



SOME METABOLIC AND PHYSIOLOGICAL RESPONSES OF BROILER CHICKS AS AFFECTED BY DIFFERENT SOURCES AND LEVELS OF DIETARY MANGANESE

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ABSTRACT

The aim of this study was to determine how manganese forms, inorganic or nano, at different levels, would affected the digestibility coefficients of nutrients and some blood biochemical parameters in broiler chicks. A total number of 180 one day - old broiler chicks (Arbor Acres plus) were used in factorial experiment (2x3) two supplementation forms (inorganic and nano) and three supplementation levels (0, 6 and 12 mg Mn/Kg diet). The 35-day experiment was carried out. Results of this study showed that, birds fed diets incorporated with nano manganese recorded significantly higher digestibility of ($P \leq 0.01$) dry matter (DM), organic matter, (OM) crude protein (CP), nitrogen free extract (NFE) and total protein concentration (TP). No significantly effects were detected in albumin, globulin or glucose concentration values. Birds received diet incorporated with 12 mg Mn/kg diet recorded significantly increased in concentration ($P \leq 0.01$) of triglycerides and VLDL cholesterol. Birds fed basal diets enriched with 12 mg nano Mn/kg diet presented the lowest level ($P \leq 0.01$) of GPT value. In conclusion, the effect of Mn in nano form was positively more pronounced than the inorganic in broiler chicks.

Key words: broilers, manganese, nano, digestibility, blood parameters.

INTRODUCTION

Manganese (Mn), which is the fifth most bountiful element on earth, is measured as trace elements. Since the

1930s, Mn has been known to be imperative in animals health (Suttle, 2010). Mn is necessary for the formation

of bones in chicken nutrition (Underwood, 1977). Additionally, it promotes several metabolic processes through the activation of enzymes such as pyruvate carboxylase, glycosyl transferase, and superoxide dismutase (Suttle, 2010).

The development of the embryo, proper bone and body growth, reproduction, and the metabolism of lipids and carbohydrates all depend on manganese (Mn) (Underwood, 1977). Moreover, Mn has a crucial role in maintaining the quality of eggshells and preventing perosis (Suttle, 2010).

Microminerals are components of many enzymes that are essential for normal biochemical reactions and take part in a variety of biochemical processes directly. Nanoparticles formula of trace minerals has been successfully added to chicken diets recently to meet their mineral needs. The nanoparticles are anticipated to differ from typical forms because of their incredibly small size and unique physical characteristics (Vinus, 2017).

Nanotechnology is a talented and evolving technology that has enormous possible to remodel poultry sector in all over the globa. The word nano technology is derivative from the Latin word “nanus” connotation dwarf. Nanoparticles generally have sizes ranging from one to one hundred nanometers. Because of this, these nanoparticles have better bioavailability at the target regions and can avoid the physiological pathways involved in nutrient distribution and transport across tissue and cell membranes. At the molecular level, nanotechnology is a subversive modernization that ultimately creates materials and enhances food's

structure, texture and quality. This technology has significant effects on food production, processing, transportation, storage, safety, and security for use by both humans and animals (Vinus, 2017).

This study was designed to evaluate the effect of manganese addition to the diet of broiler chicks at three different levels (0, 6, and 12 mg Mn/kg food) for both inorganic or nano forms on digestibility coefficients of nutrients, and some blood biochemistry of broiler chicks.

MATERIALS AND METHODS:

Study architecture and management:

This study was conceded at the Agricultural Farm of Animal and Poultry Production, Faculty of Agriculture, Minia University. One hundred eighty, unsexed, Arbor Acres plus broiler chicks one-day -old were randomly divided into 6 treatment groups of 30 chicks each. Each treatment group had 3 replicates of 10 broiler birds each. The average initial body weight was nearly comparable. Chicks were purchased and kept in ground floor (10 chicks/ m²) in open housing system. Feed and water available *ad-libitum* during the whole experiment period (0 to 35 days of age). All treatments preserved sanitary conditions and parallel management practices for the chicks. Two feeding phases were used in this experiment, starter period from 1-21 days of age then, switched to finisher period from 22-35 days of age. Birds of all trial groups were fed a basal diet as recommended by the National Research Council, NRC, (1994), chicks were randomly divided into two groups: - the 1st group received basal diets incorporated with inorganic

manganese, while the 2nd group received basal diets incorporated with nano manganese. Each group was divided into three subgroups, the 1st subgroup was fed diets contained 60 mg manganese/Kg diet (NRC,1994), while the 2nd and the 3rd subgroups were fed diets supplemented with 6 and 12 mg manganese/Kg diet, respectively. The Ingredients and chemical analysis of basal diet of broiler chickens are shown in **Table (1)**.

Applying green tomato aqueous extracts, eco- friendly (green synthesis) Mn nanoparticles from its oxide (MnO₂) were synthesised using the method of (Ogunyemi *et al.*, 2023).

Chemical composition was determined by A.O.A.C. methods (2006). DM, OM, CP, EE, CF, ash, and NFE.

Collecting blood samples:

Blood samples were collected at the time of the slaughter at seven in the morning at the end of the experiment (35 days of age). The blood samples that were collected from each experimental group were placed in only one blood collection tube. The serum was separated from this sample, which was obtained in an unheparinized state, and some serum biochemical parameters were then stored at (-20°C) after the sample was 15 minutes were devoted to centrifuging at 3000 rpm. The analyses of the chemicals

concluded at laboratories of the Department of Animal and Poultry Production, Faculty of Agriculture, Minia University, Egypt. Kits were purchased from Biodiagnostic Company in order to estimate blood biochemistry.

Statistical analysis:

The factorial experiments were designed as 2 supplementation forms x 3 supplementation levels. Data statistically analyzed using the general linear model (GLM) procedure of statistical analysis system (SAS, 2003). The model of statistical used in each experiment as follows:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Where:

Y_{ijk} = The observation value on the concerned trait.

A_i : Fixed effect of addition form ($i = 1,2$).

B_j : Fixed effect of the supplementation levels ($j = 1,2,3$).

AB_{ij} : Fixed effect of interaction between supplementation form and levels.

e_{ijk} = A random error.

Among treatments the significant differences were arranged by Duncan's multiple range test at level 5% (Duncan, 1955).

Table (1): Ingredients and chemical analysis of basal diet of broiler chickens.

Ingredient, %	Starter diet (1-21 days of age)	Finisher diet (22-35 days of age)
Yellow corn	55.23	54.30
Soybean meal, 44% crude protein	32.82	36.61
Corn gluten meal	5	0
Sun flower oil	3.05	6
Dicalcium phosphate	1.65	0
Monocalcium phosphate	0	1.05
Limestone	1.15	1.2
Sodium chloride	0.25	0.25
DL-methionine	0.15	0.14
Lysine	0.25	0.2
Cystine	0.2	0
Vitamin-mineral premix *	0.25	0.25
Calculated chemical analysis		
Crude protein,	22.98	20.96
Metabolizable energy(Kcal/ kg)	3050	3172.65
Crude fiber	3.78	3.76
Calcium	0.91	0.75
Avail phosphorus	0.45	0.38
Methionine & Cystine	1.0	0.8
Mn**	0.001	0.001

* Vitamins and minerals mixture of each kilogram contains; 800 mg Vitamin K3; 40 mg Vitamin B1; 1600 mg Vitamin B2; 600 mg Vitamin B6; 4800.000 IU of Vitamin D3; 4.000 mg Vitamin E acetate; 4.00 mg of Vitamin B12; 4.00 mg pantothenic acid; 8.00 mg Nicotinic acid; 400 mg Folic acid; 20 mg Biotin; 200 mg chorine; 4.000 Copper; 400 mg Iodine; 120.00 mg Iron; 22.000 mg Manganese; 22.000 mg Zinc, and 40 mg Selenium.. **To adjust the Mn contents in the diets extra addition of Mn was added to the diets after Mn calculation.

RESULTS AND DISCUSSION:

Impact of manganese supplementation forms and levels on digestibility of nutrients:

Data showed in Table (2) revealed that, the effect of manganese supplementation forms recorded highly significant effects ($P \leq 0.01$) on digestibility values of DM, OM, CP, EE, and NFE %. In general, chickens

received diets complemented with nano manganese recorded the highest values of the previous parameters except ether extract compared with inorganic form. While, chickens received diets complemented with inorganic manganese observed higher value of ether extract. Also, chickens received diets incorporated with inorganic Mn

achieved higher insignificant ($P > 0.05$) digestibility value of crude fiber.

These data are in agreement with **Jankowski et al., (2018)** reported that, digestibility value of (DM, OM, and CP) were increased when NP-Mn₂O₃ was added to the diet in place of MnO.

On the other hand, Lv et al., (2023) observed a linear and quadratic decline in the apparent and true availability of nutrients (DM, OM, and CP) in broiler chicks fed a meal supplemented with inorganic trace minerals (Fe, Zn, Cu, and Mn).

The data of Mn supplementation levels indicated that, there were highly substantial effects ($P \leq 0.01$) on digestibility values of dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen free extract %. Birds fed diets incorporated with 6 mg Mn / kg diet recorded higher digestibility values of dry matter, organic matter, crude fiber and nitrogen free extract % compared with other supplementation level of Mn. While, birds fed diets incorporated with 12 mg Mn /kg diet recorded a significant increase in digestibility value of ether extract and crude protein.

The present data of interaction between Mn supplementation forms and levels revealed that, there was a discernible effect ($P \leq 0.01$) on dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen free extract among all treatments. Birds fed diets supplemented with 12 mg nano Mn /kg diet recorded the highest value of dry matter, organic matter, crude protein and nitrogen free extract. While, birds fed diets supplemented with 12 mg inorganic Mn /kg diet recorded the highest significant value of ether extract.

Furthermore, chickens received diet complemented with 6 mg nano Mn /kg diet achieved the highest digestibility value of crude fiber.

The incorporation of Mn forms to the broiler diet may have improved the digestibility of some nutrients, ensuring that Mn remains essential for the metabolism of amino acids and carbohydrates as well as for many other enzymes and proteins, including superoxide dismutase, transferases, and hydrolases (**Moomaw et al., 2009**).

In addition, the better absorption of insoluble nanoparticles (NPs) over their soluble counterparts may account for the predominant role of nano Mn in enhancing the digestion of some nutrients. In general, the bioavailability of the nutrients may be enhanced by the effective delivery of active molecules to target sites (**Chen et al., 2006**).

Impact of manganese supplementation forms and levels on some blood serum biochemical parameters:

Total Protein, albumin, globulin and glucose:

Data listed in Table (3) showed that, manganese supplementation forms (inorganic or nano) had highly substantial effects ($P \leq 0.01$) on total protein. Birds fed nano form recorded significantly higher value of total protein. Also, the data of Mn supplementation forms showed insignificant ($P > 0.05$) differences in albumin, globulin and glucose concentration. Birds fed nano manganese achieved lower insignificant ($P > 0.05$) value of glucose concentration.

Matuszewski et al., (2020) speculated that, administration of nanoMn₂O₃ had no effect on parameters such as total protein, glucose (Glu), and albumin (Alb).

The results of Mn supplementation levels indicated that, no substantial effect ($P>0.05$) observed on total protein, albumin, globulin or glucose concentration. Insignificant decrease was detected in glucose value for birds fed diets incorporated with 12 mg Mn /kg diet compared with other supplementation level.

These data are concur with **Ghosh et al., (2016)** who fed broiler chicken on diet enriched with Mn at levels 50, 75, and 100 mg/kg diet and found that, adding Mn had no influence on glucose concentration.

The present data of interaction between Mn supplementation forms and levels, showed that, chickens received diets accompanied with 0, 6 and 12 mg nano manganese/kg diet recorded significantly greatest concentration ($P<0.05$) of total protein. However, there were no discernible effects ($P> 0.05$) were observed in values of albumin, globulin and glucose among all treatments.

Ognik et al., (2019) fed turkey on diet accompanied with two forms of Mn (NP-Mn₂O₃ or MnO) at three dosages: 10, 50 and 100 mg/kg. They found that, using Mn at 10 and 50 mg/kg significantly decreased the content of albumin.

Lipid profiles

Data listed in Table (4) showed that, broiler chickens received diet enriched with nano manganese or its

inorganic form, recorded insignificant differences ($P>0.05$) in values of triglycerides, total cholesterol, HDL, LDL and VLDL. Birds fed nano manganese recorded insignificant decrease in values of triglycerides, total cholesterol, LDL and VLDL. Also, birds fed nano form recorded insignificant increase in value of HDL compared with inorganic form.

These results concur with **Ghosh et al., (2016)** who found that, adding Mn had no impact on the serum concentrations of total cholesterol. Also, **Matuszewski et al., (2020)** speculated that, administration of nanoMn₂O₃ had no effect on parameters such as triglycerides (TG) or total cholesterol.

On the other hand, Pieřová et al., (2019) revealed that, adding inorganic Mn to the diet significantly reduced the concentration of total cholesterol in the hens' blood as compared to the group receiving the basal diet.

Noticeably, birds received diets complemented with 12 mg Mn/kg diet achieved significantly higher ($P\leq 0.01$) values of triglycerides or VLDL. Hence, there were no discernible effects ($P> 0.05$) of Mn levels on cholesterol, HDL and LDL concentration.

The present data of interaction between Mn supplementation forms and levels, showed that, chickens received diets complemented with 6 mg nano Mn /kg diet recorded significantly the lowest ($P\leq 0.01$) values of triglycerides or VLDL. However, there were no discernible effects ($P> 0.05$) in values of total cholesterol, HDL and LDL concentration were showed among all dietary treatments.

Table (2): Impact of manganese supplementation forms and levels on digestibility of nutrients in broiler chickens.

Items	Dry matter	Organic matter	Crude Protein	Ether Extract	Crude Fiber	Nitrogen Free Extract
Mn supplementation forms (A):						
Inorganic Mn	77.62^b	78.36^b	91.03^b	90.97^a	39.24	78.94^b
Nano Mn	80.75^a	80.99^a	92.46^a	89.29^b	38.52	80.69^a
±SEM	0.41	0.29	0.16	0.20	0.30	0.55
Significance	**	**	**	**	NS	**
Mn supplementation levels (B):						
Control (0.0)	74.63^b	75.04^b	90.90^b	91.02^b	37.46^b	75.85^b
6' mg /kg diet	81.63^a	82.20^a	92.00^a	87.07^c	41.09^a	82.36^a
12'' mg/kg diet	81.29^a	81.78^a	92.34^a	92.31^a	38.08^b	81.25^a
±SEM	0.51	0.36	0.19	0.25	0.37	0.68
Significance	**	**	**	**	**	**
Interaction between Mn supplementation forms and levels (A*B):						
Inorganic Mn (0.0) (control)	73.53^d	74.44^d	90.70^c	91.32^b	37.76^c	75.05^c
Inorganic Mn (6 mg /kg diet)	80.88^b	81.58^b	91.76^b	87.76^c	39.67^b	82.27^a
Inorganic Mn (12 mg /kg diet)	78.46^c	79.05^c	90.63^c	93.83^a	40.28^b	79.50^b
Nano Mn (0.0) (control)	75.73^d	75.64^d	91.10^c	90.72^b	37.16^c	76.65^c
Nano Mn (6 mg /kg diet)	82.39^{ab}	82.82^{ab}	92.24^b	86.38^d	42.52^a	82.45^a
Nano Mn (12 mg /kg diet)	84.13^a	84.50^a	94.05^a	90.79^b	35.88^c	82.99^a
±SEM	0.73	0.55	0.26	0.38	0.50	0.85
Significance	**	**	**	**	**	**

^{a-d}Means within the columns with different superscript are significant difference (P<0.05). The means ± standard error are used to express values. ' = 10 % (6 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. '' =20 % (12 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet.. N.S.= not significantly different (P≥ 0.05). . (**)= highly significant (p<0.01).

Table (3): Impact of manganese supplementation forms and levels on serum protein profile and glucose levels of broiler chickens.

Items	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Glucose (mg/dl)
Mn supplementation forms (A):				
Inorganic Mn	6.45 ^b	3.83	2.62	101.43
Nano Mn	6.75 ^a	3.79	2.96	96.65
±SEM	0.07	0.14	0.15	1.9
Significance	**	NS	NS	NS
Mn supplementation levels (B):				
Control (0.0)	6.63	3.73	2.91	99.67
6' mg /kg diet	6.64	3.98	2.65	101.83
12" mg/kg diet	6.53	3.72	2.81	95.62
±SEM	0.04	0.17	0.19	2.33
Significance	NS	NS	NS	NS
Interaction between Mn supplementation forms and levels (A*B):				
Inorganic Mn (0.0) (control)	6.43 ^b	3.76	2.67	99.97
Inorganic Mn (6 mg /kg diet)	6.54 ^{ab}	4.26	2.28	102.45
Inorganic Mn (12 mg /kg diet)	6.38 ^b	3.46	2.92	101.87
Nano Mn (0.0) (control)	6.83 ^a	3.70	3.13	99.37
Nano Mn (6 mg /kg diet)	6.73 ^a	3.70	3.03	101.21
Nano Mn (12 mg /kg diet)	6.69 ^a	3.98	2.70	89.37
±SEM	0.13	0.24	0.27	3.71
Significance	*	NS	NS	NS

^{a-b}Means within the columns with different superscript are significant difference (P<0.05). The means ± standard error are used to express values.. ' = 10 % (6 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. " =20 % (12 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. N.S= not significantly different (P≥ 0.05). (*)= significant (P< 0.05). (**)= highly significant (p≤0.01).

Table (4): Impact of manganese supplementation forms and levels on serum lipid profile of broiler chickens.

Items	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)
Mn supplementation forms (A):					
Inorganic Mn	131.69	205.45	55.65	184.47	26.20
Nano Mn	118.82	197.44	63.27	175.04	23.90
±SEM	5.97	20.86	5.28	13.64	1.19
Significance	NS	NS	NS	NS	NS
Mn supplementation levels (B):					
Control (0.0)	112.54 ^b	225.46	67.18	164.59	22.51 ^b
6' mg/kg diet	106.75 ^b	164.90	52.22	163.91	21.35 ^b
12'' mg/kg diet	156.47 ^a	213.98	58.97	210.78	31.29 ^a
±SEM	7.31	25.55	6.47	16.70	1.46
Significance	**	NS	NS	NS	**
Interaction between Mn supplementations forms and levels (A*B):					
Inorganic Mn (0.0) (control)	124.54 ^{dc}	229.71	62.19	168.88	24.50 ^{dc}
Inorganic Mn (6 mg /kg diet)	125.34 ^{bc}	175.79	51.44	174.74	25.07 ^{bc}
Inorganic Mn (12 mg /kg diet)	145.18 ^{ab}	210.85	53.32	209.80	29.04 ^{ab}
Nano Mn (0.0) (control)	100.54 ^{dc}	221.21	72.17	160.28	20.52 ^{dc}
Nano Mn (6 mg /kg diet)	88.15 ^d	154.01	53.01	153.07	17.63 ^d
Nano Mn (12 mg /kg diet)	167.76 ^a	217.11	64.62	211.76	33.55 ^a
±SEM	9.81	30.05	7.97	21.20	1.96
Significance	**	NS	NS	NS	**

^{a-d}Means within the columns with different superscript are significant difference (P<0.05). The means ± standard error are used to express values. ' = 10 % (6 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. '' = 20 % (12 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. N.S= not significantly different (P≥ 0.05). (**)= highly significant (p≤0.01).

Liver enzymes, glutamic–pyruvic transaminase (GPT) and glutamic – oxaloacetic transaminase (GOT):

The data presented in Table (5) revealed GOT and GPT values in broiler chickens received diet enriched with nano manganese or its inorganic form. These results showed that a substantial ($P < 0.05$) difference was detected in GPT value between forms while, no substantial effects ($P > 0.05$) was detected in the value of GOT. Birds fed diets complemented with nano form achieved lower ($P < 0.05$) significant value of GPT. Also, nano form had lower insignificant GOT value.

Matuszewski et al., (2020) speculated that, administration of nanoMn₂O₃ had no effect on parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The data of Mn supplementation levels indicated that, all supplementation level of Mn and basal diet were substantially different ($p < 0.05$) in the

values of GPT and GOT. Birds received control diet achieved significantly lower ($P < 0.05$) value of GOT. While, birds fed the higher supplementation of Mn (12 mg Mn /kg diet) achieved significantly lower ($P < 0.05$) value of GPT compare with other supplementation.

The present data of interaction between Mn supplementation forms and levels revealed that, there was a substantial decrease ($P \leq 0.01$) in GOT value owing to those fed on the basal diet. However, chickens received control diet accompanied with 12 mg nano Mn/kg diet recorded the lowest value ($P \leq 0.01$) of GPT.

CONCLUSIONS:

From this study, it could be concluded that adding nano manganese to broilers diet enhanced nutrient digestibility and no harm effect on blood criteria.

Table (5) Impact of manganese supplementation forms and levels on liver enzymes of broiler chickens.

Items	GOT (IU/L)	GPT (IU/L)
Mn supplementation forms (A):		
Inorganic Mn	98.64	53.97 ^a
Nano Mn	95.05	46.60 ^b
±SEM	2.13	2.57
Significance	NS	*
Mn supplementation levels (B):		
Control (0.0)	89.48 ^b	67.69 ^a
6' mg /kg diet	94.92 ^b	45.90 ^b
12" mg/kg diet	106.13 ^a	37.22 ^b
±SEM	2.61	3.15
Significance	*	*
Interaction between Mn upplementation forms and levels(A*B):		
Inorganic Mn (0.0) (control)	92.48 ^c	69.79 ^a
Inorganic Mn (6 mg /kg diet)	94.54 ^{bc}	52.34 ^b
(Inorganic Mn (12 mg /kg diet	108.89 ^a	39.69 ^c
Nano Mn (0.0) (control)	86.48 ^c	65.59 ^a
Nano Mn (6 mg /kg diet)	95.31 ^{bc}	39.46 ^c
(Nano Mn (12 mg /kg diet	103.36 ^{ab}	34.76 ^c
±SEM	3.81	3.79
Significance	**	**

^{a-c}Means within the columns with different superscript are significant difference (P<0.05). The means ± standard error are used to express values. ' = 10 % (6 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. " = 20 % (12 mg/kg diet) of Mn above NRC recommendation of Mn in broiler's diet. N.S= not significantly different (P≥ 0.05). (*)=significant (P< 0.05). (**)= highly significant (p≤0.01).

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الملخص العربي

بعض الإستجابات الأيضية والفسولوجية لكثايت اللحم المتأثرة بمصادر ومستويات مختلفة من علائق المنجنيز

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قسم الإنتاج الحيواني والداجنى- كلية الزراعة-جامعة المنيا- مصر

اجريت هذه التجربة بهدف دراسة تأثير بعض الإستجابات الأيضية والفسولوجية لكثايت اللحم بمصادر ومستويات مختلفة من علائق المنجنيز. وفي هذه التجربة تم استخدام 180 ككتوت تسمين غير مجنس عمر يوم من سلالة اربرايكرز بلس وصممت التجربة كتجربة عاملية (2×3) حيث تم استخدام المنجنيز في صورتين هما المنجنيز غير عضوي و المنجنيز في الصورة النانو تحت ثلاث مستويات هي صفر و 6 و 12 ملجم منجنيز لكل كيلو عليقة لكلا الصورتين وتم تقسيم الطيور الى مجموعتين غذيت المجموعة الأولى على عليقة مضاف اليها منجنيز غير عضوي بينما غذيت المجموعة الثانية على عليقة مضاف اليها نانو منجنيز تم تقسيم الكثايت في كل مجموعة الى ثلاث تحت مجموعات غذيت تحت مجموعة الأولى على عليقة كترول تحتوى على 60 مجم منجنيز/كجم علف بينما غذيت تحت مجموعات الثانية والثالثة على علائق مضاف اليها 6 و 12 مجم منجنيز/كجم علف على التوالي. واستمرت التجربة من عمر يوم وحتى عمر خمسة أسابيع. أظهرت النتائج التي تم الحصول عليها أن إضافة المنجنيز في الصورة النانو أدت إلى زيادة معنوية في معاملات هضم المادة الجافة و المادة العضوية و البروتين الخام و المستخلص الخالي من النيتروجين وكذلك زيادة محتوى السيرم من البروتين الكلي ولكن لم يلاحظ أي اختلافات معنوية في محتوى السيرم من الألبومين و الجلوبيولين والجلوكوز. كذلك وجد أن إضافة التركيز العالي من المنجنيز (12 ملجم منجنيز لكل كجم عليقة) أدت إلى زيادة معنوية في محتوى السيرم من الجليسيريدات الثلاثية و البروتين الدهني منخفض الكثافة جدا. وجد أن الطيور التي تغذت على عليقة كترول مضافا اليها 12 ملجم نانو منجنيز لكل كجم عليقة أدت إلى انخفاض معنوي في انزيم الكبد ناقلة أمين الجلوتاميك-بيروفيك (GPT).