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RESEARCH ARTICLE

Effects of dietary organic and inorganic manganese supplementation on performance, carcass and hematological parameters in broiler reared under stocking density stress

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Sıklık stresine maruz bırakılmış broylerlerde rasyona organik ve inorganik manganez ilavesinin performans, karkas ve kan parametreleri üzerine etkisi

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Öz

Amaç: Bu araştırma sıklık stresi altındaki broylerlerde rasyona farklı düzeylerde organik ve inorganik manganez (Mn) ilave edilmesinin performans, karkas ve kan parametreleri üzerine etkilerinin incelenmesi amacıyla yapıldı.

Gereç ve Yöntem: Araştırmada toplam 1000 adet günlük yaşta broyler civciv her biri 100 civcivden oluşan 1 kontrol ve 9 deneme grubuna ayrıldı. Kontrol grubu temel rasyonla, deneme grupları ise temel rasyona sırasıyla 6.25, 12.5 ve 25 mg/kg düzeylerinde organik Mn (Mn-metiyonin), 12.5, 25 ve 50 mg/kg inorganik Mn (Mn-oksit), 3.125 + 6.25, 6.25 + 12.5 ve 12.5 + 25 mg/kg organik + inorganik Mn (Mn-metiyonin+Mn-oksit) ilave edilerek beslendi.

Bulgular: Araştırmada yaşama gücünün organik ve inorganik Mn ilavesi ile 29-49 ve 0-49 günlük dönemlerde arttığı belirlendi (P<0.001). Canlı ağırlık artışının 15. güne kadar 3.125 + 6.25 mg/kg Mn-metiyonin + Mn-oksit grubunda arttığı bulundu (P<0.05). Relatif dalak ağırlığının 49. gün yemlerine 12.5 mg/kg Mn-metiyonin ve 6.25 + 12.5 mg/kg Mn-metiyonin + Mn-oksit gruplarında diğer gruplardan daha yüksek olduğu (P<0.05), 6.25 mg/kg + 12.5 mg/kg Mn-metiyonin + Mn-oksit grubunda abdominal yağ miktarının kontrol grubuna göre daha düşük olduğu (P<0.05) bulundu. Heterofil/lenfosit oranının ise Mn-metiyonin grupları ile 25, 50 mg/kg Mn-oksit ve 3.125 Mn-metiyonin + 6.25 mg/kg Mn-oksit karışımı eklenen gruplarda 49. gün düşük olduğu bulundu (P<0.05).

Öneri: Barındırma stresinde bulunan broyler piliçlerde 12.5 mg/kg organik Mn ile 3.125 + 6.25 mg/kg ve 6.25 + 12.5 mg/kg organik ve inorganik Mn karışımı ilave edilmiş yemlerle beslenmesinin performans, yaşama gücü ve stres yanıtı üzerine olumlu etkiler oluşturduğu kanaatine varıldı.

Anahtar kelimeler: Sıklık stresi, organik Mn, inorganik Mn, performans, broyler

Abstract

Aim: Aim of this study was to determine the effects of organic and inorganic manganese (Mn) at different levels supplemented to diets of the broilers under stocking density stress on performance, carcass and hematological parameters.

Materials and Methods: A total of 1000, day-old broiler chicks were divided into one control group and nine treatment groups, each consisting of 100 chicks. The control group was fed the basal diet only, whereas experimental groups were fed with basal diet supplemented with organic Mn (6.25, 12.5 and 25 mg/kg of Mn-methionine) and inorganic Mn (12.5, 25 and 50 mg/kg Mn-oxide), organic + inorganic Mn (3.125 + 6.25, 6.25 + 12.5 and 12.5 + 25 mg/kg Mn-methionine + Mn-oxide).

Results: The livability increased by the supplementation of both organic and inorganic Mn on days 29 to 49 and 0 to 49 (P<0.001). Live weight gain until 15 days of age increased in the groups receiving 3.125 + 6.25 mg/kg Mn-methionine + Mn-oxide (P<0.05). On day 49, the relative weights of spleen increased in the 12.5 mg/kg Mn-methionine and 6.25 + 12.5 Mn-methionine + Mn-oxide groups compared with the other groups (P<0.05), whereas the abdominal fat quantity in the same days decreased in the 6.25 + 12.5 Mn-methionine + Mn-oxide group compared with the control group (P<0.05). The heterophil/lymphocyte ratio decreased in the in the group receiving Mn-methionine, 25 and 50 mg/kg Mn-oxide and 3.125 + 6.25 mg/kg Mn-methionine + Mn-oxide groups on day 49 (P<0.05).

Conclusion: Supplementation of 12.5 mg/kg organic Mn, or a mixture of organic and inorganic Mn (3.125 + 6.25 mg/kg and 6.25 + 12.5 mg/kg) have positive effects on the performance, livability and stress response of broiler chicken subjected to housing stress.

Keywords: Stocking density stress, organic Mn, inorganic Mn, performance, broiler

Introduction

Numerous studies on the effect of stocking density on broiler growth and carcass quality have been reviewed (Dozier et al 2006, Skrbic et al 2009, Buijs et al 2012). Some studies showed substantial advantages to reduce stocking density, others indicated that the difference was minimal (Dozier et al 2006, Buijs et al 2012) or inexistent (Blokhuis 1984). Current intensive broiler production systems use high-yield modern broiler genotypes that very rapidly reach a weight of about 2 kg. As such animals experience a rapid muscular growth out of pace with bone development, the increasing live weight and insufficient exercise negatively impact the locomotors system, especially the feet and legs (Lynch et al 1992), leading to serious health and welfare problems directly caused by cardiovascular conditions (Henry et al 1992, Martrechar et al 2002). Some studies (Blokhuis 1984, Dawkins et al 2004, Elson 2010) have indicated that welfare is affected more by the housing environment created for broiler chicken than by stocking density. Such situation is of crucial significance for the welfare of broiler chicken and the future economics of the white meat industry (Jones et al 2005, European Commission 2010). If the adverse effects of high stocking density could be reduced by improving the housing environment, the regulations imposing a legal upper limit on stocking density might be further relaxed (Jones et al 2005).

Table 1. Composition of the basal diets used in different periods (g/kg).

Ingredients	Starter (0 to12 day)	Grower (12 to 28 day)	Finisher (28 to 49 day)
Corn	47.37	50.44	51.85
Soybean meal	24.65	17.70	14.82
Full fat soybean	15.00	18.00	17.00
Poultry meal	4.00	4.00	5.00
Meat bone meal	2.50	4.00	4.00
Vegetable oil	3.50	3.52	4.91
Limestone	0.73	0.59	0.61
Dicalcium phosphate	0.86	0.46	0.35
DL-Methionine	0.36	0.33	0.34
Sodium bicarbonate	0.30	0.25	0.31
Vitamin premix[1]	0.20	0.20	0.20
Mineral premix[2]	0.10	0.10	0.15
Salt	0.14	0.12	0.07
L-Lysine	0.13	0.13	0.23
Enzyme[3]	0.10	0.10	0.10
Anticoccidial	0.06	0.06	0.06
Chemical composition			
ME[4] (kcal/kg)	3050	3200	3360
Crude protein	232.0	227.0	218.0
Calcium	10.0	10.0	10.0
Phosphorus (available)	4.95	4.95	4.95
Lysine	15.00	14.00	14.00
Methionine	7.50	7.00	7.00
Threonin	9.97	8.84	9.02

[1]Vitamin premix (Rovimix 124-F) provides per 2.5 kg of diets: 15000000 IU vitamin A, 1500000 IU vitamin D3, 50000 vitamin E, 5000 mg vitamin K3, 3000 mg vitamin B1, 6000 mg vitamin B2, 25000 mg niacin, 12000 mg Ca-D Pantothenate, 5000 mg vitamin B6, 30 mg vitamin B12, 1000 mg folic acid, 125 mg D-biotin, 300000 mg choline chloride, 300000 L-lysine. [2]Mineral premix provides per kg of diets: 80000 mg manganese, 30000 mg ferric, 60000 mg zinc, 5000 mg cupper, 500 mg cobalt, 2000 mg iodine, 235680 mg calcium carbonate. [3]Enzyme (Optimise M): 5000 BU/g endo-1.3 beta-glucanai 5000 BXU/g endo-1.4-ksilinase, 500 FYT/g phytase. [4]ME content was estimated according to the Carpenter and Clegg equation (Leeson and Summers 2001).

In recent years, modifications of broiler feed consumption (FC) to support metabolic and immunologic system function (Yang et al 2011, Conly et al 2012) or skeletal development and welfare have been researched (Su et al 1999, Duncan 2001, European Commission 2010). The especial importance of supplementing feed with manganese (Mn) has been emphasized in this connection (Henry et al 1989, 1992, Conly et al 2012). A positive effect on bone resistance of added Mn in the broiler diet has been reported; Mn, a component of enzymes such as arginase, pyruvate, carboxylase and Mn superoxide dismutase, has been indicated as one of the minerals that may be used to counter the increase of oxidative stress and the deterioration of welfare (Mertz 1993, Moonsie-Shageer and Mowat 1993). Even though published reports has been indicated that organic Mn sources are more readily available than inorganic sources and also there are not so many studies on the levels of Mn sources added to the feed.

Therefore, the objective of this study was to determine the effects of organic and inorganic Mn supplementation to the diet at different levels in broilers grown under high stocking density stress on performance, carcass and hematological parameters.

Material and Methods

Birds and housing environment

This study was conducted from May to July at the Afyon Kocatepe University Animal Research and Application Center with the approval of the University Ethics Committee (AKU-HADYEK-37-2008). A total of 1000, day-old broiler chicks were used in this study. They were divided into one control group and nine treatment groups, each consisting of 100 chicks. All the chicks were housed at a density of 0.064 kg/m^2 until reaching seven days of age. After that, the chicks were subdivided into four subgroups with 25 chicks; all were randomly placed in the compartments with 2 m^2 space and they were hold in these compartments at a density of 60 kg/m^2

(25 chicken/m²). Feed and water were provided ad libitum. Birds were raised until 49 days of age under the same housing conditions and in conformity according to the management guide for Hubbard broilers (Hubbard 2004).

Dietary treatments

The control group was fed a basal diet including predominantly of corn, soybean meal and full fat soybean (Table 1). The experimental groups were fed basal diets with supplementation of organic Mn (6.25, 12.5 and 25 mg/kg Mn-methionine) and inorganic Mn (12.5, 25 and 50 mg/kg Mn-oxide), organic + inorganic Mn (3.125 + 6.25, 6.25+12.5 and 12.5 + 25 mg/kg Mn-methionine + Mn-oxide), respectively. The nutrient composition of the basal diet (Table 1), including crude protein and calcium was determined according to the AOAC (AOAC, 2000). The metabolisable energy (ME) of the basal diet was estimated using the Carpenter and Clegg equation (Leeson and Summers 2001). The available phosphorus, lysine, methionine and threonine levels of the basal diet were calculated according to the diet guide for Hubbard broilers (Hubbard 2004).

Performance and carcass parameters

The broilers were weighed individually at the beginning of the experimental period, and live weight gain (LWG) was weekly recorded throughout the study. FC was recorded weekly and expressed as g per bird per week. Feed conversion ratio (FCR) was calculated as kg feed per kg LWG. Deaths during the study were recorded daily. At the age of 25 and 49 days, 8 birds were slaughtered (4 males and 4 females) from each group. The liver, spleen, bursa of fabricius, proventriculus + gizzard, abdominal fat and whole carcass were weighed and the relative organ and carcass weights was calculated by dividing them by live weight at slaughter. Chilled carcass weights were determined after 18 hours at 4^0C and carcass yield was calculated.

Hematological parameters

Blood samples obtained from 8 birds (4 male and 4 female) of each group were collected at 25 and 49 days of age in heparinized tubes. The hematological analyses were performed according to Feldman et al (2000). Red blood cell (RBC) and white blood cell (WBC) counts were determined manually using a hemocytometer and Natt and Herrick's stain solution. Haemoglobin concentration was determined by the cyanmethaemoglobin method.

The Packed cell volume (PCV) was determined with micro haematocrit capillary tubes that were centrifuged at 12000 rpm for 5 min. Differential WBC counts were made on blood films stained with May-Gruenwald-Giemsa, using an average of 100 cells. Heterophils/lymphocytes (H/L) ratio was determined by dividing the number of heterophils by the number of lymphocytes.

Statistical analysis

Differences among group values with regard to all parameters were examined by analysis of variance for all values and the significance of differences between groups tested by Duncan's test. Considering the number of data points in each group, the same comparisons were also performed among the experimental groups using the Kruskal-Wallis H test as an alternative to analysis of variance for nonparametric values. The two test results gave parallel results. Descriptive statistics of the study groups are presented in the tables. A value of $P<0.05$ was considered as the limit for statistical significance.

Results

Performance and carcass parameters

The effects of the supplementation of organic and inorganic Mn between 0 and 49 day in broiler diets on livability and performance are shown in Table 2. As presented, livability increased in the groups in which organic and inorganic Mn ($P<0.001$) were supplemented, whereas LWG, FC and FCR were not affected by organic and inorganic Mn levels in the diets during the entire period of experiment ($P>0.05$). Furthermore, livability on days 0-49 was 93.62% in control group, whereas the groups supplemented with Mn-methionine, Mn oxide and Mn-methionine + Mn oxide had livability values such as 97.55%, 96.91%, 98.05%, 95.97%, 96.97%, 98.09%, 97.99%, 97.56% and 96.66%, respectively ($P<0.01$). During the periods 29 to 49 days, livability was the highest in the group fed 6.25 + 12.5 mg/kg Mn-methionine + Mn-oxide ($P<0.01$). LWG up-to day 15 of age was 21.02, 22.03, 20.92, 22.98, 22.08, 23.03, 23.24, 24.02, 22.82, 21.18 g for control and experimental groups, respectively ($P<0.05$). LWG up-to day 15 of age was 21.02, 22.03, 20.92, 22.98, 22.08, 23.03, 23.24, 24.02, 22.82, 21.18 g for control and experimental groups, respectively ($P<0.05$). LWG of broilers until this days increased in the 25 mg/kg Mn-methionine, 25 or 50 mg/kg Mn-oxide and 3.125 + 6.25 mg/kg Mn-methionine + Mn-oxide groups compared with the control group ($P<0.05$).

The Live weight (LW) at slaughter and relative weights of liver, bursa of fabricius and proventriculus + gizzard showed no changes between the control group and experimental groups on days 25 and 49 of the experiment ($P>0.05$). The relative weight of spleen did not change between the experimental groups on day 25 ($P>0.05$), whereas it was determined on day 49 that the relative spleen weight increased ($P<0.05$) in the groups receiving 12.5 mg/kg Mn-methionine and 6.25 + 12.5 mg/kg Mn-methionine + Mn-oxide. Carcass yield did not show any significant difference between groups with any

Table 2. Effects of organic and inorganic Mn supplementation to diets of the broilers under stocking density stress on livability and performance during the entire period.

		Mn-methionine (mg/kg)			Mn-oxide (mg/kg)			Mn-methionine + Mn-oxide (mg/kg)				
Parameters	Control	6.25	12.5	25.0	12.5	25	50	3.125+6.25	6.25+12.5	12.5+25.0	SEM	P
Livability (%)	93.62[b]	97.55[a]	96.91[a]	98.05[a]	95.97[a]	96.97[a]	98.09[a]	97.99[a]	97.56[a]	96.66[a]	0.21	***
LWG (g/day	49.15	49.03	47.83	51.05	50.91	48.73	50.10	49.32	49.19	50.69	0.473	NS
FC (g/d)	94.22	86.22	87.07	86.78	85.21	87.24	90.37	87.32	91.30	87.39	0.84	NS
FCR (g/g)	1.97	1.76	1.82	1.72	1.68	1.80	1.80	1.77	1.86	1.73	0.028	NS

LWG: Live weight gain, FC: Feed consumption, FCR: Feed conversion ratio. Letters ([a, b]) in the same line indicate significant differences between different letters. NS: Not significant, ***P<0.001.

Table 3. Effects of organic and inorganic Mn supplementation to diets of the broilers under stocking density stress on relative organ weights (%) and carcass traits.

		Mn-methionine (mg/kg)			Mn-oxide (mg/kg)			Mn-methionine + Mn-oxide (mg/kg)				
Parameters	Control	6.25	12.5	25.0	12.5	25	50	3.125+6.25	6.25+12.5	12.5+25.0	SEM	P
Slaughter weight (g)												
25th day	920.6	958.0	862.0	1001.2	925.1	970.2	1000.3	1023.2	972.5	939.5	12.72	NS
49th day	2483.2	2543.4	2524.2	2340.7	2520.4	2424.8	2488.9	2361.4	2319.0	2422.3	32.89	NS
Liver												
25th day	2.91	2.91	2.64	2.75	2.77	2.58	2.67	2.72	2.63	2.79	0.03	NS
49th day	1.94	2.05	1.98	1.928	2.08	1.72	1.98	1.84	2.08	2.09	0.03	NS
Spleen												
25th day	0.11	0.12	0.11	0.13	0.14	0.12	0.17	0.14	0.11	0.12	0.00	NS
49th day	0.11[bc]	0.14[abc]	0.17[a]	0.13[abc]	0.15[ab]	0.12[abc]	0.10[c]	0.14[abc]	0.17[a]	0.13[abc]	0.01	*
Bursa of fabricius												
25th day	0.20	0.23	0.22	0.15	0.15	0.20	0.16	0.21	0.17	0.19	0.01	NS
49th day	0.05	0.05	0.07	0.07	0.05	0.08	0.07	0.06	0.07	0.06	0.00	NS
Proventriculus+gizzard												
25th day	3.68	4.05	3.93	4.17	3.97	3.99	3.928	4.01	4.07	4.25	0.05	NS
49th day	2.71	2.57	2.57	2.50	2.40	2.23	2.56	2.60	2.79	2.56	0.04	NS
Abdominal fat												
49th day	1.86[a]	1.46[ab]	1.47[ab]	1.63[ab]	1.38[ab]	1.94[a]	1.60[ab]	1.83[a]	1.07[b]	1.84[a]	0.06	*
Carcass yield (%)												
49th day	60.27	60.21	60.86	61.76	64.07	63.54	61.64	63.94	64.18	60.77	2.04	NS

Letters ([a, b, c]) in the same line indicate significant differences between different letters. NS: Not significant, *P < 0.05.

level of organic or inorganic Mn, whereas the abdominal fat amount decreased (P<0.05) at the end of the experiment in the group receiving 6.25 + 12.5 mg/kg Mn-methionine + Mn-oxide (Table 3).

Hematologic parameters

The lowest level of haemoglobin concentration was found at day 25 in the group given 12.5 mg/kg Mn-methionine (P<0.05). Haemoglobin concentration increased in the 50 mg/kg Mn-oxide, 3.125 + 6.25 mg/kg Mn-methionine + Mn-oxide and 6.25 + 12.5 mg/kg Mn-methionine + Mn-oxide groups compared with the control group at day 49 (P<0.01). RBC count increased in the all groups receiving Mn except 12.5+25 mg/kg Mn methionine + MnO group and the most favorable result was found in 12.5 Mn methionine group. H/L ratio did not change between the groups until day 25, whereas it was determined at 49 days that H/L ratio reduced in the groups given organic Mn and 25 or 50 mg/kg of Mn oxide and 3.125 + 6.25 mg/kg Mn-methionine + Mn oxide compared with the control group (P<0.05). PCV and other blood cell counts showed no changes in any of the groups in this study (Table 4).

Table 4. Effects of organic and inorganic Mn supplementation to diets of the broilers under stocking density stress on hematological parameters.

		Mn-methionine (mg/kg)			Mn-oxide (mg/kg)			Mn-methionine + Mn-oxide (mg/kg)				
Parameters	Control	6.25	12.5	25.0	12.5	25	50	3.125+6.25	6.25+12.5	12.5+25.0	SE	P
RBC (10^6/mL)												
25th day	291.1	308.3	310.5	301.6	306.2	302.6	296.7	310.8	309.0	323.3	2.20	NS
49th day	275.6^{d}	308.7^{ab}	324.1^{a}	312.0^{ab}	285.5^{c}	298.2^{bc}	305.8^{abc}	313.6^{ab}	307.1^{abc}	291.8^{bcd}	2.65	**
Hemoglobine (g/dL)												
25th day	26.00^{ab}	26.37^{ab}	25.87^{b}	27.37^{ab}	26.25^{ab}	26.25^{ab}	27.62^{ab}	27.62^{ab}	27.87^{a}	26.25^{ab}	0.18	*
49th day	25.50^{b}	27.12^{ab}	26.25^{b}	27.12^{ab}	25.62^{b}	27.00^{ab}	28.75^{a}	28.62^{a}	28.50^{a}	27.12^{ab}	0.24	**
PCV (%)												
25th day	9.27	9.42	9.42	9.67	9.73	9.85	9.50	9.83	9.75	9.81	0.076	NS
49th day	8.88	9.42	9.60	9.36	9.20	9.30	9.36	9.47	9.51	9.15	0.058	NS
WBC (10^4/mL)												
25th day	31.62	30.00	29.25	32.00	31.25	29.12	33.50	29.37	31.37	33.00	0.566	NS
49th day	37.62	34.62	36.37	36.25	32.00	32.37	32.87	35.12	35.37	33.25	0.517	NS
Heterophils (%)												
25th day	31.62	30.75	28.37	28.62	29.12	28.87	28.62	30.00	30.37	28.75	0.29	NS
49th day	37.25^{a}	32.12^{bc}	29.75^{d}	30.87^{cd}	34.87^{b}	31.12^{bc}	31.37^{c}	31.50^{c}	33.25^{b}	32.75^{bc}	0.52	*
Lymphocytes (%)												
25th day	59.12	59.75	60.50	60.87	60.62	59.87	61.12	59.25	59.75	61.25	0.29	NS
49th day	54.00	59.12	59.12	59.87	55.62	59.12	59.25	58.62	57.75	57.37	0.46	NS
Monocytes (%)												
25th day	5.00	5.50	6.00	5.75	5.87	6.25	5.62	5.87	5.00	5.62	0.10	NS
49th day	4.62	5.37	6.75	5.00	4.75	5.75	4.750	5.12	4.50	5.12	0.17	NS
Eosinophils (%)												
25th day	3.37	3.50	4.25	4.00	3.75	4.00	4.12	3.87	4.37	3.75	0.09	NS
49th day	3.62	2.62	3.50	3.62	4.00	3.37	4.37	4.25	4.37	3.62	0.08	NS
Basophils (%)												
25th day	0.87	0.50	0.87	0.75	0.62	1.00	0.50	1.000	0.500	0.62	0.06	NS
49th day	1.20	1.00	1.28	1.00	1.00	1.00	1.20	1.200	1.000	1.00	0.03	NS
H/L ratio												
25th day	0.53	0.51	0.47	0.4	0.48	0.48	0.47	0.50	0.50	0.47	0.00	NS
49th day	0.69^{a}	0.54^{b}	0.50^{b}	0.52^{b}	0.63^{ab}	0.53^{b}	0.53^{b}	0.55^{b}	0.58^{ab}	0.57^{ab}	0.01	*

Letters ($^{a, b, c, d}$) in the same line indicate significant differences between different letters. RBC: Red blood cell (Erythrocyte), WBC: White blood cell (Leucocyte), PCV: Packed cell volume, NS: Not significant, *P < 0.05, **P<0.01.

Discussion

There are several recommendations on usage of different Mn forms in commercial poultry diets, to prevent adverse effects of high stocking density on broiler performance. The organic and inorganic manganese doses which were used in this study were decided according to the results of many studies that showed the effects of Mn on broilers hosted in stressfully conditions. In this study, LWG did not change between groups on days 15-49, whereas it was determined on days 0-15 that LWG increased in the groups fed Mn compared with the control group. In the last three weeks of the study, LWG in the 50 mg/kg Mn-oxide group was better than the control group. Generally, it was determined in this study that LWG in broilers of the groups given Mn-supplemented diet highest than the control group. This result showed that Mn supplementation to the diet supported the performance of broilers mainly the suplementation of 50 mg/kg inorganic manganese. The current study findings are consistent with published results (Henry et al 1989, Conly et al 2012), because Mn acts as an activator in multiple physiologic processes, such as carbohydrate, lipid and protein metabolism, collagen formation, antioxidant activity and respiration; it enters the structure of several enzymes. Because of this significant role, Mn supplementation to the fast-growing broiler diet was reported to have become a widespread practice (Henry et al 1989, Scheideler 1991, Conly et al 2012).

In the present study, no statistical significance was recorded in all days for FCR, even though it was notably different between the groups. This may be due to the considerable variation observed in the values between LWG and FCR. It has been though that the high stocking density used in the study may have exerted a progressively increasing negative effect on the growth of the chicken along with the continuation of the experiment, especially by increasing heterogeneity in FC as a result of the interactions between socially superior and inferior individuals. However, large values are seen, in effect, for the mean standard error values for live weights in this period. Moreover, daily LWG was higher, and FC and FCR lower, in chicken fed a Mn-supplemented diet, especially with Mn-methionine, compared with the animals of control group which did not receive any Mn supplementation, even though the results are based on rather small numbers (Deo et al 2006).

Conclusively, although there wasn't any significant effect, numerically decrease was determined for FC in chicken fed a Mn-supplemented diet, especially with organic as opposed to inorganic Mn under high stocking density. Higher amount of abdominal fat in the control group was observed, which had the highest FC, compared with the experimental groups, suggested that high FC may led to the deposits of abdominal fat. The low abdominal fat content of broilers fed with Mn supplementation may be an effect of Mn. Also, Sand and Smith (1999) indicated that the supplementation of Mn to the diet of broilers under heat stress may reduce abdominal fat deposits. Atherton (1993) reported that the supplementation of Mn to the diet reduced fat deposits in the carcass of pigs. Conly et al (2012) have also reported similar results. The results of our study are consistent with those of Henry et al (1992) who reported that Mn-methionine supplementation yielded more positive results than Mn-oxide, and Deo et al (2006) observed that the availability of organic Mn forms are higher than the inorganic ones, resulting higher performance and better health in animals.

Similarly Smith et al (1995) have reported that organic Mn has higher availability than the inorganic Mn. Atherton (1993) pointed out that Mn is a basic mineral for the growth and performance of birds. The results obtained with the 12.5+25.0 mg/kg Mn-methionine + Mn-oxide encouraged the speculation that this level supported performance more effectively than the other two combinations at lower doses, a result that may be consistent with a maximal effect of Mn in the group receiving the maximum amount of both sources of the mineral. In this study, no significant differences in the H/L ratio were found between groups up to day 25, but it was higher in the control group than the experimental groups subjected to an identical stocking stress. The H/L ratio is widely seen as a stress marker in birds (Maxwell 1993). The results showed that no significant stocking density was present because the birds were small (700-800 g), but this developed along with their growth, creating stress from that days forward. However, the livability of the chicken was also observed to increase with age, becoming more noticeable from the 4th week.

The mortality in the control group reached 11%, but it remained at an acceptable level of 3-5% in the experimental groups. The stocking density used for the study was supposed to substantially affect the hematological parameters. Haemoglobin concentration and RBC count were significantly lower in the control group at the end of the study on day 49 as compared with the Mn-supplemented groups. Specifically, there were remarkable positive effects on RBC and HG for some supplementations such as of 12.5 mg/kg organic manganese and 50 g/kg Mn-oxide and the combinations of organic and inorganic manganese, respectively.

Even though no statistical significance was observed for PCV value, lymphocyte, monocyte, eosinophil and basophil counts and the H/L ratio were lower in control group than in experimental groups; total leucocyte and heterophil counts were higher. These results confirmed the stress created in the control group, fed with the basal diet only, by a high stocking density (Maxwell 1993). When comparing different Mn-supplemented groups, among them, the Mn-methionine-fed birds had higher PCV and haemoglobin values and total RBC and WBC counts and absolute lymphocyte, monocyte and basophil counts with a lower H/L ratio ($P<0.05$). As Bulbul et al (2008) reported the positive effects of Mn-methionine, this study also indicated that the supplementation of Mn-methionine to the diet of broiler chicken under high-density housing conditions may have reduced the stress produced by the stocking density.

Conclusion

Stocking density at the rate of 60 kg/m^2 creates increasingly apparent stress in broilers, especially after the age of 25 days and Mn-supplemented diet in chicken has positive effects against stress, whereas no effect was observed on other organ weights, performance and carcass yield, except for relative weights of spleen and abdominal fat. Overall among the different treatments the effect of the Mn-methionine was more positive than those of Mn-oxide. Keeping in view the overall effects supplementation of 12.5 mg/kg Mn-methionine is more effective and favorable and could be used for better performance.

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