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Effects of Different Manganase Sources and Concentration in the Diets on the Performance, Reproductive Characteristics and Some Blood Parameters of Breeder Japanese Quail

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ABSTRACT

An experiment with breeder Japanese quail was conducted to determine the effects of different manganese (Mn) sources and levels in diet on the performance, reproductive characteristics and blood constituents during for five-28 day periods. In the experiment, a total of three hundred and sixty quail (female:male ratio, 2:1) at seven weeks of age was fed on diets containing 0, 60, 120, 180 and 240 mg kg^{-1} Mn levels from inorganic ($MnSO_4.H_2O$) or organic (Mn-amino acid chelate, Glycinoplex-Mn) source. Ten treatments combination of 2 Mn source and 5 Mn levels in 2X5 factorial arrangement were used with six replicate consisting of 4 female and 2 male quail each. The diets in mash form and water were given as adlibitum and 16 hours ligthting was provided in a day. In the experiment, final body weight (BW), body weight gain (BWG), livability, hen day egg production (EP), egg weight (EW) and egg mass (EM), feed consumption (FC) and feed conversion ratio (FCR), chick weights at hatching, fertility, hatchability of eggs set (%), hatchability of fertile eggs (%), embryonic mortalities and glucose, total cholesterol and protein, albümin, BUN, GGT, SGOT, SGPT, calcium, phosphorus and magnesium content of blood serum were measured.

Dietary Mn source and levels as the main factor did not significantly affect the performance and reproductive traits which was measured in the experiment. Nevertheless the interaction between source and levels of Mn had a significant effect on average EW and chick weights at hatching over the experimental period. Egg weight of quail fed diet with 60 mg kg^{-1} Mn from inorganic form was higher ($P<0.01$) than those of quail fed with 0, 180 and 240 mg kg^{-1}. Mn from inorganic and 0, 60, and 240 mg kg^{-1} Mn from organic form, again chick weights at hatching of quail fed diet with 60 mg kg^{-1} inorganic Mn, was significantly higher ($P<0.05$) compared with quail fed with all other levels of inorganic and organic Mn. In the experiment, while dietary Mn levels did not significantly affect any blood parameters, the serum glucose level of the quail fed with organic Mn was significantly higher than that of the quail fed with inorganic Mn and also serum phosphorus level of the quail fed with inorganic Mn was higher than that of the quail fed with organic Mn ($P<0.01$). The interaction groups between source and levels of Mn significantly affected the total protein, albumin and calcium levels of blood serum ($P<0.05$). These parameters were found to be higher in quail fed diets containing 60 mg kg^{-1} Mn in inorganic form than quail with some other diets. These results have shown that addition of 60 mg kg^{-1} Mn in inorganic form to breeder quail diets (containing 21.56 mg kg^{-1} Mn, found in analysis) based on corn + soybean meal may be beneficial.

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1. Introduction

As a group of nutrients for poultry, trace elements must be provided in appropriate quantities and available forms to maintain normal health of the animals, for optimum growth, yield and breeding performance and for quality egg shell production (McDonald, 2011; Suttle, 2010; Richards et al., 2008) and these trace elements play a critical role in embryo development (Wilson, 1997). In breeding diets, trace element deficiencies such as zinc (Zn), manganese (Mn), copper (Cu) that are componenets of eggs may result in a decrease in egg production, shell thickness, fertility and hatchability and an increase in embryo bone abnormalities and deficiencies such as weak feathering and dermatitis (Kienholz et al., 1961; Bird et al., 1963),.

Mn is essentially element in terms of nutrition and potentially toxic and has play role in many biological processes. Mn has an important role in appropriate growth, skeletal development, breeding functions, embryonic development, formation of collagen, which constitutes nearly half of the proteins in animal tissues and is the main protein of skeleton-binding tissues, in the function of the immune system as well as shell formation (Suttle, 2010; Scott et al., 1982). Mn is important for the activation of enzymatic systems attendant in the metabolism of carbohydrates, lipids, proteins and nucleic acids as well as enzymes attendant in oxidative phosphorylation in mitochondria, and has a critical role in glycosaminoglycan synthesis (Anonymous, 2002; Hurley, 1981; Hurley et al., 1984). For example, it functions in the activation of glycosyl transferases enzyme attendant in the production of squalene that is the precursor of cholesterol and in skeletal development (Hansen and Spears, 2008).

For birds, manganese deficiency can naturally occur in diets consisting of normal feed materials. The Mn requirement for poultry is significantly higher than for other domestic animals and Mn deficiency may be seen mainly in these species (McDowell, 2003). In practice, the main reason for this deficiency in effectively usage of Mn is probably antinutritional factors such as phytic acid, cellulose in feeds. The excess of calcium, potassium, iron, magnesium, phosphorus and cobalt in diets also negatively affects the absorption of Mn (Klasing, 1998, Collins and Moran, 1999). Mn deficiency may causedecline in growth in young chicks, perosis and ataxia, a decrease in egg yield at laying hens, a decrease shell quality, fertility rate and output power, an increase in bone abnormalities in the embryo and parrot beak (McDowell et al. , 2003, Anonymous, 2002, Leach and Gross, 1983, Scott et al., 1982).

Depending on the the bird's species, age, type of diet, chemical form of the element added to diet and criteria used to determine adequacy, Mn requirement in the diet may vary. Diets prepared with feed materials commonly used for bird nutrition may contain about 20-30 mg kg^{-1}Mn. Although NRC (1984) reported Mn requirement in diets is 30 mg kg^{-1} for Leghorn laying hens, NRC (1994) reported Mn requirement in diets for white laying hens with feed consumption of 100 g day^{-1} and breeder white hens is respectively 17 and 20 mg kg^{-1}; 60 mg kg^{-1} for young and breeder quail; 70 and 60 mg kg^{-1} for young and breeder pheasants respectively. But there are no recommendations for breeder broilers. In addition, NRC (1994) does not take into account mineral availability. However, the availability of the element may affect the performance of breeder birds and offspring (Favero et al., 2013). The recommended Mn requirement for different breeder hens is different and is 35, 70 and 65 mg kg^{-1} for Babcock, Bovans, Hylinerespectively; the mean is 56.7mg kg^{-1}. A similar situation is present forbreeder broilers and is 100; 100; 100; 120 ve 60 mg kg^{-1} for Avian, Cobb 100, Ross, Hybro and Hubbard respectively and the mean is 96 mg kg^{-1} (Anonymous, 2002). Also, the minimum, optimum and maximum Mn levels were reported as 30, 40 and 1000 mg kg^{-1}, respectively, for laying hens (Larbier and Leclercq, 1994).

In the literature, there are very limited information on the effects of organic and inorganic Mn sources on quail, and the results obtained from experiments done on laying hens are also inconsistent. In a study (Gravena et al., 2011) in which the effect of trace minerals in organic form (Se, Zn and Mn) on the performance and egg quality of Japanese quail were measured, it was reported that the addition of organic Mn (Mn-Bioplex) to the basal diet at a rate of 0, 60, 120 and 180 mg kg^{-1} reduces egg weight; does not significantly affect egg yield, feed consumption, feed conversion ratio (feed / egg mass) and survival; improves egg shell quality. Swiatkiewicz and Koreleski (2008) reported that the addition of organic Mn as amino acid complex instead of 50% and 100% of Zn and Mn (30 mg kg^{-1} Zn and 50 mg kg^{-1} Mn) in inorganic form in the diets of 25-70 week old laying hens did not affect performance. In another study (Yıldız et al., 2011) in which 49-week-old laying hens fed diets containing 15, 30, 45, 60 and 75 mg kg^{-1} Mn in organic (Mn-Bioplex) and inorganic ($MnSO_4$) forms for 12 weeks, it was determined that Mn sources did not affect egg yield, egg mass, feed consumption and utilization of feed (feed / egg mass) significantly; whereas, increase of egg weight and live weight were significantly higher with diets containing organic Mn than diets containing inorganic Mn ($P < 0.01$). Xiao et al. (2015) reported that diet Mn source and level did not significantly affect egg yield, feed consumption and feed evaluation coefficient of 50 week old laying hens fed with diets containing 0, 25, 50, 100 and 200 mg kg^{-1} Mn in organic or inorganic form. In other studies (Lim and Paik, 2003; Mabe et al., 2003) it was reported that there is no difference in performance characteristics between organic and inorganic Mn sources. However, Klecker et al. (2002) reported that addition of Mn and Zn in chelate form instead of 20% or 40% of inorganic Mn and Zn in diets

increased egg yield (P<0.05), egg weight (P<0.01) and shell quality.

In a study (Favero et al., 2013) in which breeder broilers (22-68 weeks old) were fed with diets containing the mixture of inorganic and organic trace elements (Zn-Mn-Cu) at different levels, treatments did not significantlyaffect egg yield and weight, fertility, hatching ratio from incubated eggs (%), hatching weight, salable chick and physical defective chick ratio (%). In a similar study (Gheisari et al., 2011), it was reported that for commercial laying hens, trace element additions that is 50% to 75% lower than levels of Zn-Mn-Cu adviced by NRC to the diets based on corn-soybean meal in organic form is enough to maintain egg-laying performance.

The aim of this study is to assess the effects of the manganese addition in organic (manganese-glycine amino acid complex) and inorganic (manganese sulfate) forms to the diets of breeder quail at different levels on performance, reproductive characteristics and some blood parameters.

2. Materials and Methods

This research was carried out at the quail house with the window at Selçuk University Faculty of Agriculture Department of Animal Science Prof. Dr. Orhan DÜZGÜNEŞ Livestock Research and Application Farm. In the study, 360 (female / male ratio, 2: 1) Japanese quail (Coturnix coturnix Japonica) at 7 weeks of age were used.

The quail were raised rearing cages with double-sided and five-tier stacked battery system that were locally manufactured, and had 12 division with 40x50x20 cm size on each tiers (totally 60 divisions).

Each cage compartment was taken as a replicate. A total of 6 quail were placed, each of which had 4 female and 2 male quail. The distribution of quail and experimental diets to the cage compartment was done by randomly. The experiment was carried out in the form of 5 periods of 28 days and lasted a total of 140 days. During the research, feed and water were given as ad-libitum and 16 hours lighting was done.

Raw material and calculated nutrient composition of the experimental diets are given in Table 1. Feed materials not available in the farm and trace minerals without manganese and vitamin mix were obtained from market. Experimental diets were prepared at the unit offeed preparation in the Research and Application Farm.

In the experiment, manganese sulfate monohydrate ($MnSO_4$, H_2O; Tekkim Chemical Industry and Trade Limited Company) containing 31% Mn as inorganic manganese source and Mn-amino acid chelate containing 22% manganese as organic manganese source (Glycinoplex-Mn, ANC Animal Nutrition and Health Services Inc.) were used. In the study, basal diet unsupplemented with Mn was prepared and inorganic and organic Mn sources were added to this diet at 0, 60, 120, 180 and 240 mg kg^{-1} levels. Thus, a total of 10 diets (treatments) were prepared. The experiment of 2 different Mn source X 5 Mn levels in 2X5 factorial arrangement plan were carried out with six replicate. All experimental diets except Mn were prepared with nutrients at recommended levels by NRC (1994) or some more for breeder quail.

Table1

Feedstuffs and calculated nutrient composition of basal diets used in the experiment, (as fed)

Feedstuffs	%
Corn	41.3
Barley	7.8
Soybean meal (%46)	29.5
Sunflower seed meal (%28)	7.1
Vegetable oil	5.7
Limestone	6.0
Dicalcium phosphate	1.55
Salt	0.40
Vitamin premix[1]	0.25
Mineral Premix[2]	0.10
DL-Methionine (98%)	0.15
L-Lysine HCL (78%)	0.15
Total	100
Calculated nutrient composition	
Metabolisableenergy, kcal/kg	2911
Crude protein %	20.16
Ca %	2.85
Total P %	0.69
Available P %	0.402
Methionine %	0.485
Cystine %	0.319
Methinonine+ cystine	0.804
Cu mg/kg *	9.28
Mn mg/kg *	21.56
Zn mg/kg *	89.81

*Analyzed value

[1] Vitamin premix provided per kilogram of the diet: vitamin A, 8.800 IU; vitamin D_3, 2.200 IU; vitamin E, 11 mg; nicotinic acid, 44 mg; Calcium –D- Pantothenate, 8.8 mg; riboflavin 4.4 mg; thiamine 2.5 mg; vitamin B_{12}, 6.6 mg; folic acid, 1 mg; D-Biotin, 0.11 mg; choline, 220 mg.

[2] Mineral premix without Manganese provided per kilogram of the diet: Iron, 60 mg; Zinc, 60mg;Copper, 5.0 mg; Cobalt, 0.20 mg; Iodine, 1 mg; Selenium, 0.15 mg.

2.1 Determination of Performance Characteristics

The live weights (LW) of the quail were determined by weighting as group at the beginning and end of the experiment and the live weight gain (LWG, g/ quail) was calculated from these data.

The egg yield of quail in each subgroup was recorded on a daily basis and theegg yield% was calculated in aperiod of 28 days. Egg yield (%) = ((Total number of eggs in the period / number of female animals in the group) / 28)) x 100

Eggs were collected on the 25th and 26th days of each period during the experiment and egg weights were determined by randomly weighing 5 eggs from these eggs.

For each period, egg mass was found by multiplying the average egg weight with egg yield (%) and then dividing by 100.

Feed consumption (FC) of quail was determined as a group and the feed conversion ratio (FCR) was calculated as g feed/ g egg mass.

The livability was determined by recording the deaths in all subgroups during the experiment and the necessary corrections were made for the dead animals while the performance values were calculated.

2.2. Determination of Incubation Characteristics

Incubation characteristics were determined in all uncracked, normal-sized eggs collected during the first 6. 7. 8. days of all other periods except the first period of study. The collected eggs were placed in a commercial incubator (Cimuka Kuluçka, Ankara) and standard incubation conditions were provided.

The incubation period lasted after 432 hours (18 days) and the eggs in the hatchery were checked and hatching weights were determined by weighing hatching chicks.

Following detection of fertility in non-hatching eggs, embryo deaths and stage were determined by the method as specified by Aygün and Sert (2012). The fertile egg ratio was calculated by multiplying the ratio of the fertilized egg to the incubated eggs by one hundred (fertility,% = number of fertile eggs / number of total eggs set) X100

Hatchability of fertile eggs was calculated by dividing the number of live chicks hatched to the number of fertile eggs or hatchability of incubated eggs was calculated by dividing the number of live chicks hatched from the incubated eggs to the the number of eggs set in the incubator and then by multiplying the ratio by 100. Hatchability of fertile eggs, %=(number of chicks hatched/number of fertile eggs)X100; Hatchability of eggs set,%=(number of chicks hatched/number of eggs set)X100

2.3. Determination of Blood Parameters

At the end of the study, 3 cc blood samples were taken from 2 females from each subgroup, a total of 120 quail, from jugular vein to the anticoagulant-free air taken tubes during slaughter. The feeding ended, 3 hours before the blood samples were taken. Blood samples were clotted by waiting it for 4 hours at 20-22 ^{0}C. The serums were then separated by centrifugation for 10 minutes (3000 rpm). Serums were kept at -20 ^{0}C until analysis. Serum glucose, cholesterol, total protein (TP), albumin, blood urea nitrogen (BUN), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), Ca, P and Mg values were determined by using auto-analyzer (BT 3000 Plus, Biotechnica Instruments, S.p.A., Italy).

2.4. Statistical Method

The data obtained from study were analyzed by using the General Linear Model (GLM) procedures of the MINITAB statistical package program with analysis of variance (ANOVA) according to the 2x5 factorial experiment in a randomized design and in cases where F values were significant, the comparison of the averages was made by using the Duncan test (Düzgüneş, 1975).

The mathematical model of the study is given below and Mn sources in the diet (inorganic and organic Mn) and Mn concentration in the diet (0, 60, 120, 180 and 240 mg kg^{-1}) are the main effects and their combinations are taken as interaction effects.

$Yijk = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$

μ = overall average

α_i= i. Effect of Mn source

β_j = j. Effect of Mn concentration

$(\alpha\beta)_{ij}$ = Effect of the interaction

e_{ijk} = Error

3. Results and Discussion

In this study, the effects of Mn-containing diets with inorganic and organic forms at various levels on livability and productive characteristics, reproductive traits and some blood parameters of the breeder quail were determined and results are given below.

3.1. Livability and Productive Characteristics

Effects of the treatments on live weight of quail at the beginning and end of experimental period and live weight gain, livability, egg production, egg weight, egg mass, feed consumption and feed conversion during all experimental period are shown at Table 2.

The fact that initial live weight of the experiment was similar in all treatment groups (P>0.05) indicates that the experiment animals were distributed homogeneously to subgroups. The initial live weight of the treatment groups ranged from 198.9 g in the group fed with diet containing 120 mg kg^{-1} inorganic Mn and 209.8 g in the group fed with diet containing the 60 mg kg^{-1} inorganic Mn. The treatments did not have a significant effect on the live weight at the end of experiment and the live weight gain and livability of the quail during all experimental period. The highest and lowest mean live weights at the end of experiment were observed in the groups fed with diet containing 60 mg kg^{-1} inorganic Mn (259.7g quail^{-1}) and diet containing 0 mg kg^{-1} organic Mn (240.8 g quail^{-1}) respectively. The highest and lowest live weight gain in the treatment groups were in groups fed with diet containing 180 mg kg^{-1} of organic Mn (53.9 g) and diet containing 240 mg kg^{-1} of inorganic Mn (42.5 g), respectively.

Table 2.
The effects of dietary manganese source and levels on performance of breeder quail (means±standart error)

Treatments		Initialy Body Weight (g)	Final Body Weight (g)	Livability (%)	Egg Production (%)	Egg Weight (g)	Egg Mass (g/quail/day)	Feed Intake (g/day/quail)	Feed Conversion (g/g)
Sources									
Inorganic Mn		204.80±1.52	251.90±1.54	97.87±1.24	86.54±0.87	12.62±0.06	9.45±0.12	27.87±0.23	2.98±0.04
Organic Mn		202.90±1.65	252.30±2.34	94.44±2.01	86.41±0.80	12.51±0.07	9.35±0.11	28.31±0.25	3.06±0.04
P		0.353	0.899	0.356	0.913	0.194	0.535	0.216	0.129
Levels (mg/kg)									
0		199.20±3.00	245.40±2.74	97.22±2.78	88.34±0.74	12.36±0.10^{b}	9.51±0.15	28.16±0.37	2.98±0.05
60		205.30±2.12	256.70±2.63	97.22±1.87	87.00±1.10	12.70±0.10^{ab}	9.61±0.20	28.31±0.34	2.97±0.05
120		204.30±3.19	253.10±3.91	97.44±2.37	87.23±0.68	12.79±0.13^{a}	9.62±0.13	28.28±0.44	2.98±0.06
180		203.50±2.14	253.70±3.05	97.22±1.87	86.07±1.55	12.52±0.10^{ab}	9.25±0.20	27.58±0.43	3.01±0.06
240		206.90±1.58	251.70±2.50	91.67±3.84	83.75±1.85	12.46±0.09^{ab}	9.03±0.20	28.14±0.34	3.17±0.07
P		0.201	0.112	0.489	0.152	0.016	0.105	0.692	0.139
Source x Level Interaction									
Inorganic Mn	0	203.60±4.53	250.00±3.84	100.00±0.00	89.18±1.00	12.26±0.12^{B}	9.50±0.22	27.66±0.49	2.93±0.06
	60	209.80±2.75	259.70±3.27	97.22±2.78	86.35±1.95	13.12±0.16^{A}	9.92±0.26	28.42±0.22	2.90±0.07
	120	198.90±3.15	249.40±2.68	97.67±3.73	86.77±0.93	12.87±0.16^{AB}	9.55±0.20	28.00±0.64	2.96±0.10
	180	204.40±2.92	251.00±3.57	100.00±0.00	87.43±1.30	12.42±0.09^{B}	9.38±0.26	27.34±0.61	2.95±0.06
	240	207.30±2.70	249.70±2.81	94.44±3.51	82.99±3.21	12.44±0.12^{B}	8.93±0.35	27.95±0.54	3.17±0.13
Organic Mn	0	201.80±3.35	240.80±3.13	94.44±5.56	87.50±1.06	12.45±0.15^{B}	9.52±0.23	28.66±0.51	3.04±0.07
	60	200.90±2.08	253.70±4.02	97.22±2.78	87.65±1.16	12.28±0.08^{B}	9.31±0.26	28.20±0.67	3.04±0.06
	120	209.60±4.82	256.80±7.38	97.22±2.78	87.69±1.05	12.71±0.19^{AB}	9.68±0.20	28.55±0.64	3.00±0.08
	180	202.50±3.35	256.40±5.04	94.44±3.51	84.71±2.85	12.61±0.17^{AB}	9.13±0.32	27.83±0.65	3.08±0.11
	240	206.50±1.91	253.70±4.24	88.89±7.03	84.51±2.11	12.48±0.14^{B}	9.13±0.20	28.33±0.46	3.16±0.08
P		0.208	0.200	0.489	0.697	0.001	0.514	0.874	0.872

$^{A-B}$Means within the same column with different superscripts are significantly different at (*P<0.01*).
$^{a-b}$Means within the same column with different superscripts are significantly different at(*P<0.05*).

The livability of breeder quail varied between 100 % and 88.9 % for different treatments and lowest values were observed in groups fed with diet containing 240 mg kg^{-1} Mn in inorganic or organic form (94.4% and 88.9%, respectively).

Treatments did not significantly affect other performance characteristics measured in this study, except egg weight. However, egg weight increased with diets of containing Mn and the egg weight of quail, fed with diet containing 120 mg kg^{-1} Mn, was significantly higher than the control group (P<0.05). However, the effect of interaction on this character is also important. The addition of 60 mg kg^{-1} Mn in inorganic form to basal diet increased egg weight significantly compared to groups containing control (0), 180, 240 mg kg^{-1} inorganic and 0, 60 and 240 mg kg^{-1} organic Mn (P<0.01). However, the addition of Mn in organic form at different levels to basal diet did not significantly affect egg weight. In accordance with the present study results, in a study (Gravena et al., 2011) in which the effects of organic forms of Zn, Mn and Se (Zn and Mn-Bioplex, Sel-Plex, respectively) on the performance and egg quality of quail were evaluated, it was reported that treatments did not significantly affect egg yield, FC, FCR and livability. However, these researchers reported that, unlike the current study, egg weight was reduced by addition of 60 and 120 mg kg^{-1} Mn levels to diet but egg weight was increased by 180 mg kg^{-1} Mn level, but it was not significantly different from the control group. In this study, the quadratic effect of diet Mn level on egg weight was found significant (P<0.05). Yıldız et al. (2011) reported that egg weight and LWG at chickens fed with organic Mn were significantly (P<0.01) higher than chicken fed with inorganic Mn, that diet Mn levels did not affect other performance characteristics except feed consumption and that, as a 75 mg kg^{-1} Mn level, feed intake was significantly (P <0.05) higher than all other groups. These researchers also reported that diet Mn source x level interactions did not affect any performance characteristics significantly. Favero et al. (2013) reported that mixtures containing inorganic and organic forms of Zn-Mn-Cu at different levels for breeder broiler didnot significantly affect egg yield, egg weight, livability and live weight at the end of experiment. Other studies also reported that there is no significant difference in performance characteristics between organic and inorganic Mn sources (Xiao et al., 2015, Swiatkiewicz and Koreleski, 2008, Lim and Paik, 2003, Mabe et al., 2003). However, Klecker et al. (2002) reported that mixtures of organic and inorganic forms of Mn and Zn significantly (P<0.05 and P<0.01, respectively) increased egg yield and egg weight.

It was also shown in other studies that increased Mn levels in diet did not significantly affect performance in laying hens. For example, Sazzad et al.

(1994) reported that the addition of 0, 20, 40, 80 mg kg^{-1} Mn in the form of manganese oxide (MnO) to basal diet containing 25 mg kg^{-1} Mn based on corn + soybean pulp in two different poultry did not significantly affect egg yield, egg weight, feed consumption and FCR. However, Hossain and Bertechini (1998) reported that, in 42-52 week old chickens, the addition of Mn at 25, 50, 75 mg kg^{-1} levels in the form of Mn-sulphate pentahydrate to basal diet containing 12 mg kg^{-1} Mn did not affect FC as independent of diet phosphorus level and FCR but diets containing 50, 75 mg kg^{-1} Mn increased egg yield and egg weight significantly ($P<0.05$).

The different effects of different sources of Mn on same performance may be attributed to their effects on the chemical conditions in the digestive tract and that their stability (Pang and Applegate, 2007) and biological availability are different in low pH at the anterior parts of the digestive tract (Favero et al., 2013; Guo et al., 2001, Cao, 1998). In addition, the fact that organic minerals form less complex with other nutrients or compounds in diet such as phytate, phosphate, and fiber in the intestinal tract may help organic minerals to be absorbed more from the digestive tract (Nollet et al., 2007, Renema, 2004).

In the present study, the fact that the addition of Mn in inorganic and organic form at 0, 60, 120, 180, 240 mg kg^{-1} levels to basal diet based on corn + soybean pulp containing 21, 56 mg kg^{-1} Mn (found in analysis) did not affect the performance of breeder quail except egg weight shows that basal diet Mn level is sufficient to meet the minimum Mn requirement. This amount is about 36% lower than the 60 mg kg^{-1} Mn level recommended for breeder quail and pheasants by NRC (1994). Similarly, in another study carried out with commercial chickens (Gheisari et al., 2011), Zn, Mn, Cu, additions in organic form at levels of 50% to 75% lower than levels recommended by NRC (1994) to laying hence diets based on corn + soybean meal containing Zn, Mn and Cu levels of, respectively, 30.2, 19.2 and 4.2 mg kg^{-1} has been reported to be sufficient to maintain the laying performance.

As stated earlier, Mn requirement may change according to the species of the wing, its age, the type of diet, the chemical form and the level of the element involved in the diet and the criteria used to determine the requirement.

3.2. Reproductive Performance

Chick weights at hatching, fertile egg ratio, total hatchability of fertile eggs and incubated eggs set, early-mid-late embryo and outer pips mortalities are given in Table 3.

As a main factor, dietary Mn sources did not significantly affect any reproductive characteristics measured in the study. Though the second major factor, dietary Mn level, did not affect fertile egg ratio, hatchability of total incubated eggs and hatchability of fertile eggs significantly, it affected chick weights at hatching and hatchability of fertile eggs significantly ($P<0.05$). Chick weights at hatching of quail fed with diets containing an additional 60 mg kg^{-1} Mn was found to be significantly ($P<0.05$) higher than the ones of quailfed with diet containing 0,180 and 240 mg kg^{-1} Mn. However, the effect of the interactions on the hatching weight is also important.

The addition of Mn up to 240 mg kg^{-1} to basal diet increased hatchability of fertile eggs ($P>0.05$). However, hatchability (%87.3) of fertile eggs of quail fed with diet containing 240 mg kg^{-1} Mn was significantly ($P <0.05$) lower than the ones (94.5%) of quail fed with diet containing 180 mg kg^{-1} Mn. This may be related to the toxicity of relatively high Mn levels in the diet.

The diet Mn source and level interaction did not significantly affect any reproduction parameters measured in this study, except hatching weight. The average post-incubation hatching weight (9.45 g $quail^{-1}$) of quailfed with diet containing 60 mg kg^{-1} of additional Mn in inorganic form was found to be significantly ($P<0.05$) higher than all other levels of inorganic and organic Mn.

Superoxide dismutase containing manganese (MnSOD) is the major oxidant enzyme in mitochondria and catalyzes the conversion of free radicals to hydrogen peroxides. The formed hydrogen peroxides are then converted to water by other antioxidant enzymes. Thus, MnSOD protects embryos against harmful effects of free radicals having formed as a result of cell respidiet during embryo development (Leach and Harris, 1997; Johnson et al., 1992). In the present study, The positive effect of additional Mn on the hatching weight may possibly be related to the antioxidant characteristics of Mn. In addition, proteoglycans are needed for healthy cartilage and bone formation. In the synthesis of proteoglycans, enzymes called glycosyltransferases activated by manganese have functions (McDowell, 2003; Leach and Harris, 1997). This may be another possible cause of the positive effect of Mn on hatching weight.

The effect of the treatments on early, middle, late embryo stage and outer pips mortalities also did not show a consistent course. While the highest early embryo mortalities were observed in quail (4.35%) fed with diet containing 240 mg kg^{-1} of additional Mn in inorganic form, the highest outer pips mortalities were observed in quail (5.62%) fed with diet containing 240 mg kg^{-1} of additional Mn in organic form ($P>0.05$).

The treatments did not have a significant effect on embryo deaths. There are not enough studies on the effect of different trace element sources and levels in diets on the reproductive performance of breeder poultry and different results have been obtained from the existing studies in terms of various reproduction parameters. In a study in which the effects of diets containing mixture of inorganic and organic forms of Zn-Cu-

Mn for breeder broilers on reproductive performance were researched, Favero et al. (2013) reported that treatments did not significantly affect fertility, total hatchability of eggs, middle, late embryo and outer pips mortalities. These results are consistent with the Table 3.

present study results. These researchers reported that, unlike the present study, treatments increased hatchability of fertile eggs significantly (P<0.05) and reduced early embryo mortalities (P<0.05).

The effects of dietary manganese source and levels on reproductive performance of breeder quail (mean±standart error)

Treatments		Chick weights at hatching (g)	Fertility (%)	Hatchability of fertile eggs (%)	Hatchability of eggs set (%)	Embryo mortality (%)			
						Early dead	Middle dead	Late dead	Outer-pips
Sources									
Inorganic Mn		9.01±0.049	98.02±0.738	89.57±1.128	88.54±1.076	2.45±0.406	1.10±0.398	1.53±0.322	2.67±0.460
Organic Mn		8.91±0.048	98.18±0.676	90.56±1.197	88.87±1.426	1.96±0.386	1.12±0.294	1.19±0.579	2.56±0.570
P		0.144	0.874	0.527	0.849	0.375	0.979	0.608	0.877
Levels (mg/kg)									
0		8.86±0.080^{b}	97.03±1.610	87.80±2.746^{ab}	86.94±2.660	2.30±0.699	0.70±0.391	2.49±1.380	2.71±0.657
60		9.17±0.074^{a}	98.77±0.480	90.94±1.953^{ab}	89.84±1.983	2.26±0.788	1.43±0.559	1.05±0.478	2.09±0.850
120		8.99±0.081^{ab}	98.35±0.594	89.78±1.287^{ab}	88.31±1.669	2.16±0.471	2.34±0.879	0.55±0.379	2.63±0.826
180		8.91±0.068^{b}	98.07±1.368	94.51±0.806^{a}	92.75±1.654	1.60±0.377	0.56±0.290	0.53±0.271	1.96±0.610
240		8.86±0.074^{b}	98.28±1.185	87.29±1.118^{b}	85.69±1.334	2.72±0.751	0.53±0.271	2.19±0.496	3.70±1.077
P		0.017	0.859	0.038	0.089	0.786	0.095	0.208	0.555
Source x Level Interaction									
Inorganic Mn	0	8.87±0.105^{b}	98.96±0.712	87.80±4.524	90.64±1.390	1.46±0.710	0.35±0.346	2.19±0.799	2.57±0.714
	60	9.45±0.101^{a}	99.27±0.460	88.98±2.181	88.31±2.327	2.78±1.161	2.15±0.987	1.05±0.710	3.13±1.497
	120	8.95±0.133^{b}	97.40±0.997	87.82±2.259	85.54±2.891	1.89±0.733	2.58±1.491	1.08±0.719	3.73±1.379
	180	8.91±0.079^{b}	97.22±2.778	93.97±0.744	91.50±2.949	1.77±0.354	0.43±0.415	0.70±0.437	2.16±0.762
	240	8.87±0.086^{b}	97.25±2.359	89.26±0.919	86.73±2.018	4.35±1.086	0.01±0.000	2.65±0.769	1.78±0.648
Organic Mn	0	8.85±0.123^{b}	95.11±3.069	87.81±3.566	83.24±4.872	3.13±1.171	1.05±0.710	2.79±2.776	2.84±1.176
	60	8.89±0.082^{b}	98.26±0.836	92.91±3.238	91.37±3.309	1.74±1.129	0.70±0.437	1.05±0.710	1.05±0.710
	120	9.04±0.094^{b}	99.31±0.439	91.73±0.808	91.07±0.919	2.43±0.639	2.09±1.074	0.01±0.000	1.53±0.781
	180	8.92±0.112^{b}	98.93±0.481	95.04±1.480	94.00±1.648	1.43±0.699	0.70±0.437	0.36±0.345	1.77±1.021
	240	8.86±0.121^{b}	99.31±0.439	85.31±1.760	84.65±1.822	1.08±0.479	1.05±0.464	1.74±0.639	5.62±1.791
P		0.016	0.287	0.486	0.131	0.071	0.491	0.933	0.060

$^{A-B}$Means within the same column with different superscripts are significantly different at (*P<0.01*).
$^{a-b}$Means within the same column with different superscripts are significantly different at (*P<0.05*).

3.3. Blood Parameters

Serum glucose, cholesterol, TP, albumin, BUN, GGT, SGOT, SGPT and Ca, P and Mg values are given in Table 4 and Table 5 respectively.

The dietary Mn level as the main factor did not significantly affect any blood parameters measured in this study. However, the dietary Mn sources affected the content of serum glucose (P<0.01),TP (P<0.05), P (P<0.01) significantly. While blood glucose levels of quail fed with diets containing organic Mn were found to be significantly (P<0.01) higher than those fed with diets containing inorganic Mn, TP and P contents were found to be significantly (P<0.05 and P<0.01, respectively) lower. However, the effect of interactions on serum TP content was also significant (P<0.01). In addition, while serum total cholesterol, albumin, GGT, Ca and Mg contents decreased with organic Mn source, BUN, SGOT and SGPT levels increased insignificantly (P>0.05, Table 4 and Table 5). In accordance with the present study, Makarski and Gortat (2011) reported that blood glucose levels in turkeys, at 0-19 weeks of age, fed with organic copper (Cu-lysine, 20 mg dm^{-3}) in drinking water were significantly (P <0.5) higher than control group to which additional copper was not given and than the group to which 10 mg dm^{-3} copper in organic form was given and than the groups to which copper in inorganic form ($CuSO^{4}$, 10 and 20 mg dm^{-3}) was given.

Similar results were obtained from another study (Makarski and Polois, 2001) and plasma Cu, glucose and hematocrit values of turkeys, at 8 weeks of age, to which organic copper (Bioplex-Cu, 0.5 gl^{-1}) was given in drinking water were significantly (P<0.05) higher than those of control group to which additional copper was not given. However, Seyfori et al. (2018) reported that the level of serum glucose was not affected in ostrichs to which 100 mg, 1 g and 2 g / day / animal chelate trace mineral (Fe, Zn, Mn, Cu) was given in drinking water. In another study with layer chicks at 0-8 weeks period (Das et al., 2014),in basal diet containing Zn, Cu, Mn (80, 15 and 80 mg kg^{-1}, respectively) in inorganic form, instead of entire or half of each one of Zn, Cu ve Mn or third of them (100% or 50%), their organic forms (amino acid chelate) were substituted. In contrast to the present study, researchers reported that

substitution of organic Zn instead of Zn in basal diet reduced blood glucose and cholesterol levels significantly (P<0.05), but no treatment significantly affected serum TP, albumin, urea, SGPT, SGOT, Ca and P levels.

Table 4.

The effects of dietary manganese source and levels on the some serum parameters of breeder quail (mean±standart error)

Treatments		Glucose (mg/dl)	Total cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	GGT (U/I)	SGOT (U/I)	SGPT (U/I)
Sources									
Inorganic Mn		189.60±8.27^{B}	180.60±7.78	4.59±0.10^{a}	1.72±0.04	3.93±0.26	5.07±0.25	256.30±10.77	11.60±0.76
Organic Mn		240.70±9.25^{A}	171.90±8.03	4.17±0.14^{b}	1.60±0.05	4.03±0.33	4.50±0.13	260.70±13.36	13.17±1.25
P		0.000	0.429	0.010	0.125	0.627	0.060	0.725	0.191
Levels (mg/kg)									
0		199.30±16.89	174.10±10.18	4.50±0.12	1.69±0.07	4.59±0.59	4.67±0.23	295.60±19.69	10.75±1.30
60		202.10±13.36	189.80±12.06	4.28±0.27	1.63±0.11	3.58±0.45	5.00±0.33	240.30±13.73	10.00±0.90
120		231.20±16.25	172.60±12.73	4.42±0.16	1.67±0.06	3.95±0.43	4.73±0.20	249.80±12.09	12.92±0.87
180		203.70±15.13	174.50±15.42	4.42±0.29	1.69±0.10	3.95±0.47	5.08±0.56	271.10±28.49	14.33±2.31
240		239.50±14.90	170.10±12.71	4.28±0.15	1.61±0.05	3.86±0.40	4.42±0.15	235.70±13.63	13.92±2.09
P		0.129	0.801	0.874	0.828	0.545	0.641	0.362	0.115
Source x Level Interaction									
Inorganic Mn	0	184.50±27.96	180.80±12.43	4.48±0.15^{AB}	1.70±0.10^{abc}	4.39±0.78	5.17±0.31	276.20±28.11	12.00±2.31
	60	174.50±10.99	196.50±19.90	4.92±0.18^{A}	1.88±0.10^{ab}	3.50±0.55	5.17±0.48	241.80±11.95	10.17±0.98
	120	191.70±19.02	150.70±13.36	4.40±0.23^{AB}	1.58±0.10^{bc}	3.18±0.48	4.60±0.25	242.00±14.10	12.83±1.58
	180	177.00±15.65	204.30±16.44	5.08±0.17^{A}	1.88±0.07^{a}	4.48±0.56	6.00±1.00	297.30±35.43	12.83±1.94
	240	220.20±14.26	170.50±19.77	4.05±0.14^{BC}	1.55±0.04^{bc}	4.13±0.55	4.33±0.21	224.00±16.44	10.17±1.64
Organic Mn	0	214.20±19.64	167.30±16.82	4.52±0.19^{AB}	1.68±0.12^{abc}	4.75±0.92	4.17±0.17	315.00±27.65	9.50±1.20
	60	229.70±19.00	183.20±15.02	3.65±0.36^{C}	1.38±0.14^{c}	3.66±0.78	4.83±0.48	238.80±26.19	9.83±1.60
	120	270.70±13.27	194.50±18.50	4.43±0.24^{AB}	1.75±0.07^{ab}	4.59±0.60	4.83±0.31	257.50±20.50	13.00±0.93
	180	230.30±21.87	144.70±20.48	3.75±0.40^{BC}	1.50±0.14^{bc}	3.50±0.71	4.17±0.17	244.80±45.17	15.83±4.34
	240	258.80±24.99	169.70±17.89	4.52±0.23^{AB}	1.67±0.08^{abc}	3.55±0.64	4.50±0.22	247.30±22.21	17.67±3.30
P		0.755	0.073	0.001	0.013	0.344	0.242	0.190	0.146

$^{A-B-C}$Means within the same column with different superscripts are significantly different at (*P<0.01*).

$^{a-b-c}$Means within the same column with different superscripts are significantly different at (*P<0.05*).

Blood glucose is regulated by nutrition, breakdown of glycogen and gluconeogenesis and is an essential energy source for almost every cell in the body. Many enzymes activated by manganese (hydrolases and kinases) play an important role in cabohydrate, amino acid and cholesterol metabolism. Pyruvate carboxylase that is an enzyme containing Mn and phosphoenolpyruvate carboxykinase (PEPCK) that is an enzyme by activated Mn are critical enzymes in gluconeogenesis (McDowell, 2003, Higdon, 2001). That increase the activity of these enzymes by organic Mn may be possible reason for organic Mn to increase blood glucose levels. That organic

Mn stimulates the glucose synthesis (gluconeogenesis) from glucogenic amino acids in the liver may also be a possible reason for the decrease in blood serum total protein and alanine levels.

Interactions having formed from combinations of different levels of inorganic and organic Mn did not significantly affect other parameters except serum TP, albumin and Ca levels. Although the serum TP content (4.92 and 5.08 g dL^{-1}, respectively) of quail fed with diet containing 60 and 180 mg kg^{-1} Mn in inorganic form was not significantly different from those of quail fed with diet containing Mn at 0 mg kg^{-1} level of both sources (control groups), it is significantly (P<0.01) higher than the quail fed with diet containing 240 mg kg^{-1} (4.05 g dL^{-1}) in inorganic form and 60 mg kg^{-1} (3.65 g dL^{-1}) and 180 mg kg^{-1} (3.75 g dL^{-1}) in organic form.

The serum TP content (3.65 g dL^{-1}) of quail fed with diet containing 60 mg kg^{-1} Mn in organic form was significantly (P <0.01) lower than the control and all other groups except groups fed with diet containing 240 mg kg^{-1}in inorganic form and 180 mg kg^{-1} in organic form.

Although the serum albumin level of quail fed with diet containing 180 mg kg^{-1} Mn in inorganic form was not different from those of quail fed with diet containing Mn at 0 mg kg^{-1} level of both sources, it is significantly (P<0.01) higher than those of quail fed with diet containing 120 and 240 mg kg^{-1} in inorganic form and 60 and 180 mg kg^{-1} in organic form. Although the albumin level of quail fed with diet containing 60 mg kg^{-1} Mn in organic form was lower (P>0.05) than those of quail fed with diet containing Mn at 0 mg kg^{-1} level of both source, it was significantly (P<0.05) lower

groups fed with diet containing 60 and 180 mg kg^{-1} Mn in inorganic form and 120 mg kg^{-1} Mn in organic form.

Table 5.
The effects of dietary manganese source and levels on the some serum minerals of breeder quail (mean±standart error)

Treatmens		Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)
Sources				
Inorganic Mn		25.58±0.613	12.76±0.393^{A}	7.21±0.170
Organic Mn		24.67±1.015	9.50±0.399^{B}	6.68±0.226
P		0.449	0.000	0.075
Levels (mg/kg)				
	0	27.05±1.373	12.21±0.530	7.59±0.181
	60	23.39±1.690	11.09±0.902	6.90±0.439
	120	24.66±0.797	10.54±0.597	6.84±0.252
	180	25.30±1.583	11.31±1.129	6.87±0.415
	240	25.03±1.017	10.50±0.606	6.54±0.210
P		0.571	0.165	0.149
Source xLevel Interaction				
	0	25.62±1.905^{ab}	12.88±0.539	7.46±0.325
	60	26.50±0.888^{ab}	13.38±0.874	7.64±0.255
Inorganic Mn	120	23.68±0.688^{ab}	11.78±0.718	6.61±0.344
	180	28.23±1.459^{a}	14.15±1.338	7.58±0.504
	240	24.02±0.948^{ab}	11.58±0.426	6.77±0.332
	0	28.48±1.960^{a}	11.53±0.874	7.71±0.179
	60	20.80±2.801^{b}	8.80±0.845	6.16±0.750
Organic Mn	120	25.63±1.395^{ab}	9.30±0.660	7.08±0.373
	180	22.37±2.336^{ab}	8.47±0.765	6.17±0.552
	240	26.05±1.800^{ab}	9.42±0.982	6.30±0.247
P		0.025	0.132	0.204

$^{A-B}$Means within the same column with different superscripts are significantly different at (*P<0.01*).
$^{a-b}$Means within the same column with different superscripts are significantly different at (*P<0.05*).

The serum Ca level of quail fed with diet containing 60 mg kg^{-1} Mn in organic form was found to be significantly (P<0.05) lower than quail fed with diet containing 0 mg kg^{-1} Mn in organic form and 180 mg kg^{-1} Mn in inorganic form. In chickens, approximately 25% of Ca in blood is present in the form of free-ionized Ca2+ ion, the remaining part is present depending on the proteins such as albumin and also some part is present in chelated form with citrate and phosphate. Many pathological or non-pathological conditions can significantly affect blood Ca level. However, the main determinant of the amount of serum bound Ca is serum proteins (Klasing, 1998; Leeson and Summers, 2001). Since the level of Ca in blood is directly related to the level of albumin (Tully et al., 2000), in the present study, the possible reason of Ca level in blood with diet containing 60 mg kg^{-1} Mn in organic form to be significantly lower (P<0.05) than the group (control) fed with diet not containing additional Mn may be that blood total protein and albumin level is lower than the control group with the same diet.

In general, blood values obtained in the present study are consistent with the values reported for laying hens (Altintas and Fidancı, 1993), but some of the results are not consistent with the literature reports. Dikmen et al. (2015) reported that, in contrast to present study, diets containing zinc and manganese in the form of amino acid chelate at 1.0, 2.0 and 4.0 g kg^{-1} level forold chickens did not significantly affect plasma glucose, total protein, Ca, P levels. However, these researchers reported that the treatments did not significantly affect plasma total cholesterol levels, which is consistent with the present study results. Attia et al. (2011) reported that diets containing organic (bioplex Cu-lysine) and inorganic ($CuSO_4.5H_2O$) copper at 60 and 120 mg kg^{-1} levels for breeder laying chickens did not significantly affect plasma albumin and triglyceride levels but plasma total protein and globulin levels with diet containing 120 mg kg^{-1} copper in inorganic form andtotal cholesterol level with diet containing 60 mg kg^{-1} copper in organic form were significantly (P<0.05) lower in other groups. Seyfori et al. (2018) reported that serum total protein, albumin, cholesterol, iron, manganese and magnesium levels werenot affected at ostrichs to which chelate trace mineral (Fe, Zn, Mn, Cu) at different levels were given in drinking water but the serum triglyceride level at the group to which 2 g / day /animal organic trace mineral were given were significantly (P<0.05) higher than the control group.

As mentioned above, there are differences between the results of the present study and the results obtained from other studies in terms of various characters. Possible reasons for these differences in the results may be differences in the species, genotype and age of the animals used in the study, the size of the animal's body trace mineral deposits before experiment, the trace element levels of the basal diets used in the study, the stability of the trace mineral resources and the diffrences at the level of antagonists present in diet as well as the amounts used in the diet (Xiao et al., 2015, Leeson, 2009; Richard et al., 2008; Spears and Hansen, 2008; McDowell, 2003; Cao et al., 2002).

As a result, it was observed that for breeder quail, diets containing approximately 22 mg kg^{-1} Mn (21.56 mg kg^{-1} found in the analysis) based on corn+soybean meal were sufficient in terms of other performance and reproduction characteristics except egg weight and hatching weight but Mn requirement for maximum egg weight and hatching weight were higher. It was observed that addition of 60 mg kg^{-1} Mn in inorganic form to diets of breeder quail is sufficient for maximum egg weight and hatching weights.

4.References

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