Effect of Feed Restriction and Manganese Supplementation on the Immunological and Physiological Traits and Glycogen Level of Broiler Ross 308

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Abstract

The addition of manganese to the feed was applied to support the immune system of chickens and improve physiological characteristics and energy levels during and after the period of feed restriction of broiler (Ross 308). Using 180 chicks, divided into five groups with 45 chicks in each group and distributed into three replicates, each replicate contains 15 chicks. The groups were 0 manganese (Mn) + (*ad libitum* diets), 0 Mn + Feed restriction 8 h/day, 75, 100, and 125 mg Mn/ kg feed+ feed restriction 8 h/day for groups G1, G2, G3, G4, and G5 respectively, and were subjected to feed restriction (8 hours/day) for 8-14 days of the experiment. The results were: significantly ($P \leq 0.05$) superior to group G4 in the relative weight of the Bursa of Fabricius at the age of 21 days and for the groups G3 and G5 in the antibodies of Newcastle and IBD at the age of 21 and 35 days and group G3 in the relative weight of the thymus gland at the age of 35 days. In liver glycogen superiority of groups G1, G4, and G5 group increase in the muscle glycogen and red blood cells, the groups G1, G4 and G5 were significantly superior at the age of 21 days, and the group of G4 was superior at the age of 35 days compared with the rest of the groups , also significant increase in the ratio of H/L, triglycerides, uric acid, AST and ALT enzymes of G2 group at 21 and 35 days of age, while the cholesterol concentration of G2 and G4 groups increased at 21 days of age compared to the rest of the groups.

Keywords: Immunological, Glycogen, Manganese, Feed Restriction.

Introduction

Feed restrictions have been resorted to food restriction systems to get rid of the defects of the prepared carcass and reduce the content of abdominal fat in it while increasing the rate of protein sedimentation, which leads to improving the quality of the carcass and the use of feed restriction in raising broiler has an important role to reduce the amount of feed consumed and avoid the rate The rapid growth of modern breeds, in addition, restriction feed in the early stage of chick development is beneficial to improve the feed conversion factor and thus reduce the cost of rearing (1), and although restriction feed at an early age reduces growth performance, it will be Achieving compensatory growth in the complete post-feeding period to accelerate the growth of chicks in order to reach the required market weight, Some studies have shown that dietary restriction can reduce metabolic diseases and skeletal disorders that lead to economic losses due to reduced growth performance of birds, high mortality rates and rejection of marketed birds at slaughterhouses (1 and 18), Manganese is one of the main trace minerals with many physical and chemical properties, which is involved in many metabolic pathways and activities inside the body (18). Manganese and iron are also absorbed by binding to the same divalent metal ion carriers, so the absorption of iron is related to the level of available manganese and may cause an imbalance in anemia on the other hand, a high level of manganese inhibits iron metabolism via the synthesis of aminolevulinic acid which has a special role in heme synthesis, and manganese also participates in the activation of mineral enzymes that contribute to the metabolism of Carbohydrates, fats, and amino acids, Additionally, it is a crucial part of the enzyme Mn-SOD, which guards cell against oxidative stress. Reduced oxidative defense system, malformation of the skeleton, and cartilage malformation are all symptoms of manganese deficiency that are linked to skeletal and physiological disorders, and impaired reproductive and immune susceptibility (17). The current study aims to show the effect of the conditions of food restriction (time restriction) in conjunction with adding manganese to the diet and observing its subsequent effect on the birds represented in the immune response, physiological changes in the blood and the level of body glycogen until the end of the experiment at the age of 35 days.

Materials and Methods

This study was conducted on the farm of Jaflawi Poultry Company for the period from 25-2-2020 to 31-3-2020, one -day - old 225 chicks were used, which were divided into five groups, and the experimental parameters were as follows:

G1: control group without additives and free diets.

G2: feed restriction 8 hours/day.

G3: Add 75 mg manganese sulfate/kg feed with a feed restriction of 8 hours/day.

G4: Add 100 mg manganese sulfate/kg feed with a feed restriction of 8 hours/day.

G5: Add 125 mg manganese sulfate/kg feed with a feed restriction of 8 hours/day.

The dietary restriction application for the time from 8 to 4 pm for the period from 8 to 14 days of the experiment.

Feed group:

Diet ingredients are shown in the table below (Table 1).

Studied traits

The relative weight of immune organs

Three chicks from each group were randomly chosen when they were 21 and 35 days old, and the feed was cut off from them for 3 hours to stabilizing their weights. Then they were slaughtered and dissected to extract the lymphatic organs (thymus, Fabricius and spleen) and weighed using a sensitive scale for four decimal places, then extracted the

percentage of their weights to the weight of the living body according to the following equation:

$$\text{The relative weight of organs (\%)} = \frac{\text{Weight of the member eaten (gm)}}{\text{Live body weight (gm)}} \times 100$$

-antibodies against Newcastle and IBD (Infection bursal disease)

The titer of serum antibodies directed against Newcastle disease and IBD was measured by selecting an ELISA (Enzyme Linked Immune Sorbant Assay).

Glycogen in breast muscle and liver

The sections represented by chest muscle and liver tissue were collected for the times on day 10 of the experiment and at three times (8 am, 4 pm and 12 am) with a weight of approximately 1 g and preserved in 5 ml glass tubes by freezing. The glycogen concentration was measured according to Kadhim *et al*. (12).

Blood cellular and biochemical characteristics

Fresh blood tests and blood biochemical characteristics were conducted in the laboratory of the College of Sciences / University of Babylon according to Zaki and Al-Jebory(20).

Statistical analysis

The statistical Analysis was conducted according to the following mathematical model (7 and 16).

$$Yij=\mu+Ti+eij$$

Table 1. Percentages of diet components and their chemical composition

Ingredients %	Grower (1-3 W)	Finisher (4-5 W)
Corn	50.85	54.84
Wheat	10	10
Soybean Meal 44 % protein	28	24
Protein concentration*	5	5
Sun flower fat	4.15	4.3
Dicalcium phosphate (DCP)	0.5	0.4
Salt	0.1	0.1
Limestone	1.14	1.1
Methionine	0.13	0.13
Lysine	0.13	0.13
Total	100	100
**Chemical composition		
Metabolic Energy kcal/kg	3177.52	3227
Crude protein %	20.91	19.33
Crude fiber %	2.6	2.6
Lysine %	1.3	1.18
Methionine + cysteine %	0.96	0.91
Calcium %	0.88	0.84
Phosphors%	0.44	0.42

*The protein concentrate used by a Dutch company (imported) Brocon.

**Chemical composition according to (4) .

Results and Discussion

1- Immune traits

Table (2) shows the impact of the study on the immune traits of birds at the age of 21 days. The G4 group was significantly superior ($P\leq0.05$) compared to the other groups e except G1 non-significant with G4in Fabricius gland and spleen relative weight, and there was no significant difference between the groups, thymus

gland. Group G3 and G5 had significantly increased (P≤0.05) in antibodies of Newcastle disease, while a significant increase (P≤0.05) in antibodies against IBD disease to the G3 compared to the other groups except G1.

The relative weight of the Fabricius gland and the spleen did not significantly change between the groups at the conclusion of the experiment (35 days of bird age), (Table 3), The G3 group was significantly superior (P≤0.05) to the G3 group in terms of the relative weight of the thymus gland and the level of antibodies against Newcastle disease, while the G5 group was significantly increased (P≤0.05) compared to the other groups in the level of antibodies against IBD disease.

2- The glycogen level

The impact of the dietary restriction period on the amount of liver and muscle glycogen is shown in Table (4). The G3 and G4 groups were significantly superior (P≤0.05) in the liver glycogen compared with the G1, G2 and G5. As well as G1, G4, and G5 significant height in the muscle's glycogen level compared with the G2 and G3 groups.

Table 2. Effect of feed restriction and manganese supplementation in relative weight of the lymphoid organs (%) and the antibodies level for Newcastle and IBD disease at 21 days of age for broiler (mean ± standard error)

Groups	Bursa of fabricius	Thymus gland	Spleen	Newcastle disease antibody	IBD Antibody
G1	0.16±0.01 ab	0.18±0.02	0.08±0.01 ab	2928± 6.13 ab	2347±8.53 ab
G2	0.14±0.02 c	0.17±0.05	0.06±0.04 b	2457±5.88 b	1915±10.18 c
G3	0.15±0.04 b	0.18±0.03	0.08±0.00 ab	3100±5.50 a	2639±11.10 a
G4	0.17±0.04 a	0.16±0.07	0.09± 0.01 a	2878±9.24 ab	1810±9.22 c
G5	0.15±0.06 b	0.17±0.12	0.08±0.01 ab	3112±4.00 a	2159±6.32 b
Significant	*	N.S	*	*	*

Means with different letters differ significantly with level * (P≤0.05), N. S: Not significant The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

Table 3. Effect of dietary restriction and manganese supplementation on the relative weight of lymphoid organs (%) and antibodies level for Newcastle and IBD disease at 35 days of age (mean ± standard error) for broiler

Groups	Bursa of fabricius	Thymus gland	Spleen	Newcastle disease antibody	IBD Antibody
G1	0.09±0.03	0.24±0.10 b	0.20±0.05	3130±14.32b	2509±8.36b
G2	0.10±0.04	0.20±0.11 c	0.18±0.05	2980±12.50c	2310±16.25b
G3	0.09±0.04	0.28±0.15 a	0.19±0.02	4155±10.62 a	2387±20.67b
G4	0.09±0.05	0.23±0.32bc	0.21±0.01	3654±15.00 b	2471±19.51b
G5	0.08±0.01	0.25±0.13 b	0.20±0.04	3960±17.65 b	2953±13.26a
Significant	N.S	*	N.S	*	*

Means with different letters differ significantly with level * (P≤0.05), N. S: Not significant The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

3- Cellular characteristics of blood

The impact of food restriction and manganese on cellular blood traits is shown in Table (5), in 21 days the chickens in G1, G4, and G5 groups was significant improvement (P≤0.05) in red blood cells compared to G2, and G3 groups, while no significant difference in the number of white blood cells, as well a significant increased (P≤0.05) for G2 compared to the other groups in H/L ratio.

At 35 days old the chickens in the G4, group was significantly superior (P≤0.05) in red blood cells compared G2,G3 and G5 groups, while no significant difference in the number of white blood cells, in the H/L ratio significantly increased (P≤0.05) for G2, and G3 group compared other groups.

Table 4. Effect of dietary restriction and manganese supplementation on the level of liver and muscle glycogen (mg/g tissue) at 10 days of age (mean ± standard error) for broiler

Groups	Liver	Breast muscles
G1	19.48±4.82 b	20.00±2.55 a
G2	17.59±10.40 c	16.74±8.39 c
G3	20.19±2.97 a	19.19± 2.03 b
G4	21.78±5.39 a	20.68± 4.32 a
G5	19.12±6.87 b	21.29±7.04 a
Significant	*	*

Means with different letters differ significantly with level * (P≤0.05), The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

Table 5. Effect of dietary restriction and manganese supplementation on some cellular values of blood at 21 and 35 days of age (mean ± standard error) for broiler

Period		21 Day			35 Day	
Groups	RBC× 10^6 /ml^3	WBC 10^3 /ml^3	H/L%	RBC× 10^6 /ml^3	WBC 10^3 /ml^3	H/L%
G1	3.19±1.88a	31.17±4.12	0.21±0.03c	3.60±0.33ab	30.08±1.36	0.16±0.01bc
G2	2.58±1.00b	30.54±3.62	0.26±0.01a	2.94±0.58c	30.11±1.84	0.19±0.01a
G3	2.76±2.55b	32.69±1.26	0.20±0.30c	3.41±0.91b	29.91±1.11	0.17±0.02b
G4	3.11±3.62a	31.74±0.50	0.23±0.02b	3.78±0.64a	30.14±2.00	0.15±0.01c
G5	3.24±1.25a	32.38±1.09	0.21±0.05c	3.52±0.75b	30.22±1.45	0.15±0.01c
Significant	*	N.S	*	*	N.S	*

Means with different letters differ significantly with level * (P≤0.05), N. S: Not significant The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

4- Biochemical characteristics

At 21 days of age, (Table 6) noted a high significant for the G2 group in triglyceride; Uric acid and ALT enzyme compared other groups, chickens in G1, G2, G3, and G4 groups increased significantly (P≤0.05) in cholesterol compared to the G5, as well chickens in G1, and G2 groups were significantly superior (P≤0.05) in AST enzyme compared to the other groups.

At 35 days old G2 group was a significantly increased (P≤0.05) in

triglyceride; Uric acid and AST compare to other groups, while no significant difference in cholesterol and ALT levels between groups studied.

The improvement in the relative weight of the Fabricia gland and the thymus gland and the level of antibodies against Newcastle and IBD for the restriction groups with the addition of manganese to the diet at the age of 21 days and the continued superiority in the level of antibodies until the age of 35 days may explain the role of dietary restriction in supporting the immunity of birds and preventing diseases Metabolic diseases and Sudden death syndrome (3), indicating the effect of dietary restriction of feed by 20% at 7-15 days of age in lowering the antibodies directed against Newcastle disease, but it raised the level of Immunoglobulin A (IgA) compared with the control group. The improvement of the immune status of birds may also be due to the effectiveness of manganese added to the restriction diets in developing the health status of broiler compared to the control groups manganese, Being a co-factor and activator of numerous enzymes, including Galactosyltransferase, agmatinase, arginase, glutamine synthetase, pyruvate carboxylase, and superoxide dismutase (19) ,which are responsible for the proper development of body organs and tissues, cell structure, and metabolism, manganese is one of the mineral elements required for the growth and development of living organisms and the progression of metabolic, the mitochondrial antioxidant system and regulation of apoptosis (10), and as a result may be the reason for the improvement in the relative weight of the lymphatic organs and raising the level of antibodies against Newcastle and IBD diseases, and manganese deficiency in poultry diets can lead to an imbalance in the absorption of other mineral elements necessary for the immune system such as copper, zinc and iron, which leads to immunosuppression Increased intracellular oxidative stress, and continued deficiency can induce cell death For a programmer (6) and this explains the improvement in the immune status and the level of antibodies to NK and IBD patients in dietary restriction groups as a result of adding manganese to it, Mwangi *et al*., (14) found that the low level of manganese In the diet, lead to decrease immune response and the level of IgA and IgM antibodies, as well as the addition of manganese to the feed increases the phagocytic activity of microphage cells and natural killer cells as well as the expression of the pro-inflammatory cytokines IL-1β, IL-6, IL-8 and IFN and the antibody titer and this explains the improvement of the immune status in manganese addition groups , Our results agree with Babaei *et al*., (4) who found that supplementation of manganese at a concentration of 60, 72, and 84 mg/kg feed in comparison with the probiotic improved the relative weight of Fabricius gland and the level of antibodies against Newcastle disease as well as the concentrations of IgG, IgM and total immunoglobulin produced Against SRBC in the group of adding 84 mg manganese.

The absorbed manganese is transported to the liver cells and then to the mitochondria and the cell nucleus for use as a cofactor in the metabolic processes and in the intracellular protein and carbohydrate synthesis processes, and thus may improve the level of liver and muscle glycogen stores, as both the increase and decrease in the availability of manganese affect carbohydrate metabolism (9) within the body and manganese also activates multiple enzymes related to metabolism Carbohydrates such as pyruvate carboxylase (PC) (EC 6.4.1.1) and phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) were stored, Pyruvate carboxylase (16) is a manganese metalloenase enzyme containing 4 moles of manganese which explains the improvement The level of glycogen in

manganese addition groups and its clear role in reducing the effect of dietary restriction through the role of manganese in activating enzymes of carbohydrate synthesis as a cofactor on the one hand and its entry into the formation of some enzymes on the other hand (11).

The reason for the increase in the number of red blood cells for most of the restriction groups compared with the control group may be due to the clear role of manganese in mitigating the effects of dietary restriction, as it is known that dietary restriction reduces the number of red blood cells (8), erythropoiesis pathway due to a decrease in the energy level in the red blood cell manufacturing pathways. Inside the body (5), and the positive effect of manganese can also be inferred from the decrease in the ratio of lymphocytes to heterotrophic cells (H/L) in the blood of most birds of the restriction groups compared to the control except for the G2 group, to which no fodder was added, where H/L is the best evidence that birds are exposed to any stress (2), which shows the clear role of manganese as an antioxidant in reducing the severity of stress.

The decrease in the level of uric acid and ALT enzyme in the serum of birds of control and restriction-treated birds fortified with manganese powder is probably due to the antioxidant role of manganese (14) which reduced the level of uric acid and ALT in the serum of birds fed manganese as the dietary restriction Alone, without feed additives, may cause an increase in uric acid and ALT enzyme in the blood serum. It is known that AST and ALT are liver enzymes, and their high levels indicate damage to liver cells, and the level of uric acid is an indicator of kidney function. It was found that adding manganese to a broiler diet affects the gene expression activity of liver enzymes, which is very important as a defense system. Enzymatic antioxidant in activate free radicals caused by metabolic disturbance, which may reduce metabolic diseases caused by dietary restriction (13). Elevated levels of ALT and AST in the blood and tissues can be an important indicator of damage to the body. Tissue that can result from an increased level of reactive oxygen species, while it was found Mwangi et al., (14) the addition of 60 mg/kg feed of manganese improved the level of T-SOD and ALT enzyme activity, The changes observed in the dietary restriction parameters and the addition of manganese from the improvement in the level of cholesterol and triglycerides and the decrease in the activity of liver enzymes indicate the ability of manganese to improve the antioxidant system well and this improves better when adding manganese in the form of nanoparticles as it stimulates more than the enzyme SOD and catalase, and the addition of manganese to the diet is associated with an increase in the efficiency of absorption and representation of other mineral elements from iron, copper and zinc, and thus the activity of enzymes and oxidation systems associated with these elements, and this explains the improvement in the number of red blood cells, as these elements stimulate the process of The formation of red blood cells erythropoiesis.

Table 6. Effect of dietary restriction and manganese supplementation on the values of some cellular traits of blood and biochemical traits of blood serum at 21 days of age (mean ± standard error) for broiler

Groups	Triglyceride (mg/100 ml blood)	Uric acid(mg/100 ml blood)	Cholesterol (mg/100 ml blood)	AST (UI/L)	ALT (UI/L)
G1	193.72±6.37b	4.55±0.19b	135.79±6.40b	88.20±1.20a	6.35±0.12ab

G2	232.37±21.72a	5.68±0.21a	148.00±2.50a	89.61±6.72a	6.50±0.07a
G3	182.82±15.37c	4.09±0.16b	131.66±2.46b	80.26±2.94b	5.71±0.02b
G4	187.35±10.95c	4.23±0.25b	132.45±2.95a	78.42±2.30b	5.70±0.20b
G5	190.75±6.55b	4.49±0.33b	126.87±1.32c	79.09±0.90b	5.58±0.57b
Significant	**	**	*	*	*

Means with different letters differ significantly with level * (P≤0.05), **(P≤0.01), The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

Table 7. The effect of dietary restriction and manganese supplementation on the values of some cellular traits of blood and biochemical traits of blood serum at 21 days of age (mean ± standard error) for broiler

Groups	Triglyceride (mg/100 ml blood)	Uric acid(mg/100 ml blood)	Cholesterol (mg/100 ml blood)	AST (UI/L)	ALT (UI/L)
G1	202.67±3.33ab	3.61±0.50b	134.66±2.33	83.39±2.38b	6.25±3.60
G2	210..85±3.35a	5.01±0.35a	135.28±2.28	94.71±2.54a	6.21±1.04
G3	197.05±7.85b	3.84±0.32b	132.65±1.35	82.59±11.90b	5.99±0.69
G4	191.65±9.65b	4.96±0.48ab	132.58±0.28	86.63±0.74b	6.37±1.63
G5	193.62±3.77b	2.89±0.25c	129.97±4.82	88.05±0.57b	6.25±0.45
Significant	*	*	N.S	*	N.S

Means with different letters differ significantly with level * (P≤0.05), N. S: Not significant The groups G1, G2, G3, G4, and G5, are control groups without addition, feed restriction 8 h/day, adding 75, 100, 125 mg/kg manganese and feed restriction 8 h/day respectively.

Conclusion

In general, the results of the above experiment showed an improvement in the immune system of the chickens and some physiological characteristics, as well as an improvement in the level of liver and breast muscle glycogen when chicks being exposed to fasting during the period of feed restriction, and this was the result of adding manganese to the diet. That is why it is recommended to add it to commercial rations in conjunction with feed restriction because of their important economic role.

Conflicts of interest

The authors have no conflict of interest.

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