The Physiological Response of Broilers Chickens (Ross 308) to Adding Nano-Boron to the Diet

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Abstract

This study was conducted at Poultry Farm of Animal production Dept., College of Agriculture, University of AL-Qasim Green to investigate the effect of adding different levels of nano boron to the diet of broiler chickens Ross 308 on some blood parameters. 180 broiler chicks Ross 308 day-old were randomly assigned to four treatments (with 3 replicates per treatment and 15 chicks per replicate). The treatments were as follows: The first treatment was control group, which was free from any addition. The second treatment represented the addition of 40 mg nano-boron/kg feed, while the third treatment represented the addition of 60 mg nano-boron/kg feed and the fourth treatment represented the addition of 80 mg nano-boron/g feed. The studied characteristics were i.e, red blood cell account, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC), corpuscular volume, white blood cells account and differential leucocyte count. The results of the study did not show significant differences between the experiment treatments in the number of red blood cells account, hemoglobin concentration, percentage of haematocrit, MCH and MCHC at 21 days age. The value of MCV, recorded the highest concentration for the first treatment (control). The fourth treatment (addition of 80 mg nano-boron particles/kg feed) showed an increase in the red blood cells account and MCHC significantly ($P<0.05$) at 35 days age. As for the hemoglobin concentration and percentage of haematocrit, no significant differences were recorded between all experiment treatments. The results showed the superiority of the first treatment (control) in both the value of MCV and the value of MCH compared to the fourth treatment. The treatments of nano-boron had a role in raising the immune traits for the bird at 35 days age, where a significant improvement ($P<0.05$) in white blood cells account and the highest percentage of Lymphocytes (L) and the lowest percentage of (H/L) recorded compared to control treatment, while no significant differences in the percentage of Heterophil cells (H) between bird groups. It concluded from this experiment, that the addition of nano-boron to the ration can lead to improve in some blood parameters of the broiler.

Keywords: Nano-boron, Blood parameters, Broiler.

Introduction:

The modern strains of commercial broiler chickens were distinguished by their high growth and efficiency in converting fodder into meat, and this ability resulted in the growth rate as a result of genetic selection, and this was negatively affected by the immunity of birds. Modern strains most vulnerable to treatment for poultry from rare mineral elements became very high due to their rapid growth and rate, besides it has a high metabolism to be highly productive of both meat and eggs (Nbras and Nihad, 2019), as well as trace mineral elements in reproduction, immunity, and other vital processes (Zahra *et al*., 2018). Therefore, nutrients, lack of nutrients, deficiency of nutrients, and deficiency of elements have tended to be nutritional and deficiency of the basic elements of plants since 2014. In 1923 and by the eighties, a role in human and animal nutrition emerged through its influence on a large number of metabolic processes and vital activities such as the metabolism of enzymes rooted in Sadat, and steroids (Al-Awadi, and Al-Nadawi, 2020). It also has a large and effective role. In the metabolism of calcium, phosphorous and magnesium and in the development and growth of domestic birds (Zahra *et al*., 2018). Boron is found in meat and plants, and in many types of grains, vegetables and nuts as well (Zahra *et al*., 2018), that compounds containing boron have anti-bacterial properties and are often present in the form of boric acid or borates (Ali and Al-Shuhaib, 2021) and the poultry industry witnessed in recent years, multiple technologies, the most important of which is nanotechnology, which is a promising and emerging technology that has enormous potential for a revolution in the poultry sector around the world, and nanoparticles in general have dimensions between (1-100) nanometers approximately. Nanoparticle's bypass some of the physiological methods of distributing and transporting nutrients through tissues and cell membranes, and nanotechnology is working to develop new products and the possibility of reformulating traditional materials to give effective results while the size of the material is greatly reduced, which leads to the formation of physical properties. A new chemical, and these properties allow nano materials to be used in a wide range of fields, including health, pharmacy, industry and other unlimited fields (Ali and Al-Shuhaib, 2021), and nano-boron was used as a new source for boron because of its characteristics with high catalytic efficiency, large surface area, and high absorption capacity compared to boron. Due to the lack of studies on the use of nano boron in the diets of poultry, this study came to determine the best concentrations of nano boron added to poultry diets, which we can recommend and study the extent of its effect on some of the blood characteristics of broilers.

Materials and methods:

This study was conducted in the poultry field of the Animal Production Department at Faculty of Agriculture, Al-Qasim Green University during the period started from 9/9/2018 until 14/10/2018. 180 chicks of non-naturalized Belgian broiler Ross of meat were used, with an average weight of 38 g/chick, as the chicks were purchased from Amer hatchery (Babil governorate). Chicks were raised in Aknan, and were distributed randomly into 4 treatments, each consisting of 3 replicates, as each replicate contained 15 chicks. Fodder was provided to the birds freely, as two diets were provided, one from the age of 1 to 21 days and one for the beginning of the age of 22-35 days (Table 1). The nano boron was added to the diet (manual mixing) starting from the age of one day and as follows: The first treatment: a group without any addition. The second treatment: a basic diet to which 40 mg nano boron/kg of feed is added, while the third treatment: a basic diet to which 60 mg nano boron/kg of feed is added, and the fourth treatment: a basic diet to which 80 mg nano boron/kg feed is added. The experiment studied the following characteristics: Red blood cell count, hematocrit, hemoglobin concentration, average red blood cell hemoglobin weight value, mean red blood cell hemoglobin concentration, estimation of average red blood cell volume value, white blood cell numbers and differential white blood cell count, where the blood was collected in weeks 3 and 5 from 9 birds from each treatment (3 birds from each replicate) randomly, and blood was collected from the humeral vein where tubes containing Potassium EDTA anticoagulant were used to prevent blood clotting. The blood stack was calculated using capillary tubes, especially containing an anticoagulant according to the method indicated by Archer, (1965). Hemoglobin concentration was estimated according to the method indicated by Campbell, (1995). The number of red and white blood cells was estimated according to the method indicated by Natt and Herrick, (1952). The heterophil/lymphocyte ratio was estimated using glass slides where a drop of blood is placed on the glass slide and it is spread very carefully with another glass slide placed over the drop of blood and pulled over the first slide at an angle of 45 degrees without pressing it hard and left to dry at a rate of 10 minutes, the slices were then doused with a mixture of Wright Gimsa dyes according to the method of Shen and Patteron, (1983). The counting is performed using an optical microscope under a magnification force (1000) by placing a drop of oil on the slide according to the method of Burton and Guion, (1968). After that, the ratio of heterocytes to lymphocytes is calculated. Completely Randomized Design was used to study the effect of different factors on the studied traits. The significant differences between the averages were compared using Duncan polynomial test (Duncan, 1955), and the statistical program SAS (SAS, 2016) was used to analyze the data.

The boron nanomaterial was used as a powder supplied by a company Naqaa Foundation for Scientific Research, Technology and Development. The Transmission Electron Microscope (TEM) test was performed by means of a transmission electron microscope (Fig. 1) on the boron nano sample at the Central Laboratory of the College of Education for Pure Sciences, Ibn Al Haytham.

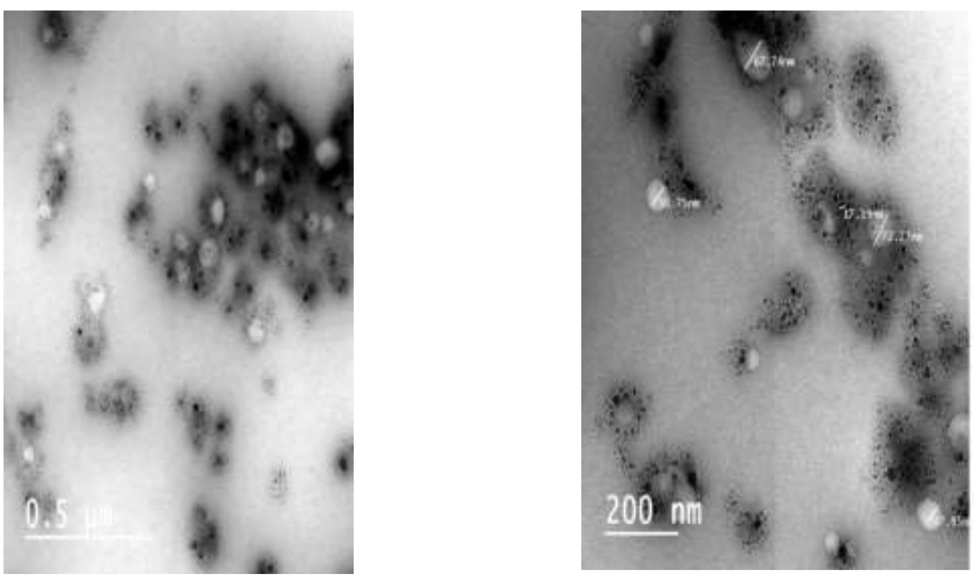

Fig. 1. The transmission electron microscope (TEM) test on the boron nano sample.

Table 1. Composition of two kinds of diets starter and final as well as the chemical analysis (calculated).

Feed material	Starter diet (1-21 days)%	Final diet%
Yellow corn	48.2	58.7
Local wheat	8	7.5
Soybean cake (44% protein)	28.5	20.5
Protein Center *	10	10
Vegetable oil (sunflower)	4	2.5
Limestone	1	0.5
Table salt	0.3	0.3
Total summation	%100	100%
Calculated chemical analysis **		
Representative energy (kilowattara/kg)	3079.85	3102.6
Crude protein (%)	21.56	18.87
Lysine (%)	1.04	0.85
Methionine + Cysine (%)	0.455	0.42
Crude fiber%	3.54	3.2
Calcium (%)	1.28	1.07
Ready phosphorous (%)	0.42	0.41

*** BROCON-5 SPECIAL W protein concentrate: Chinese origin, each kg contains: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% phosphorous available, 3.25% lysine, 3.90% methionine + cysteine 2.2% sodium, 2100 kcal/kg energy represented, 20,000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6, 300 B12 mg, 10 mg folic acid, 100 mcg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT). According to the chemical analysis of the diet according to NRC (1994).**

Results and Discussion:

It is noted from Table (2) the effect of adding different levels of nano boron to the diet on blood agglutination ratio, red blood cell counts, hemoglobin concentration, average red blood cell volume, average red blood cell hemoglobin weight and average hemoglobin concentration of red blood cells for broilers at the age of 21 days. Significant differences were found between all trial parameters in each of the percentage of hemoglobin (pcv) (%), the number of red blood cells (10^6/mm^3 blood), hemoglobin concentration (g/100 ml blood), the average weight of the hemoglobin red blood cells MCH (pico gram/Cell) and MCHC red blood cell hemoglobin concentration.

As for the average value of red blood cell volume MCV (microns^3), it was noted that the first treatment (control) recorded a significant difference ($P < 0.05$), where the highest value was (95.68 micron^3), followed by the second treatment, where the MCV value was (86.20 micron^3), and the third and fourth treatments were (79.34 and 79.07 micron^3), respectively, without significant differences with the second treatment .Table (3) indicates the effect of adding different levels of nano boron to the diet on blood agglutination ratio, red blood cell counts, hemoglobin concentration, average red blood cell volume, average red blood cell hemoglobin weight, average hemoglobin concentration of broilers at 35 days of age, but no significant differences between all groups of birds in terms of the percentage of blood accumulation (%) and hemoglobin concentration (g / 100 ml of blood). As for the number of red blood cells (10^6/mm^3 blood), the fourth treatment recorded the highest number of red blood cells, ($10^6 \times 3.03$/mm^3 blood) compared to the other treatments (the first, second and third) that recorded the following numbers (1.90, 2.04 and 2.10 × 10^6/mm^3 blood), respectively, as for the average size of the red blood cells MCV and the average weight of the hemoglobin red blood cells MCH, the first treatment (control) recorded the highest value (159.89 $micron^3$) and 53.26 pg/cell, respectively, followed by the second and third treatments which recorded (139.11 and 136.86 $micron^3$) for the average size of MCV red blood cells respectively. As for the average weight of the hemoglobin of red blood cells MCH, the following values were recorded (46.35 and 45.60 pg/cell) respectively. As for the average concentration of the hemoglobin of red blood cells MCHC, the third and fourth nano-boron parameters recorded the highest percentage (33.32 and 33.32%) respectively, followed by the second treatment, while the first (control) treatment recorded the lowest percentage (33.31%). Table (4) shows the results of the statistical analysis of the effect of adding different levels of nano boron to the diet on the number of white blood cells, the ratio of heterogeneous cells and lymphocytes, and the L/H ratio of broilers at the age of 35 days, where it was noted that the superiority of the third and fourth nano boron treatments in the numbers of white blood cells was significant (P<0.05) for the first treatment (control) and the following numbers were recorded as 32.33, 33.66 and 33.33 × 10^3/ mm^3 blood, respectively, while the first treatment recorded 27.66 × 10^3/mm^3 blood. As for the percentage of heterogeneous cells (%), the results showed that there were no significant differences between all the treatments, while the first treatment (control) recorded the lowest percentage significantly (P<0.05) of lymphocytes (%) and reached (43.66%). Boron nanoparticles (the second, third and fourth) have the highest percentage of lymphocytes (55.50, 57.16 and 52.00%, respectively). As for the ratio of heterozygous cells to lymphocytes (H/L), the control treatment recorded a ratio of (0.50%), with a significant difference (P<0.05) for nano-boron treatments (second, third and fourth), which recorded the following rates (0.34, 0.33 and 0.37%) respectively.

Table 2. The effect of adding different levels of nano boron to the diet on some blood parameters of broilers at 21 days of age

Studied traits						**Treatments**
Mean red blood cell hemoglobin concentration (MCHC)(%)	**Mean weight of MCH red blood cell hemoglobin (pg/cell)**	**Average red blood cell volume MCV $micron^3$**	**Hemoglobin Gm/100 ml) (blood**	**Red blood cells (10^3/ mm^3) DM**	**PCV(%)**	
33.33±0.002	31.25±1.98	95.68±5.94a	8.49±0.22	2.72±1.99	25.50±0.67	**T1 (Control)**
33.31±0.38	28.30±1.80	86.20±4.87ab	8.66±0.49	3.06±2.45	26.00±1.43	**T2 Addition of nano boron 40 mg / kg feed**
33.33±0.004	26.09±1.69	79.34±5.09b	±8.610.24	3.30 ±1.81	25.83±0.74	**T3 Addition of nano boron 60 mg / kg feed**

33.33±0.27	26.10±1.51	79.07±4.28b	8.83±0.36	3.34 ±1.70	26.16±1.04	**T4 Addition of nano boron 80 mg / kg feed**
NS	NS	*	NS	NS	NS	**Significant level**

Means with different letters within a single column are significantly different. * (P <0.05), NS: not significant.

Table 3. The effect of adding different levels of nano boron to the diet on some blood parameters of broilers at 35 days of age

Studied traits						**Treatments**
Mean red blood cell hemoglobin concentration (MCHC) (%)	**Mean weight of MCH red blood cell hemoglobin (pg/cell)**	**Average red blood cell volume MCV micron^3**	**Hemoglobin (Gm/100 ml blood)**	**Red blood cells (10^3/ mm^3) DM**	**PCV(%)**	
33.313± 0.004b	53.26±8.60a	159.89±25.80a	9.11± 0.14	1.90± 256039.28b	27.33±0.42	**T1 (Control)**
33.323±0.003ab	46.35±3.37ab	139.11±10.09ab	9.22±0.23	2.04±159570.60b	27.66±0.71	**T2 Addition of nano boron 40 mg / kg feed**
33.325±0.003a	45.60±3.84ab	136.86±11.55ab	9.33± 0.31	2.10± 159473.09b	28.00±0.93	**T3 Addition of nano boron 60 mg / kg feed**
33.325± 0.003a	32.77± 1.86b	98.35± 5.50b	9.83±0.29	3.03±139325.76a	29.50 ±0.71	**T4 Addition of nano boron 80 mg / kg feed**
*	*	*	NS	*	NS	**Significant level**

Means with different letters within a single column are significantly different. * (P <0.05), NS: not significant.

Table 4. Effect of adding different levels of nano boron to the diet on white blood cell counts, heterocyte and lymphocyte ratio, and L/H ratio for broilers at 35 days of age.

Studied traits				Treatments
Ratio (H/L)	**Lymphocyte ratio (%)**	**The proportion of heterozygous cells (%)**	**White blood cells (10^3/ mm^3) DM**	
0.50±0.02a	43.66 ±1.96b	21.83±0.87	27.66±1801.23b	**T1 (Control)**
0.34±0.01b	55.50±2.04a	19.16±1.01	32.33 ± 557.77a	**T2 Addition of nano boron 40 mg / kg feed**
0.33 ± 0.03b	57.16±2.49a	19.16± 1.35	33.66±760.11a	**T3 Addition of nano boron 60 mg/kg feed**
0.37±0.02b	52.00±3.08a	19.00±0.77	33.33 ± 760.16a	**T4 Addition of nano boron 80 mg/kg feed**
*	*	NS	*	**Significant level**

Means with different letters within a single column are significantly different. * (P <0.05), NS: not significant.

The significant increase in the number of red blood cells (Table 3) is due to the role of nano-boron added at a concentration of 80 mg/kg of feed, stated that boron increases the iron concentration in the blood plasma, and that iron is one of the important factors that enter in the process of forming red blood cells, the increase in body weight and weight gain due to the addition of boron increases the number of red blood cells.

Or, the reason for this rise in red blood cells may be due to the role of boron in increasing the concentration of copper as copper increases the absorption of iron from the gastrointestinal tract and releases iron from its stores in the reticuloendthelial system, and then increases the biosynthesis of red blood cells (Al-Awadi and Al-Nadawi, 2020). In addition to the role of boron in increasing the production of the hormone thyroxine and activating its role in the body and its participation in the process of formation of red blood cells, as well as in raising the rate of gene expression for the manufacture of Erythropoietin. Erythropoietin is a growth factor that mainly stimulates the production of red blood cells and Erythropoietin is also co-neutralizing the negative action of estrogen. As for the increase in lymphocytes in the nano boron treatments, it may be considered a positive indication of a decrease in the inflammatory response, as boron is an important factor in activating the immune system, stimulating it and increasing its immune response (Al-Awadi, and Al-Nadawi, 2021). Nano boron may be a result of the active and positive role of boron in raising the body's immune response (Basoglu *et al*., 2010). Several biological molecules share this effect, including the FC receptor, as it is one of the proteins that are found on the surface of some types of white blood cells and contribute to the function Preventive effects of the immune system during its association with antibodies and attacking germs and pathogens (Boross *et al*., 2008). As for MCV and MCH, it is noted that they are significantly superior in the first treatment (control) compared to the nano-boron treatments. This may be due to the decrease in the numbers of red blood cells in the control treatment, which requires increasing the cell sizes to resist the decrease in the numbers of red blood cells, as this result is consistent. With Nbras and Nihad, (2019) who noticed a negative correlation coefficient between the total number of red blood cells, average cell size, and cell hemoglobin concentration.

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