

Research Article



Immunity and Glycogen Metabolism of Laying Hens Fed Diets Supplemented with Manganese Sulfate During the Force Molting

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Abstract | This study was carried out to evaluate the effect of dietary manganese supplementation on the glycogen metabolism and immunity of laying hens during the force molting. A total of 120 Lohman brown chickens aged 80 weeks were exposed to force molting for 6 weeks using the fasting program. All laying hens were randomly assigned to five dietary treatments, each containing three replicates (8 chickens/ replicate). Hens in the first group were fed the basal diet without supplementation and served as the control (G1), while those in the 2nd (G2), 3rd (G3), 4th (G4), and 5th (G5) groups were fed the basal diets supplemented with 25, 50, 75, and 100 mg manganese/kg of diet, respectively. The results showed that the G3 group exhibited the highest ($P \leq 0.05$) antibody titers against Newcastle disease at the 3rd and 6th weeks. Total immunoglobulin titers were found to be increased ($P \leq 0.05$) in the G4 group at the 6th week, while the G3, G4, and G5 groups showed a significant increase ($P \leq 0.05$) in IgG antibody titers throughout the entire experimental period. Moreover, all manganese treatments significantly improved ($P \leq 0.05$) AST enzyme levels, while the G3, G4, and G5 groups showed significant ($P \leq 0.05$) improvements in ALT enzyme levels. Hens in G1 and G2 had significantly ($P \leq 0.05$) the lowest liver glycogen levels at one, three, and six weeks. Additionally, hens in G1 and G2 respectively had significantly ($P \leq 0.05$) the lowest heart glycogen levels at one and six weeks, while hens in G1 and G4 respectively showed significantly ($P \leq 0.05$) the lowest muscle glycogen levels at one and three weeks. These results showed that addition of manganese to chicken diet improved their immune status, and metabolic state during forced molting.

Keywords | Immunity, Glycogen, Force molting, Manganese, Laying, Hens.

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INTRODUCTION

There are several methods of molting, each of which results in a reduction in the body weight of laying hens. Many of these methods, particularly fasting, subject the chickens to stress due to starvation. This stress leads to decreased immunity and metabolic rate disruptions (Khalf et al., 2023). Recently, public concern about the practice of depriving laying hens of food to induce molting has grown (Wang et al., 2023). A new management approach ad-

ressing animal welfare standards is the non-fasting molt (Lei et al., 2023). Various techniques can induce molting, including the traditional fasting molting program (Ahmed et al., 1995) and high dietary doses of Zn (Berry and Brake, 1987).

Research indicates that the molting process weakens the bird's cellular immune system and promotes a systemic illness state (Zhang et al., 2021). Fasting during molting programs results in reduced populations of splenic leuko-

cytes and circulating leukocytes, along with elevated levels of circulatory adrenal corticoids (Akram et al., 2002). To mitigate these effects, it has become necessary to supplement the chicken diet with immunomodulatory and growth promoting agents. Manganese is crucial in chicken nutrition as it acts as a catalyst in numerous metabolic processes and enhances enzyme activity (Olgun, 2017; Al-Saeedi et al., 2023). It also plays a vital role in stimulating chicken immunity by interacting with the basement membrane of neutrophil and macrophage cells. These cells play essential roles in initiating primary immune responses and subsequent production of antibodies crucial for disease defense (Sunder et al., 2006). Therefore, the current study aimed to assess the immunological and metabolic status of laying hens fed diets supplemented with manganese sulfate during forced molting.

MATERIALS AND METHODS

BIRDS AND EXPERIMENTAL DESIGN

This experiment was carried out at the poultry farm, Faculty of Agriculture, Al-Qasim Green University, Babylon, Iraq. A total of 120 Lohman brown chickens, 80 weeks old, were randomly assigned to five groups, each group containing three replicates (8 hens/ replicate). All hens were exposed to force molting for 6 weeks using the fasting program. Hens in the first group were fed the basal diet without any supplementation and served as control, while those in the 2nd, 3rd, 4th and 5th group were fed the basal diets supplemented with 25, 50, 75, and 100 mg manganese/kg diet, respectively.

MOLTING PROGRAM

The molting program was implemented with continuous lighting (24 hours) during the first five days. After this period, the chickens underwent fasting, and the lighting hours were reduced to 12 hours per day. Before fasting, the birds weighed 2350 grams. After six days of fasting, their weight decreased by approximately 15-20%. By the 21st day of molting, the number of lighting hours was gradually increased, reaching 16 hours per day by day 35 of the molting period.

DIETS

The composition of diet fed to chickens is presented in Table 1. Before molting, hen fed 115 gm of feed with the addition of manganese at the different experimental levels. In molting, the feed was restricted during the fasting period, then 50, 80, and 90 g/hen were given for the third, fourth, and fifth weeks, respectively, then after molting (the sixth week), chickens were fed 100 gm ration per day.

Table 1: Diet ingredients and their chemical composition

Ingredient	During molting	After molting
Yellow corn	30	36.5
Wheat	15	12
Barley	20	13.9
Soybean meal	20	25
* Premix	2	2.5
DCP Calcium Diphosphate	9	8.3
Sunflower oil	4	1.8
Total**	100	100
Metabolic energy (kilocalories/kg feed)	2970	2758.68
Crud protein (%)	16	17.19
Crud fiber	5	3.24
Calcium%	2.28	3.81
Available phosphorus (%)	0.45	0.29
Methionine + cysteine (%)	0.45	0.73
Lysine (%)	0.45	0.95

*Maxcare® Belgium

** Chemical analysis computed according to NRC (1994).

MEASUREMENTS

Immunology: Blood samples were collected at the end of the first, third, and sixth weeks, and immunological markers including antibody level against Newcastle disease, total immunoglobulins and IgG antibody titer were measured, according to Synder et al. (1984).

Liver enzyme and glycogen: Glycogen levels in the liver, heart, and muscles were measured according to Plummer, (1978). Aspartate and alanine aminotransferase (AST and ALT) enzymes were measured in blood serum using a standard kit (English company Randox), according to Reitman and Frankel (1957).

DATA ANALYSIS

The data analysis was performed by SAS-System (2012), and the Duncan multiple ranges test (Duncan, 1955) was applied to calculate the statistical differences between various groups. The results were considered significant when their P-value was found ≤0.05.

RESULTS

IMMUNITY STATUS

Data in Table 2 shows the effect of experimental groups on Newcastle disease antibodies, at 1st week of force molting, G3 and G4 groups showed the highest Newcastle disease antibodies compared to the other groups. Also, G3 group had the highest (P≤0.05) Newcastle disease antibodies at 3rd and 6th week of force molting compared to G1, G2, G4 and G5, respectively.

Table 2: Effect of manganese supplementation on antibody level against Newcastle disease in laying hens exposed to force molting

Groups	Means± SE		
	1 week	3 week	6 week
G1	4142.53±10.63 b	3890.12±5.51 d	8791.11±12.07 ab
G2	4053.65±14.01 b	5041.27±9.35 b	7963.15±10.11 c
G3	5421.20±12.31 a	6007.36±11.08 a	9151.14±16.52 a
G4	5397.25±16.80 a	4204.71±8.77 c	8960.58±14.22 ab
G5	4506.79±10.08 b	5932.16±15.78 ab	8564.75±8.65 b
Significant	*	*	*

* ($P \leq 0.05$).

a, b and c letters in a column showing significant difference between groups at ($P \leq 0.05$).

Table 3: Effect of manganese supplementation on total immunoglobulin and IgG antibody titer in laying hens exposed to force molting

Groups	Means± SE		
	1 week	3 week	6 week
Total immunoglobulin titer (g/l)			
G1	2.18±0.01	2.24±0.04	2.51±0.09 b
G2	2.25±0.03	2.05±0.07	2.53±0.14 b
G3	2.41±0.08	2.55±0.02	2.49±0.03 b
G4	2.01±0.02	2.22±0.03	3.14±0.06 a
G5	2.13±0.05	2.21±0.05	2.59±0.01 b
Significant	N.S	N.S	*
IgG antibody titer (g/l)			
G1	3.24±0.25 b	4.23±0.10 c	3.31±0.15 c
G2	3.69±0.31 b	4.39±0.78 c	3.28±0.11 c
G3	4.52±1.02 a	6.88±0.13 a	4.60±1.05 b
G4	4.61±0.55 a	5.21±0.14 b	5.88±0.30 a
G5	3.70±0.21 b	5.27±0.62 b	4.69±0.24 b
Significant	*	*	*

N. S: Not significant; * ($P \leq 0.05$).

a, b and c letters in a column showing significant difference between groups at ($P \leq 0.05$).

The change in total immunoglobulin titer and IgG antibody titer were shown in Table 3. Total immunoglobulin titer noted that there were no significant differences between groups in the 1st and 3rd weeks, meanwhile, there were a significant increase ($P \leq 0.05$) in G4 group at the last experimental week compared to other groups. However, G3, G4, and G5 groups showed significant increased ($P \leq 0.05$) in IgG antibody titer during the experimental periods.

LIVER MARKERS

The liver enzyme response of laying hens during different stages of forced molting is presented in Table 4. Aspartate aminotransferase (AST) levels showed significant improvement ($P \leq 0.05$) in all manganese-supplemented

groups compared to the control group. However, no significant differences were found between groups at three and six weeks. Serum alanine aminotransferase (ALT) demonstrated significant improvement ($P \leq 0.05$) in groups G3, G4, and G5 compared to groups G1 and G2 at one week. Similarly, no significant differences were observed between groups at three and six weeks.

Table 4: Effect of manganese supplementation on liver enzymes in laying hens exposed to force molting

Groups	Means± SE		
	1 week	3 week	6 week
AST (U/L)			
G1	70.28±1.24 a	48.17±0.95	52.14±0.27
G2	50.14±3.21 c	46.51±1.75	49.78±1.17
G3	65.31±0.89 b	47.01±2.05	50.45±1.33
G4	60.24±1.13 b	48.52±1.00	51.02±2.05
G5	61.82±4.16 b	47.53±0.25	50.51±0.13
Significant	*	N.S	N.S
ALT (U/L)			
G1	5.83±0.16 a	6.24±0.02	5.12±3.55
G2	5.09±0.12 a	6.11±0.03	4.97±1.18
G3	3.69±1.54 c	6.89±0.14	5.32±1.30
G4	4.15±0.05 b	6.14±2.24	5.01±0.31
G5	4.35±0.22 b	6.47±1.55	5.78±1.00
Significant	*	N.S	N.S

N. S: Not significant; * (P≤0.05).

a, b and c letters in a column showing significant difference between groups at (P≤0.05).

Table 5: Effect of manganese supplementation on glycogen level (μmol of glucose/g tissue) in laying hens exposed to force molting

Groups	Means± SE		
	1 week	3 week	6 week
Liver			
G1	29.46±5.09 c	30.50±1.57 bc	27.85±1.88 c
G2	29.57±1.87 c	28.69±1.81 c	29.62±1.45 b
G3	36.29±4.04 ab	35.75±2.75 ab	33.38±3.19 ab
G4	34.99±2.20 b	37.82±1.07 a	35.58±2.42 a
G5	38.03±1.35 a	34.55±2.34 b	34.40±1.19 ab
Significant	*	*	*
Heart			
G1	19.10±2.10 d	20.25±1.11	20.68±1.52 c
G2	21.31±3.04 c	21.07±1.32	18.87±2.02 d
G3	24.79±1.10 b	20.71±1.43	23.06±1.27 b
G4	26.38±0.60 a	20.09±1.09	22.61±0.51 b
G5	26.09±1.48 a	20.64±1.30	25.10±1.14 a
Significant	*	N.S	*
Muscle			
G1	9.22±0.71 c	22.96±1.78 b	12.79±2.34
G2	10.46±2.35 bc	25.22±3.46 a	12.58±1.09
G3	14.70±0.81 b	24.95±4.72 ab	12.32±0.67
G4	9.77±1.52 c	20.93±1.62 c	11.84±0.32
G5	18.57±2.01 a	22.72±1.37 b	12.21±1.02
Significant	*	*	N.S

N. S: Not significant; * (P≤0.05).

a, b and c letters in a column showing significant difference between groups at (P≤0.05).

GLYCOGEN LEVEL

The effect of different dietary manganese levels on glycogen levels is presented in Table 5. The data indicate a significant increase ($P \leq 0.05$) in liver glycogen in group G5 compared to groups G1, G2, and G4 at one week. By the third week, group G4 showed a significant increase ($P \leq 0.05$) in liver glycogen.

In the heart, significant increases ($P \leq 0.05$) in glycogen were observed in groups G4 and G5 compared to other groups at one and six weeks, respectively. Similarly, muscle glycogen levels significantly increased ($P \leq 0.05$) in groups G5 and G2 compared to other groups at the first and third weeks, respectively.

DISCUSSION

The notable enhancement in immunological indices observed in manganese-supplemented groups (including increased levels of antibodies against Newcastle disease, total immunoglobulins, and IgG) may be attributed to manganese's role in stimulating neutrophils and macrophages. This stimulation occurs through interactions with these cells' membranes, thereby promoting immune responses and subsequently boosting antibody and immunoglobulin production (Sunder et al., 2006).

Additionally, manganese supports the activity of superoxide dismutase, crucial for the function of heterophils and macrophages (Sabaghi et al., 2021; Luo et al., 2007). According to Ghodh et al. (2016), supplementation with 75 mg/kg of manganese in the diet increased antibody titers against Newcastle disease compared to control groups. On the other hand, Onbasilar and Erol (2007) reported decreased immunity and elevated levels of the stress hormone corticosterone in laying hens subjected to molting using two methods. Increased corticosterone levels during stress can lead to decreased immunity in birds through receptor interactions on lymphocyte surfaces, resulting in immunosuppression (Tian et al., 2022). Research by Zhang et al. (2023) further supports this, showing that elevated corticosterone levels inhibit lymphocyte reproduction. In summary, manganese supplementation appears to play a critical role in enhancing poultry immune function, while stress-induced increases in corticosterone can negatively impact immunity in birds.

The elevated levels of liver enzymes observed in the control group may be attributed to high corticosterone levels induced by stress during the fasting period in chickens. This stress can affect various liver enzymes, including ALT and AST, increasing their activity in the bloodstream (Tang et al., 2022; Richard and Preston, 2005). ALT and AST enzymes are crucial for glucose production from glycogen

and amino acids, facilitating the transfer of amino groups from alpha-amino acids to keto acids. Glycogen serves as a vital energy source in the Krebs cycle for mitochondrial energy production (Stryer, 2000; Al-Jebory and Ibrahim, 2021). The increased activity of these enzymes correlates with a decrease in glycogen levels in chickens from these groups, aiming to elevate serum glucose levels. Additionally, thyroid hormones influence AST enzyme activity; Kaplan and Larsen (1985) noted that decreased thyroid activity is linked to increased AST activity, resulting in reduced protein and carbohydrate synthesis, thereby explaining the lower glycogen levels in the control group.

The improved glycogen levels observed in the manganese-supplemented groups may be attributed to manganese's role in numerous metabolic reactions essential for animals. In poultry nutrition, manganese is crucial for bone formation and various biochemical processes, activating enzymes such as pyruvate carboxylase, superoxide dismutase, and glycosyl transferase (Suttle, 2010). Manganese is essential for embryonic development, normal body and bone growth, reproduction, and the metabolism of carbohydrates and lipids (Olgun, 2017).

CONCLUSIONS

The inclusion of manganese in the chicken diet enhanced their immune status and metabolic health during forced molting, underscoring manganese's pivotal role in mitigating the stress severity experienced by chickens during fasting. Moving forward, efforts should focus on developing methods to further alleviate stress in chickens and promote enhanced welfare standards.

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CONFLICT OF INTEREST

There is no conflict of interest disclosed by the writers.

NOVELTY STATEMENT

This study is the first to use manganese to boost immunity and metabolism at a layer of the Iraqi/Babylon government.

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ETHICS STATEMENT

Ethics statement: Every animal used in this research was treated and handled in compliance with the necessary biosecurity protocols. Prior to commencing this study, the Ethics Committee of the College of Agriculture, Al-Qasim Green University, Iraq (Number 21 A.P. at 15/12/2022) accepted the guidelines for the care and use of laboratory animals and the Lohman layer guide.

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