detected significant difference among the groups of TWZ1, TWZ2, MWZ2 and MWZ1 for total protein and globulin. In addition, the group of TWZ0 recorded the significantly lowest value for total protein and globulin compared to the other groups.

The significantly highest IgG, IgM and IgA activity were recorded for growing male rabbits that consumed TW and MW supplied or unsupplied with Zn compared to the group drinking TW. Also, the significantly highest IgG, IgM and IgA activity was recorded for growing male rabbits that consumed MW supplied with both level of Zn (MWZ1 and MWZ2) compared with the other experimental groups. Moreover, the significantly

Table (6): Effect of water type, zinc supplementation and their interaction on total protein, albumin, albumin and globulin ratio and immunoglobulins of male V-line rabbits during growing period at 84 days of age.

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml
Effect of water type						
TW	6.20^{b}	3.28	2.92^{b}	3.97^{b}	1.32^{b}	0.50^{b}
MW	6.60^{a}	3.30	3.30^{a}	4.52^{a}	1.48^{a}	0.55^{a}
SEM	0.0617	0.0248	0.0563	0.0342	0.0342	0.0045
Effect of zinc level						
Z0	6.17^{c}	3.29	2.89^{c}	3.59^{b}	1.35^{b}	0.52^{b}
Z1	6.42^{b}	3.29	3.13^{b}	4.29^{a}	1.44^{a}	0.52^{b}
Z2	6.62^{a}	3.30	3.33^{a}	4.26^{a}	1.41^{a}	0.55^{a}
SEM	0.0602	0.375	0.0693	0.0769	0.0246	0.0051
Effect of interaction between water type and zinc level						
TWZ0	5.99^{c}	3.29	2.70^{c}	3.83^{c}	1.24^{c}	0.50^{c}
TWZ1	6.30^{b}	3.28	3.02^{b}	4.06^{b}	1.37^{b}	0.51^{b}
TWZ2	6.32^{b}	3.28	3.04^{b}	4.02^{b}	1.34^{b}	0.51^{b}
MWZ0	6.35^{b}	3.28	3.07^{b}	4.54^{a}	1.46^{a}	0.54^{a}
MWZ1	6.53^{b}	3.30	3.23^{b}	4.52^{a}	1.52^{a}	0.56^{a}
MWZ2	6.92^{a}	3.31	3.61^{a}	4.49^{a}	1.47^{a}	0.55^{a}
SEM	0.0549	0.071 8	0.4064	0.0395	0.0239	0.0068
P value						
Water type	0.0001	0.0921	0.0001	0.0001	0.0001	0.0053
Zinc level	0.0001	0.0625	0.0001	0.0335	0.0006	0.0005
Interaction	0.0001	0.0721	0.0001	0.0414	0.0056	0.0065

The letter: within Colum, different superscripts indicate that means differed significant ($P \leq 0.0001$), TW = Tape water, MW = magnetic water, Z0 = zero Zinc level, Z1 = zinc level 75 mg/ liter water, Z2 = zinc level 100 mg/ liter water, IgG = Gamma immunoglobulin, IgM = Mu immunoglobulin, IgA= Alpha immunoglobulin.

lowest IgG, IgM and IgA activity were recorded for the groups consumed TW (TWZ0).

The increasing of total protein and globulin in the group consumed magnetized water and supplied with 100 mg zinc/ liter are in agreement with Khalisa and Aous (2012) who referred that drinking MW had a beneficial effect on some physiological aspects manifested by a significant rise in serum total proteins concentration. Yacout *et al.* (2015) reported that total protein, albumin and globulin were significantly increased for lactating goats that consumed magnetized water. Meshreky and Hamdy (2006) demonstrated that Zn significantly increased serum total protein, and albumin in rabbits. Also, Al-Mousawi (2013) showed that there were significant changes (p= 0.05) in the serum levels of total protein, albumin and globulin in diabetic group treated with Zinc.

In agreement with herein results, Fortun-Lamothe and Drouet-Viard (2002) mentioned that consumed MW increased humeral immunity and further supporting evidences showed that the liver was capable of synthesize enough globulins for immune functions (immunoglobulin, IgG, IgM and IgA). Rashid *et al.* (2009) reported that MTW improved significantly the titer of antibody against newcastle and Gumbouro disease in fawbro broiler.

Contrary was found by Tenfords and Shifrine (1984) who showed that humeral and cell-mediated immune responses, including the level of serum IgM and spleen lymphocytes, sheep erythrocytes vaccination were not significantly different in the exposed mice in comparison to control animals. This result is also agrees with Fouad *et al.* (2016) who revealed that magnetically treated water have an adverse effect on immune system and this result was comparable to Al-Mufarrej *et al.* (2005) who showed that magnetic treated water have adverse effect on female broiler chickens immune response.

These results are consistent with previous reports that diets supplemented with zinc tend to improve the ability of the birds to produce antibodies. Wirth *et al.* (1989) demonstrated that zinc was essential for the activity of macrophages. Same trend was observed by Prasad (1991) who revealed that prenatal zinc deficiency in mice results in long-term suppression of IgM, IgA, and IgG. Agreement with our results, Shankar and Prasad (1998) documented that zinc is known to play a central role in the immune system and zinc-deficient persons experience increased susceptibility to a variety of pathogens. Interestingly, zinc plays an important role in immunity; it is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells. Zinc deficiency also affects the development of acquired immunity by preventing both the outgrowth and certain functions of T lymphocytes such as activation, Th1 cytokine production, and B lymphocyte help. Likewise, B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised Shankar and Prasad (1998). In addition, Bartlett and Smith (2003) referred that birds receiving 181 mg Zn/ kg diet had a significantly higher titers of total antibodies, IgM and IgG antibodies than those receiving the other diets. Wang *et al.* (2012) showed that zinc bacitracin increased concentration of IgG and IgM for immunologic function for growing rabbits.

Total lipids, cholesterol, Creatinine, AST and ALT:

Data regarding the effect of water type with zinc on total lipids, cholesterol, Creatinine, AST and ALT of blood plasma are showed in Table 7.

Main effect of water type: results noted that the plasma total lipids, cholesterol, creatinine, AST and ALT concentrations for the growing male rabbits were significantly (p=0.0001) decreased in the groups consumed MW compared with those consumed TW.

Main effect of zinc level: results showed that the plasma total lipid, cholesterol, creatinine AST and ALT concentrations were significantly (p=0.0001) decreased for the groups supplied with both levels of Zn compared with those unsupplied with Zn.

Interaction between water type and zinc level: results indicated that the significantly lowest plasma cholesterol, total lipid, creatinine and AST concentrations were recorded for growing male rabbits consumed MW supplied or unsupplied with Zn (MWZ0, MWZ1 and MWZ2) compared with those consumed TW. Moreover, the significantly highest cholesterol, total lipid, creatinine, AST and ALT concentrations were recorded for the groups that consumed TW (TWZ0). The significantly lower plasma ALT concentrations was recorded for growing male rabbits that consumed MW supplied with Zn (MWZ1 and MWZ2) compared with those consumed MW without Zn or Tw with or without Zn.

The decline of total lipids, cholesterol, creatinine, AST and ALT in MW is in agreement with Yacout *et al.* (2015) who reported that cholesterol, AST and ALT were significantly decreased in the group consumed MW compared with those that consumed unmagnetic water. Also, Khalisa and Aous (2012) referred that drinking of MW had beneficial effects on suppression in serum total cholesterol, triacylglycerol and low density lipoprotein concentrations and exhibited some physiological aspects manifested by a significant rise in serum high density lipoprotein-cholesterol concentration. Contrary with the current results, Osbakken *et al.* (1986) reported that magnetic field had no effect on cholesterol and triglyceride.

In addition, Gilani *et al.* (2014) referred that the activity of AST in serum of broilers that drank magnetized water significantly decreased, but ALT was not influenced by the MW.

Results of Zn supplementation are in agreement with Meshreky and Hamdy (2006) who elevated that serum total lipid and cholesterol concentrations were significantly decreased with increasing Zn supplementation. Reinforce our result, Al-Mousawi (2013) showed that

Table (7): Effect of water type, zinc supplementation and their interaction on total lipids, cholesterol, creatinine, AST and ALT of male V-lin rabbits during growing period at 84 days of age.

Treatment	Total Lipids (g/dL)	Cholesterol (mg/dL)	Creatinine (mg/dl)	AST (U/l)	ALT (U/l)
Effect of water type					
TW	6.36[a]	110.57[a]	1.48[a]	27.87[a]	56.63[a]
MW	5.66[b]	98.06[b]	1.28[b]	24.27[b]	53.14[b]
SEM	1.93	5.12	0.0229	0.3828	0.5844
Effect of zinc level					
Z0	6.18[a]	119.85[a]	1.44[a]	27.80[a]	57.84[a]
Z1	5.97[b]	94.80[b]	1.35[b]	25.30[b]	53.00[b]
Z2	5.89[b]	96.51[b]	1.37[b]	25.10[b]	54.30[b]
SEM	1.75	4.98	0.1348	0.6241	0.4613
Effect of interaction between water type and zinc level					
TWZ0	6.66[a]	116.74[a]	1.53[a]	30.60[a]	60.88[a]
TWZ1	6.34[b]	110.28[b]	1.42[b]	26.45[b]	54.00[b]
TWZ2	6.08[b]	108.32[b]	1.40[b]	26.60[b]	55.00[b]
MWZ0	5.70[c]	98.28[c]	1.31[c]	24.00[c]	54.8[b]
MWZ1	5.59[c]	93.77[c]	1.22[c]	24.2[c]	52.00[c]
MWZ2	5.69[c]	95.12[c]	1.30[c]	23.60[c]	52.60[c]
SEM	1.88	5.44	0.0400	0.5393	0.5217
P value					
Water type	0.0001	0.0001	0.0001	0.0001	0.0001
Zinc level	0.0002	0.0001	0.0331	0.0037	0.0001
Interaction	0.0134	0.0001	0.0088	0.0001	0.0039

The letter: within Colum, different superscripts indicate that means differed significant ($P \leq 0.0001$), TW = Tape water, MW = magnetic water, Z0 = zero Zinc level, Z1 = zinc level 75 mg/ liter water, Z2 = zinc level 100 mg/ liter water, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase.

administered orally zinc significantly reduced the serum total cholesterol and triglyceride in diabetic rabbits treated with zinc.

Disagreement with the Zn results in ours study; Gilani *et al.* (2014) mentioned that broiler chickens drinking magnetized water with zinc supplementation raises levels of cholesterol in longissimus dorsi muscle.

Same context of effect zinc supplementation, Meshreky and Hamdy (2006) and Li *et al.* (2007) revealed that zinc methionine treatment effectively remarkably decreased serum activities of AST and ALT in rabbits and human, respectively. In addition, Al-Mousawi (2013) showed that administered orally zinc significantly reduced the serum ALT and AST concentration.

Conclusively, it could be concluded that performance, biochemical, hematological traits and immune statues for weaning male rabbits improved due to consuming magnetized water supplied with low level of zinc (75 mg/ liter).

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الآداء والإستجابة المناعية وصفات الدم الطبيعية والبيوكيميائية لذكور الأرانب النامية المتأثرة بنوعية الماء مع الزنك

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**معهد بحوث الأنتاج الحيوانى – مركز البحوث الزراعية – وزارة الزراعه – مصر.

تم توزيع اثنين وسبعون أرنب من سلالة الفي لين المفطومين عمر ٣٣ يوماً بمتوسط وزن ٦٣٠±١٢.٢٣ جم توزيعاً عشوائياً إلى مجموعتين متساويتين حسب نوع الماء (ماء الصنبور والماء المعرض للمجال المغناطيسي بقوة ٤٠٠٠ جاوس تقريباً، وبكل مجموعة ثلاثة مجموعات فرعية تم معاملتها بمستويات ٠، ٧٥ و ١٠٠ ملجرام من سلفات الزنك في مياه الشرب في تصميم عاملي (نوعين من الماء × ثلاثة مستويات من الزنك).

وأظهرت النتائج ما يلي:

١- استهلاك الماء الممغنط ادى الى زيادة ملحوظة فى وزن الجسم النهائى والوزن المكتسب ،كمية العلف المأكول.وكذلك افضل معدل تحويل غذائى.

اظهرت اضافة الزنك بتركيز ١٠٠مللجرام/لتر ماء أفضل وزن نهائي بينما الوزن المكتسب كان متساويا احصائيا مع تركيزي الزنك ٧٥-١٠٠ملجرام ،من ناحية اخرى سجلت مياه الصنبور اقل القيم المتحصل عليها.

٢- اظهرت نتائج التداخل بين نوع الماء واضافات الزنك زيادة في وزن الجسم النهائي ، الوزن المكتسب ،اقل كمية علف مأكول وافضل نسبة تحويل عند استخدام تركيز ١٠٠مللجرام/لتر .

٣- زاد عدد كرات الدم الحمراء والهيموجلوبين وحجم كرات الدم وكرات الدم البيضاء مع الأرانب التي تناولت الماء الممغنط والزنك.

٤- استهلاك كل من الماء الممغنط والزنك بمستوى ٧٥.٠ و١٠٠مجم/ لتر ماء أدى إلى زيادة نسبة الخلايا الليمفاوية والأحادية بشكل ملحوظ. بينما انخفضت نسبة الخلايا المتعادلة والحامضية والقاعدية مع المجموعات التي تناولت ماء الصنبور العادي بدون زنك.

٥- أدى الماء الممغنط والزنك والتفاعل بينهما إلى زيادة معنوية في تركيزات بروتين البلازما الكلي والجلوبيولين. ومع ذلك لم يتأثر تركيز ألبيومين البلازما بنوع الماء المستهلك.

٦- انخفض تركيز الدهون الكلية وكوليسترول البلازما مع المجموعات التي تناولت الماء الممغنط بدون الزنك أو بإضافة الزنك.

٧- انخفض تركيز الكرياتينين والـ AST وALT مع المجموعات التي تناولت الماء الممغنط بدون أو بإضافة الزنك.

٨- ارتفع مستوى الجلوبيولينات المناعية الـ IgG، IgM و IgA مع المجموعات التي تناولت الماء بدون أو بإضافة الزنك.

التوصية: نستنتج من ذلك أن الماء الممغنط والمضاف اليه الزنك بتركيز ٧٥ ملجم/ لتر ماء أدى الى تحسن في الآداء الإنتاجي للأرانب وكذلك مكونات الدم الطبيعية والكيميائية والاستجابة المناعية في الأرانب المفطومة.