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Effect of Replacing Dietary $FeSO_4$ with $FeHPO_4$ Nanoparticles on Growth Performance, Carcass Characteristics and Tissue Iron Content in Broilers

Research Article

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ABSTRACT

A study was conducted to compare the effects of iron hydrogen phosphate nanoparticles ($FeHPO_4$) with iron sulfate ($FeSO_4$-$7H_2O$) on growth performance, carcass characteristics and mineral content of breast muscle in broiler chickens. A total of 200 one-day-old male Ross 308 broiler chicks were assigned randomly into four dietary groups with five replications of ten chicks per replicate. The basal diet (BD) included corn-soybean meal with 87.40, 85.13 and 82.24 mg Fe/kg diet in starter, grower and finisher feeding phases, respectively. Four dietary groups consisted of: group 1) BD + 80 mg/kg $FeSO_4$, as control group; group 2) BD + 6.4 mg/kg $FeHPO_4$ nanoparticles (FNPs); group 3) BD + 3.2 mg/kg FNPs; and group 4) BD + 1.6 mg/kg FNPs were provided during 1-42 d of age. The results showed that dietary groups did not significantly ($P>0.05$) affect the average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion ratio (FCR), mortality rate, and European production efficiency factor (EPEF). Adding FNPs in dietary group 2 significantly ($P= 0.04$) reduced relative weight of abdominal fat compared to control group. Furthermore, there were cubic ($P=0.05$) and quadratic ($P=0.02$) responses to the addition of FNPs in dietary group 3 on carcass yield and relative weight of liver, respectively. Compared with birds fed the diet supplemented with 80 mg $FeSO_4$/kg, birds fed supplemental FNPs in groups 2 and 3 had significantly increased Fe and Cu content in the breast muscle ($P<0.05$). There was no differences in Zn content of breast muscle among the dietary groups ($P>0.05$). This study concluded that, replacing $FeSO_4$ with FNPs had no significant effect on growth performance, but it could significantly increase the iron content of breast muscle.

KEY WORDS broiler chickens, $FeSO_4$, $FeHPO_4$ nanoparticles, iron content, performance.

INTRODUCTION

Iron is a vital element for all living organisms which plays an essential role in various enzyme systems in the body and also in the synthesis of haemoglobin that is required for cellular respiration (Shinde *et al.* 2011). Human and animals suffer from metabolic disorders leading to poor performance in response to Fe deficiency (Akter *et al.* 2017). Iron deficiency can cause anemia (especially microcytic hypochromic anemia in birds), reduce cognitive development and lower work capacity (Zimmermann and Hilty, 2011). Therefore, due to high requirements of oxygen capacity in broilers as a fast-growing strain, iron should be provided in the diet for to reach their optimum genetic growth potential. The NRC (1994) has recommended 80 mg Fe/kg diet for broilers. There is common concern in the poultry industry that the NRC recommendation may not be sufficient to prevent production losses due to phytate in the commercial diets of broiler chickens containing corn and soybean meal. On the other hand, competition for the common transporters of iron and other divalent metals such as zinc, copper, cobalt and cadmium in intestine leads to reduction in iron absorption through gastrointestinal tract (Miroshnikova *et al.* 2015).

Nanotechnology is defined as the understanding and control of matter at the nano-scale, at dimensions between approximately 1 and 100 nanometer (Huang *et al.* 2015). Nanoparticles (NPs) by reduction of their particle size, demonstrate unique properties. Due to its high surface area, NPs rapidly and completely dissolve in gastrointestinal tract and when reach the apical membrane of the mucosa, they may potentially be absorbed by gut-associated lymphoid tissue, and pass through the mesenteric lymphatics to the venous circulation, liver and spleen (Jani *et al.* 1990; Hussain *et al.* 2001). It has been reported that decreasing the particle size of metallic iron powders by 50-60% to a mean particle size of 7-10 µm increases Fe absorption by 50% in rats (Rohner *et al.* 2007). Iron absorption from various iron compounds is typically compared to absorption from $FeSO_4$, and expressed as relative bioavailability (Zimmermann and Hilty, 2011). Previous studies have demonstrated that nanotechnology studies about poultry breeding have indicated that NPs not only can improve animal immunity and reduce the use of antibiotics, but also can reduce the manure odor of livestock and poultry via direct impact on the intestinal microbiome of broiler chickens (Huang *et al.* 2015; Yausheva *et al.* 2018), which can lead to improved environmental conditions. Also, NPs are increasingly used to target bacteria as an alternative to antibiotics and via multiple antimicrobial mechanisms may be particularly advantageous in treating bacterial infections (Wang *et al.* 2017). Research has shown that the utilization coefficient of inorganic trace elements was about 30%, while the utilization coefficient of nano trace element was close to 100% (Huang *et al.* 2015). Thus, utilization rate of nano trace elements are much more than that of the ordinary inorganic trace elements because of its high surface area and enhanced permeability.

There are limited data on the effects of using iron nanoparticles in poultry nutrition. Nikonov *et al.* (2011) demonstrated that replacement of iron sulfate by iron NPs at levels of1.5 and 0.75 g/ton basal diet improved growth performance of broilers during 1-35 d of age, but did not significantly influence on chemical composition of breast muscle. Furthermore, Rahmatollah *et al.* (2018) reported that administration of 1.2 mg/kg Fe_3O_4-Cys NPs in basal diet are required and sufficient for quails' optimal maintenance and growth as compared to higher doses of Fe_3O_4-Cys NPs and 120 mg/kg of $FeSO_4$ during 1-42 d of age. Concerning toxic effect of nano-materials, iron NPs have biologically active properties, and they are less toxic than inorganic iron salts (Nikonov *et al.* 2011; Lee *et al.* 2014).

Also, iron NPs are more economical, physiological, compatible, safe as compared to gold, silver, and other nanoparticles (Bano *et al.* 2017).

Cytotoxicity of NPs depend on some physiochemical properties such as size, shape, aspect ratio, density, and surface and structural defects and dissolving rate which may be cause a range of acute and chronic effects in the body (Rahi *et al.* 2014).

Although, most studies have conducted on inorganic Fe, organic Fe and its replacement effects upon broiler chickens; however, there is much less information about effect of supplementation of iron nanoparticles (especially $FeHPO_4$ NPs) on broiler performance. Thus, the aim of this study was to evaluate the effects of supplementing of $FeHPO_4$ NPs at the experimental levels on growth performance, carcass characteristics and iron content of breast muscle in broilers.

MATERIALS AND METHODS

Animals, diets and experimental design

This experiment was accomplished at the Poultry Research Station of Urmia University (Urmia, Iran), and all experimental methods were approved by Urmia University Animal Care Committee (RD 1828, 15 August 2016). An experiment was performed with 200 one-day-old (Ross 308) male broilers.

All the birds were randomly assigned into 4 dietary treatments with 5 replicates of 10 chickens each. The basal corn-soybean diet (Table 1) was formulated to meet Ross 308 (Aviagen, 2014) requirements for starter (1-10 d), grower (11-24 d) and finisher (25-42 d) feeding phases. The mineral contents of the basal diets (BD) were analyzed by flame atomic absorption spectrophotometry (AA-6300; Shimadzu, Tokyo, Japan), as shown in Table 1.

The four diet groups were: group 1) BD + 80 mg/kg $FeSO_4$, as control group; group 2) BD + 6.4 mg/kg $FeHPO_4$ nanoparticles (FNPs); group 3) BD + 3.2 mg/kg FNPs; and group 4) BD + 1.6 mg/kg FNPs. The experimental levels of FNPs were based on previous findings on iron NPS in broiler chickens (Nikonov *et al.* 2011). The inorganic Fe as reagent grade $FeSO_4$-$7H_2O$ with 21% Fe sulfate heptahydrate (Merck Company, Germany) added to basal diet and nano Fe as iron hydrogen phosphate nanoparticles ($FeHPO_4$) was manufactured by reacting $FeSO_4$-$7H_2O$ and H_3PO_4 at 1:1 molar ratio. The product of $FeHPO_4$ was synthesized in Laboratory of Organic Chemistry Department of Urmia University. A mixture of 1 L (2 Molar) NaOH and 1 L (1 Molar) H_3PO_4 were stirred at room temperature for 30 min, and then 1 L (1 Molar) FeSO4 were added and stirred at 100 ˚C for 120 min. The residue was filtered, washed with water and dried at 100 ˚C.

Table 1 Composition of the experimental diets (as fed basis)

Ingredients, %	Starter (1 to 10 d)	Grower (11 to 24 d)	Finisher (25 to 42 d)
Corn	51.93	57.19	62.26
Soybean meal (44% CP)	41.05	35.65	30.49
Soybean oil	2.38	2.88	3.40
Limestone	1.02	0.95	0.88
Dicalcium phosphate	2.10	1.87	1.63
NaCl (iodized salt)	0.26	0.26	0.27
Vitamin-mineral premix[1]	0.50	0.50	0.50
L-lysine HCL	0.18	0.17	0.16
DL-methionine	0.36	0.32	0.28
Threonine	0.07	0.06	0.04
Sodium bicarbonate	0.10	0.10	0.10
Coccidiostat	0.05	0.05	-
Calculated composition			
Metabolizable energy (MJ/kg)	12.55	12.97	13.39
Crude protein (%)	23	21.5	19.5
Crude ash (%)[2]	5.65	5.43	4.87
Crude fat (%)[2]	4.91	6.35	6.84
Linoleic acid (%)	1.25	1.20	1
Calcium (%)	0.96	0.87	0.79
Available P (%)	0.48	0.43	0.39
Lysine (%)	1.44	1.29	1.16
Methionine + cysteine (%)	1.08	0.99	0.91
Methionine (%)	0.56	0.51	0.47
Threonine (%)	0.97	0.88	0.78
Fe (mg/kg)[2]	87.40	85.13	82.24
Zn (mg/kg)[2]	97.42	95.02	93.76
Cu (mg/kg)[2]	13.85	14.06	13.42

[1] Premix provided per kilogram of diet: vitamin A (retinol): 12800 IU; vitamin D_3 (cholecalciferol): 4000 IU; vitamin E (DL-α- tocopheryl acetate): 48 IU; vitamin K_3 (menadione): 4.4 mg; vitamin B_6 (pyridoxine): 6.4 mg; vitamin B_{12} (cyanocobalamin): 0.016 mg; vitamin B_3 (niacin): 22.4 mg; vitamin B_5 (pantothenic acid): 64 mg; vitamin B_9 (folic acid): 1.6 mg; Choline chloride: 400 mg; Mn (manganese sulfate): 112 mg; Fe (iron sulfate): 32 mg; Zn (zinc oxide): 128 mg; Cu (copper sulfate): 12.8 mg; Se (sodium selenite): 0.4 mg and Iodine (calcium iodate): 0.9 mg.

[2] Trace element values were analyzed by flame atomic absorption spectrophotometry.

Rearing conditions

All chicks were reared in a room with separated pens (1×1 m) under the same environmental conditions throughout the experimental phases. Room temperature was set at 33 ˚C during the first week of age and then was gradually reduced by 3 ˚C weekly until it reached 22 ˚C after 3 weeks. All birds were allowed *ad libitum* access to the experimental diets and water during of study. The lightning regimen for reared chicks was 23 L:1 D throughout the study.

Growth performance

Final body weight (at 42 day of age), and average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion ratio (FCR) for whole experimental period was calculated and mortality rate was recorded daily and used to adjust the FCR. Also, the effectiveness of chicken fattening was determined on the basis of the European production efficiency factor (EPEF) calculated according to the following formula (Akbari Moghaddam Kakhki *et al.* 2016):

EPEF= [(body weight (kg)×livability (%)) / (length of fattening period (d)×FCR)] × 100

Carcass characteristics

On the 42 day of the study, 4 birds from each replicate (pen) were randomly selected and slaughtered by decapitation. After removal of skin and feather, breast and thigh muscles, liver, pancreas, gizzard, heart, small intestine, duodenum, jejunum, ileum and abdominal fat were weighed individually. Yields were expressed as the percentage of live BW.

Chemical analysis of breast muscle

After slaughter, the contents of moisture, protein, fat and ash for breast muscle were analyzed according to AOAC (1994). Approximately 40 g of breast raw meat sample from each of 4 treatments within 5 replications was collected, ground, and homogenized. To determine moisture content meat samples completely dried for 24 h at 105 ˚C. Subsequently, crude protein (CP) and ether extract (EE) were determined using the standard Kjeldahl and Soxhlet methods, respectively.

Determination of Fe, Cu and Zn content in breast muscle

Trace minerals (Fe, Cu and Zn) in feed and breast meat samples were measured using atomic absorption spectrophotometer (AA-6300; Shimadzu, Tokyo, Japan). The samples were dried at 100 ˚C for 24 h and ashed for 10 h at 550 ˚C. The ashed samples were dissolved in a nitric acid-perchloric acid mixture (1:1) and diluted with deionized water for mineral analysis (Kwiecien *et al.* 2015).

Mineral analysis was performed by a computerized atomic absorption spectrophotometer following the instruction manual supplied by the manufacturer. The wavelengths used for iron measurement were 248, 259, and 213 nm for Fe, Zn, and Cu, respectively.

Statistical analysis

A polynomial regression (linear, quadratic and cubic) analysis was used to determine the optimal graded levels of dietary iron on different parameters tested. Data were analyzed using the generalized linear model (GLM) procedure of SAS software (SAS, 2009). The univariate test in SAS was used to assess the normality of all data. Each pen of birds was considered the experimental unit for all analysis. The polynomial regression models were selected based on the significance of the regression coefficients ($P<0.05$) and on the value of the coefficient of determination.

RESULTS AND DISCUSSION

Growth performance

Table 2 shows effects of supplemental Fe from dietary groups on growth performance of broiler chickens. Dietary Fe supplementation of both FNPs and $FeSO_4$ forms had no significant effect ($P>0.05$) on the growth performance parameters including final BW, ADWG, ADFI, FCR, mortality rate and EPEF of broiler chickens during the whole experimental period from 1 to 42 d of age. Although, ADWG quadratically ($P=0.03$) increased in birds fed supplemental Fe in dietary groups 1, 3 and 4 as compared to group 2; however, these differences were not statistically significant (Tukey test, $P>0.05$). Also, ADFI tended ($P=0.08$) to improve in birds fed on Fe supplemented dietary groups during 1 to 42 d of age.

Carcass characteristics

On d 42, dietary treatments had no significant effect ($P>0.05$) on relative weight of breast muscle, thigh muscle, pancreas, heart, small intestine, duodenum, jejunum and ileum (Table 3). However, supplementation of iron with FNPs compared to $FeSO_4$ caused a significant effect on carcass yield, and relative weights of liver and abdominal fat. Birds fed dietary supplemental Fe from dietary group 3 exhibited cubic ($P=0.05$) and quadratic ($P=0.02$) manner in carcass yield and liver weight on d 42, respectively. Regarding the abdominal fat weight on d 42, a cubic response ($P=0.04$) was observed in broilers fed 6.4 mg FNPs/kg diet in dietary group 2 compared with control group.

Chemical analysis of breast muscle

Supplementation of iron both $FeSO_4$ and FNPs forms had no significant effect ($P>0.05$) upon moisture content, CP, EE and ash content of breast muscle (Table 4). Concerning mineral deposition in breast muscle, the positive results were obtained by supplementation of FNPs in dietary groups 2 and 3 compared to $FeSO_4$ in control diet. Birds fed 6.4 mg/kg of FNPs in dietary group 2 exhibited a cubic increase ($P=0.007$) in Fe concentration of breast muscle. Furthermore, Cu deposition of breast muscle linearly ($P=0.006$) and quadratically ($P=0.007$) increased by addition of 3.2 mg/kg of FNPs in dietary group 3. Although, there is a numerical difference among dietary groups regarding Zn content in breast muscle; however, this difference was not statistically significant (Tukey test, $P>0.05$).

In the present study, supplementing basal diet with 80 mg/kg of $FeSO_4$ and different levels of nano-Fe had no significant effect on the growth performance parameters in broilers. However, the results of our study do not agree with Nikonov *et al.* (2011) who reported that replacement of $FeSO_4$ with NPs of iron (Fe_2O_3 NPs) at doses of 0.75 and 1.5 g/ton in basal diet increased the percentage of livability, live weight of birds and expenditure of feed/kg of increase in live weight for 35 days. Also, Saki *et al.* (2014) demonstrated that administration of iron NPs + Alimet (liquid methionine) in hatching fertile eggs improved performance parameters including FCR, EPEF and ADFI in broilers.

Rohner *et al.* (2007) found that use of $FePO_4$ small particle size of NPs in rats diet increased body weight gain and fortified Fe intake of rats as compared to $FePO_4$ large particle size, $FeSO_4$ and control (Fe-deficient diet) dietary treatments. It has been reported that particle size is an important determinant of Fe absorption from poorly soluble Fe compounds in foods which could result in increased Fe absorption by 50% in rats via decreasing the particle size of metallic Fe powders by 50-60% to a mean particle size of 7-10 µm (Motzok *et al.* 1975; Verma *et al.* 1977). Additionally, in another study on quails, Rahmatollah *et al.* (2018) reported that quails given 1.2 mg/kg Fe_2O_3 NPs had higher ADWG and improved FCR when compared to those fed 120 mg/kg $FeSO_4$ and high doses of Fe_2O_3 NPs, on d 42. There are contradictory and limited findings about the possible effects of iron NPs, on the performance of broiler chickens.

On the other hand, due to body weight is not more sensitive index for determination of iron requirement and iron deficiency of chickens, and considering that delay in the growth rate of the bird occurs only during the final stages of iron deficiency (Amine *et al.* 1972).

Table 2 Replacement effect of dietary supplemental $FeSO_4$ with $FeHPO_4$ nanoparticles (FNPs) on growth performance of broiler chickens during 1-42 d of age[1]

Item	$FeSO_4$ (mg/kg)	FNPs (mg/kg)			SEM	P-value		
	Group 1	Group 2	Group 3	Group 4		Linear	Quadratic	Cubic
Final body weight (g)	2261.30	2066.30	2196.80	2167.00	101.50	0.74	0.43	0.30
ADWG (g/bird/d)	50.76	45.27	47.14	48.62	1.44	0.49	0.03	0.25
ADFI (g/bird/d)	83.74	78.61	81.63	86.76	2.73	0.34	0.08	0.62
FCR (g/g)	1.65	1.74	1.73	1.79	0.06	0.17	0.81	0.57
Mortality (%)	2.92	3.49	3.12	3.29	0.95	0.86	0.83	0.73
EPEF	294.19	249.17	262.45	261.17	16.68	0.27	0.21	0.34

[1] P: significance level at 5% by the adjusted regression equations.
Group 1: BD + 80 mg/kg $FeSO_4$, as control group; Group 2: BD + 6.4 mg/kg FNPs; Group 3: BD + 3.2 mg/kg FNPs and Group 4: BD + 1.6 mg/kg FNPs.
ADWG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio and EPEF: European production efficiency factor.
SEM: standard error of the means.

Table 3 Replacement effect of dietary supplemental $FeSO_4$ with $FeHPO_4$ nanoparticles (FNPs) on carcass characteristics of broiler chickens during 1-42 d of age (% of live body weight)[1]

Item	$FeSO_4$ (mg/kg)	FNPs (mg/kg)			SEM	P-value		
	Group 1	Group 2	Group 3	Group 4		Linear	Quadratic	Cubic
Carcass yield	70.21	69.25	71.59	69.04	0.85	0.76	0.36	0.05
Breast muscle	22.61	22.92	24.09	22.63	0.67	0.71	0.23	0.30
Thigh muscle	18.96	18.80	18.60	18.59	0.46	0.53	0.87	0.92
Liver	1.92	2.13	2.14	1.91	0.08	0.95	0.02	0.95
Heart	0.52	0.51	0.49	0.49	0.04	0.58	0.82	0.85
Pancreas	0.23	0.23	0.23	0.24	0.01	0.69	0.88	0.89
Gizzard	1.40	1.41	1.33	1.62	0.09	0.16	0.13	0.26
Small intestine	4.13	4.36	4.37	4.34	0.32	0.67	0.70	0.89
Duodenum	0.71	0.81	0.78	0.79	0.05	0.29	0.37	0.42
Jejunum	1.97	1.95	2.01	1.94	0.19	0.98	0.89	0.80
Ileum	1.46	1.61	1.58	1.61	0.14	0.52	0.69	0.68
Abdominal fat	1.77	1.27	1.56	1.43	0.12	0.20	0.14	0.04

[1] P: significance level at 5% by the adjusted regression equations. Equations set for carcass yield (CY), relative liver weight (RLW) and relative abdominal fat (RAF) were $CY= 82.66 - 20.93x + 9.84x^2 - 1.36x^3$; $RLW= 1.47 + 0.55x - 0.11 x^2$ and $RAF= 4.22 - 3.82x + 1.57 x^2 - 0.20x^3$, respectively.
Group 1: BD + 80 mg/kg $FeSO_4$, as control group; Group 2: BD + 6.4 mg/kg FNPs; Group 3: BD + 3.2 mg/kg FNPs and Group 4: BD + 1.6 mg/kg FNPs.
SEM: standard error of the means.

Table 4 Replacement effect of dietary supplemental $FeSO_4$ with $FeHPO_4$ nanoparticles (FNPs) on chemical composition (%) and mineral concentration (mg/100g of tissue) of breast meat of broiler chickens at 42 d of age[1]

Item (%)	$FeSO_4$ (mg/kg)	FNPs (mg/kg)			SEM	P-value		
	Group 1	Group 2	Group 3	Group 4		Linear	Quadratic	Cubic
Moisture	75.42	76.06	76.39	76.16	0.46	0.23	0.35	0.90
Crude protein	23.56	23.88	24.00	23.93	0.19	0.18	0.33	0.98
Crude fat	3.20	2.79	2.74	2.36	0.42	0.19	0.97	0.72
Ash	3.26	3.66	3.52	3.50	0.25	0.60	0.42	0.55
Fe	22.54	31.94	24.67	29.63	2.10	0.16	0.31	0.007
Cu	2.44	2.52	2.64	2.54	0.03	0.006	0.007	0.08
Zn	17.45	20.00	23.80	20.55	1.53	0.07	0.08	0.24

[1] P: significance level at 5% by the adjusted regression equations. Equation set for $Fe= 82.66 - 20.93x + 9.84x^2 - 1.36x^3$ and Equation set for $Cu= 2.43 + 0.04x$.
Group 1: BD + 80 mg/kg $FeSO_4$, as control group; Group 2: BD + 6.4 mg/kg FNPs; Group 3: BD + 3.2 mg/kg FNPs and Group 4: BD + 1.6 mg/kg FNPs.
SEM: standard error of the means.

Therefore, it appears that growth performance parameters are not reliable indicators of the bird's status iron and do not have direct correlation with iron body reserves.

According the quadratic response of ADWG to adding FNPs in current experiment, it can be stated that inclusion of dietary FNPs possibly have caused the higher iron uptake of cells. It has been reported that higher iron levels than the needs of the cell can generate oxidative stress which is characterized by the increased basal concentration of reactive oxygen species (ROS) (Oliveira *et al.* 2014). In a similar way, Ma *et al.* (2016) reported that adding 40 to 60 mg/kg inorganic Fe into diet increased quadratically weight gain of broilers, but adding more than 60 mg Fe/kg diet reduced body weight.

In our study, liver weight of broilers quadratically increased by addition of FNPs.

The high accumulation of iron, copper and zinc in the liver of 6.4 and 3.2 mg/kg of FNPs fed birds can have increased the liver weight and metabolism. Iron is stored mainly in the liver, containing around 60% of the body's iron pool, of which around 95% is stored as ferritin in hepatocytes (Buzala *et al.* 2016).

Moreover, a reduction in relative weight of abdominal fat at 42 of day was observed in broilers given 6.4 mg/kg of FNPs as compared to those fed control diet. Human and animal studies have been showed that iron is involved in the regulation of insulin and glucose (Suliburska *et al.* 2011) and iron overload associated with the increased risk of developing type 2 diabetes and subsequently obesity through the metabolism of adiponectin (Gabrielsen *et al.* 2012). Adiponectin is a protein hormone that regulates of the different metabolic processes such as glucose regulation and fatty acid oxidation (Diez and Iglesias, 2003). This protein hormone is secreted from adipose tissue into the bloodstream (Chen *et al.* 2006), and plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes (Ukkola and Santaniemi, 2002), obesity, atherosclerosis (Diez and Iglesias, 2003). Moreover, adiponectin exerts some of its weight reduction effects via the brain that is similar to the action of leptin (Nedvidkova *et al.* 2005). Therefore, increased production of adiponectin could be related to lower weight of abdominal fat weight in 6.4 mg/kg of FNPs fed birds.

The body fat of chicken mainly deposits in regions such as the abdomen, subcutaneous and muscular tissues (Mirghelenj *et al.* 2016). According to Moon *et al.* (2000) a certain amount of intramuscular fat can enhance the traits such as the flavor and tender degrees of meat, but accumulation of abdominal fat in chickens result in increased poultry feed cost and decreased final product quality (Mirghelenj *et al.* 2016). Additionally, it has been reported that success of broiler meat production has been strongly related to improvements in growth and carcass yield, mainly by increasing breast yield and reducing abdominal fat (Zerehdaran *et al.* 2004). Consequently, the control of lipid deposition in broilers aimed at efficient lean-meat poultry production is of current interest and any reduction in the amount of abdominal fat is considered to be positive by both producers and consumers (Fisher, 1984; Hermier, 1997). Recent human studies suggest that micronutrient deficiencies may contribute to fat deposition and chronic inflammation (Garcia *et al.* 2009; Zavala *et al.* 2012). It has been reported that the high prevalence of micronutrient deficiencies such as iron, zinc, vitamin A, vitamin E and vitamin C might be contributing to the development of obesity (Garcia *et al.* 2013). It has been shown that these micronutrients decrease or inhibit the expression of leptin, in both humans and animal models (Garcia *et al.* 2013; Garcia-Diaz *et al.* 2010). Furthermore, relationship between vitamin E, iron and zinc concentrations with glucose and insulin has been studied in human and animal models (Williams *et al.* 2012).

According to this study, Nikonov *et al.* (2011) found that supplementation of 0.3, 0.75, 1.5 and 3 g/ton of Fe_2O_3 NPs in broilers diet had no effect on DM, CP, EE and ash content of breast muscle. However, supplementing basal diet with 6.4 mg/kg of FNPs increased Fe content in breast muscle. In the present study, the iron content of breast meat ranged from 22.54 (in control group) to 31.94 mg/100 g of tissue (in dietary group 2) which indicates the positive effect of supplementing FNPs in broilers diet on the iron content in breast muscle compared with the 80 mg/kg of $FeSO_4$ in control group. The results of Nikonov *et al.* (2011) study showed that trace elements (including Fe, Zn, Cu and Mn) deposition in the breast muscle of broilers did not influenced by dietary Fe supplementation. Regarding Cu and Zn deposition of muscle tissue in our study, addition of 50% of highest dose of FNPs (3.2 mg/kg of FNPs) increased Cu content in breast muscle compared to control group, but Zn deposition of breast muscle did not affect by dietary groups. It has been well documented that there is a negative interaction between trace elements including Fe, Cu and Zn (Underwood, 1977). This competitive antagonism between Fe, Cu and Zn may affect their absorption by brush borders from GIT pathway and led to reduce bioavailability of intestinal iron in bird. It has been reported that copper deficiency decrease iron absorption in animal models, which is believed to be due to intestinal iron transports being copper dependent (Collins *et al.* 2010). On the other hand, the interaction between iron, copper and zinc absorption may be explained by competitive binding to a transporter called divalent metal transporter 1 (DMT-1), which participates in divalent metal transport such as Fe, Cu, Zn, Mn, Co and Pb by a proton-coupled mechanism (Gunshin *et al.* 1997). It has been demonstrated that DMT1 is the main Fe^{2+} transporter that it participated actively in Cu^{1+} transport (Arredondo *et al.* 2003). According to Seo *et al.* (2008) such interactions among divalent trace elements occur when a certain mineral component is supplied at excessively high level and other mineral(s) is marginally deficient, whereas Fe, Cu and Zn levels in the experimental diets of current study were supplied at the amount of standard requirement for broilers according to NRC (1994).

CONCLUSION

The results of present study showed that replacing $FeSO_4$ with FNPs did not affected the growth performance of broiler chickens, but including dietary 6.4 mg/kg FNPs increases the meat Fe and Cu contents and reduce the relative abdominal weight and consequently proposed for enrichment of broiler meat trace elements especially iron for human consumption.

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