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Relative Bioavailability of Different Zinc Sources Based on Tissue Zinc Concentration in Broiler Chickens

Research Article

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ABSTRACT

This research project was conducted to evaluate bioavailability and chemical properties of different zinc sources in broiler chickens when fed by practical diets. For that, 312 day-old male Ross 308 broiler chicks were fed from 8-28 day to estimate the biological availability of four zinc sources. Chicks were allocated randomly to 13 experimental diets in a 3 × 4 factorial design. Diets included an unsupplemented corn-soybean meal basal diet, or the basal diet supplemented with 100, 150 or 200 mg/kg of DM as either zinc sources. Supplementation of basal diets by different zinc levels and sources had no effects on feed intake and body weight gain of chicks during the first and the second weeks of experimental periods, but feed conversion ratio in the first and the second week, feed intake, body weight gain, feed conversion ratio in the third week and the total experimental periods were significant ($P<0.05$) affected by supplementary levels of zinc sources. Tibia and liver zinc content was increased linearly by adding different levels of zinc sources except zinc oxide A in the basal diet ($P<0.05$). The highest zinc concentration in the pancreas was observed only in the basal diet supplemented with 200 mg zinc oxide B per kg of diet in compare to basal diet ($P<0.05$). Using the slope ratio methods from the regression of zinc concentration in the tibia, liver and pancreas on zinc intake from different supplementary zinc levels showed that the highest relative bioavailability value based on tibia bone zinc concentration was estimated when used 150 mg different zinc sources in kg of basal diet, but relative bioavailability value was also estimated based on pancreas zinc content showing a linear increase by increasing different level of zinc sources. In conclusion Bioplex Zn in the same level was more bioavailabe than others.

KEY WORDS bioavailability, broiler chicks, tibia, zinc.

INTRODUCTION

The physiological functions of zinc are numerous. It is required for the functional and structural integrity of more than 300 zinc-dependent enzymes and a great number of functional zinc proteins. Zinc plays role in gene expression, appetite control, fat absorption and antioxidant defense. In all species zinc deprivation is characterized by in appetence, retardation of growth, skeletal or reproductive disorders (Suttle, 2010). The NRC (1994) zinc requirement for broilers is 40 mg/kg, that based upon research conducted more than 40 years ago with animals of markedly different productive potential than those existing today. In addition the requirement was based on a few research reports, most of which were carried out using purified or semi purified diets with growth as the only response criterion (Zeigler *et al*. 1961; Emmert and Baker, 1995).

As genetic improvements continually change the commercial broiler strains and nutritionists start to question if currently used trace mineral levels and sources will be suitable in the future when feeding these fast-growing and highly productive birds for meat and eggs (Nollet *et al.* 2008). Poor bioavailability of dietary zinc, especially from diets rich in fiber and phytate, is a major factor contributing to the high prevalence of nutritional zinc deficiency in poultry farms (Attia *et al.* 2013). Dietary modification to increase intake of components that promote zinc absorption from low-bioavailability meals is an effective strategy for combating nutritional zinc deficiency. Therefore, the attempted researches using organically bounded trace elements in animal nutrition is justified, taking into account not only humans and animals safety but also environment protection. The utilization of organic trace elements in animal nutrition are nowadays increasing and can improve trace elements utilization and decrease environmental pollution (Attia *et al.* 2010a; Attia *et al.* 2010b; Attia *et al.* 2011). The bioavailability of minerals from the diet depends on its content, chemical form, solubility and interactions with other components of ratio (Ammerman *et al.* 1998; Underwood and Suttle, 1999; Attia *et al.* 2013). Also, it was reported that dietary Zn from organic sources was more effective in increasing tissue Zn concentration in broilers than that of inorganic sources (Bao and Choct, 2009).

Zinc is added to broiler diets usually as inorganic feed grade zinc sulfate, zinc chloride, zinc oxide or one of the organic forms complexed to amino acids, proteins or carbohydrates. The nutritional value of mineral sources depends on the concentration in the feed; potential interactions with, for example, calcium; the amount of the element that is absorbed, the bioavailability of the element to the bird (Salim *et al.* 2008) or a combination of these. In recent years, organic zinc sources have been used increasingly due to their potentially higher zinc bioavailability (Kidd *et al.* 1996; Salim *et al.* 2010). However, some studies indicated only small or no differences in bioavailability of Zn between organic and inorganic sources (Pimentel *et al.* 1991; Ammerman *et al.* 1995). It seems that accurate values for the bioavailability of different organic and inorganic zinc sources are important to ensure adequate zinc supplementation of broiler diets. Therefore this work was conducted to determine bioavaiability of different zinc sources such as zinc sulfate, zinc oxide A, zinc oxide B and Bioplex Zn based on tissue zinc concentration as chickens fed practical diet.

MATERIALS AND METHODS

Birds, diets, experimental design and management

This study was conducted in the poultry research farm of Tabriz university (Tabriz, Iran). A total of 312 day-old Ross 308 male broiler chicks were randomly divided into 13 dietary treatments in a 3 × 4 factorial arrangement plus unsupplemented corn-soybean meal basal diet. Each treatment was represented by 6 birds per replicate and 4 replicates per experimental diet. Diets included an unsupplemented corn-soybean meal basal diet, which it was formulated using Zn-free mineral premix, so that contained minimum amount of zinc contained 25.50 mg Zn/kg as fed basis (by analysis), or the basal diet supplemented with 100, 150 or 200 mg/kg of DM as either zinc sources as feed grade zinc sulfate, zinc oxide feed grade A, zinc oxide feed grade B and Bioplex Zn (as organically Zn compounds). The basal corn-soybean meal diet (Table 1) was formulated to meet or exceed nutritional requirements of broiler chicks (NRC, 1994).

Table 1 Composition of the basal diet without added zinc sources (as fed basis)

Ingredients (%)	(8-28 d)
Corn	55.85
Corn starch	0.05
Soybean meal	36.60
Soybean oil	3.60
Di-calcium phosphate	1.50
$CaCO_3$	1.35
Salt	0.25
DL-methionine	0.15
L-lysine	0.10
Vitamin premix[a]	0.25
Zn free mineral premix[b]	0.25
Total	100
Nutrient composition[c]	
Metabolizable energy (kcal/kg)	3000
Crude protein (%)	21.00
Calcium (%)	0.95
Available P (%)	0.44
Lysine (%)	1.19
Methionine (%)	0.49
Fe (mg/kg)	97
Cu (mg/kg)	32
Mn (mg/kg)	69
Zn (mg/kg)	25.50

[a] Supplied per kg of diet: vitamin A: 11025 IU; vitamin D_3: 3528 IU; vitamin E: 33 IU; vitamin K: 0.91 mg; vitamin B_6: 5 mg; vitamin B_{12}: 28 µg; Thiamin: 2 mg; Riboflavin: 8 mg; Niacin: 55 mg; Ca pantothenate: 18 mg; Biotin: 0.221 mg; Folic acid: 1 mg and Choline: 478 mg.

[b] Zinc free mineral premix: provided per kg of diet: Mn (from $MnSO_4 \cdot H_2O$): 60 mg; Fe (from $FeSO_4 \cdot 7H_2O$): 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$): 6 mg; I (from Ca $(IO_3)2 \cdot H_2O$): 1 mg and Se: 0.20 mg.

[c] Diet nutrient composition except ME, lysine and methionine others determined based on chemical analysis.

Each pen had a similar as initial weight and weight distribution. A single batch of basal feed was mixed and divided into 13 aliquots according to the experimental treatments; each Zn source was premixed with corn starch to the same weight and mixed with each aliquot of the basal diet. All of the diets were calculated to contain equal concentrations of methionine, lysine and other nutrients except of Zn. Chicks were housed in the cage pens by covered plastic layer, which placed at thermostatically-controlled room. Chicks were maintained on a 24 h constant lighting regimen and had free access to feed and tap water containing no detectable Zn in all times. Feed and water were provided using plastic instruments to minimize environmental Zn contamination.

Data collection

Body weight, body weight gain, feed conversion ratio and zinc intake were recorded weekly and overall experimental period. At the end of the experiment, 12 birds per group (3 birds per cage) were selected according to average body weight after a 12 h fast, individually weighed, and slaughtered by cervical dislocation. Slaughtering process was done based on the animal welfare guidelines of Iran. The liver, pancreas and right tibia were collected and frozen (-20 ˚C) prior to ash and Zn analyses.

The right tibia was excised and frozen in an individual heat-sealed polyethylene bag for Zn analysis. The samples from 3 individual chicks in each cage were pooled before analysis.

Chemical analysis

Prior to formulating the diets, the ingredients and Zn sources were analyzed for dry matter, crude protein, calcium, phosphorus, iron, copper and manganese content according to standard procedures of AOAC (1995). Zinc concentrations in zinc sources, tibia ash, liver, pancreases, diets and water were determined by atomic absorption spectrophotometry, approximately 0.1 g of Zn sources and 0.5 g of feed, pancreas and liver sample were weighed in triplicate and digested with 10 mL of HNO_3 and 0.4 mL of $HClO_4$ at 200 ˚C in a 50 mL calibrated flask until the solution became clear, and it was evaporated to almost dryness and diluted 1:20 (vol/vol) with 2% HNO_3 before analyses. Before ashing tibia was autoclaved for 20 min and cleaned of soft tissue (Hall *et al.* 2003), then the bones were dried for 12 h at 105 ˚C and finally ashed in a muffle furnace at 550 ˚C for 16 h.

The samples of 3 individual chicks from each cage were pooled before analysis. Approximately 0.2 g of ash sample from each replicate was solubilized in 5 mL of 50% HCl and the mineral extract was filtered into a volumetric flask. The extract was then diluted using deionized water to the required volume and Zn contents were determined by atomic spectrophotometry (Anonymous, 1982).

Statistical analysis

Data were analyzed by GLM procedure of SAS (1999). The model included main effects of Zn source, Zn added levels and their interaction.

The relative Zn bioavailability was estimated by slope ratio techniques from regression of zinc concentration in tibia ash, liver and pancreas on Zn intake with $ZnSO_4.H_2O$ as the standard source at 100% (Littell *et al.* 1997). Because feed intake differences among treatments could affect Zn intake, regressions were calculated using dietary Zn intake (based on Zn assays of diets) as the independent variable rather than added Zn concentrations (Wedekind *et al.* 1992). Regression equations for the $ZnSO_4.H_2O$ standard curves were derived using Minitab 10. Zinc concentration in tibia ash, liver and pancreas was regressed on supplemental Zn intake from $ZnSO_4.H_2O$ and Zn bioavailability in the test sources of zinc were determined using standard-curve methodology. Relative bioavailability estimates were calculated for each of the four replicate pens that were fed each experimental zinc source. Tukey Kramer test was used to separate treatment means at $P<0.05$ significant level.

RESULTS AND DISCUSSION

Evaluation of the chemical characteristics of the different zinc sources are reported in Table 2. Dry matter percentage varied from 90.8 in Bioplex Zn to 100 in zinc oxide B, and the concentrations of zinc in organic and inorganic zinc sources had remarkable differences, with values from 15% in Bioplex Zn to 75% in zinc oxide A. The concentration of other micro- and macro-elements except iron was very low. The effects of source and supplemental zinc level on performance parameters are shown in Table 3 and 4. The zinc sources and theses concentration did not affect feed intake (FI) or weight gain (WG), in the first and the second week and body weight (BW) in 14 d, 21d and 28 d. But feed conversion ratio (FCR) in whole experimental periods, feed intake (FI) or body weight gain (BWG) in the third week and the overall trial periods were significantly ($P<0.05$) affected by zinc source supplementary levels (Table 3 and 4). In the first week of trials, the best FCR was observed in the 100 mg/kg levels of Bioplex Zn, 200 mg/kg levels of zinc oxide FG A, 100, 150 and 200 mg/kg levels of zinc sulfate.

Table 2 Characterization of zinc sources used in the experimental diets

Zinc sources	Color	DM g/kg	Zn mg/kg	Ca g/kg	P g/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg
$ZnSO_4.H_2O$ (FG)	White	98.5	32	2.10	0.01	0.14	50.43	0.62
ZnO (FG A)	White	99.7	75	2.48	0.03	2.14	59.22	1.20
ZnO (FG B)	White	100	72	2.44	0.007	3.07	112.6	0.71
Bioplex Zn[a]	Yellow	90.8	15	1.88	0.017	0.53	7.29	1.53

FG: feed grade

[a] Supplied by Alltech biotechnology.

DM: dry matr.

Table 3 Effects of different zinc sources and levels on growth performance of broilers fed with conventional corn-soybean meal diets

Zinc source	Zinc added level mg/kg	8-14 d			BW 14d	15-21 d			BW 21d
		FI	BWG	FCR		FI	WG	FCR	
Control (basal diet)	0	270.75^{a}	177.83^{a}	1.52^{b}	317.16^{a}	393.99^{a}	204.95^{a}	1.92^{a}	522.11^{a}
	100	253.54^{a}	178.99^{a}	1.41^{a}	314.74^{a}	412.83^{a}	239.12^{a}	1.72^{b}	553.86^{a}
Sulfate FG	150	243.16^{a}	172.99^{a}	1.40^{a}	320.03^{a}	385.66^{a}	218.78^{a}	1.76^{b}	538.82^{a}
	200	251.16^{a}	176.87^{a}	1.42^{a}	329.87^{a}	408.45^{a}	229.33^{a}	1.78^{b}	559.20^{a}
	100	254.79^{a}	176.37^{a}	1.44^{ab}	314.47^{a}	408.35^{a}	229.45^{a}	1.78^{b}	543.93^{a}
Oxide FG A	150	275.33^{a}	192.45^{a}	1.43^{ab}	335.24^{a}	430.24^{a}	237.45^{a}	1.81^{b}	572.69^{a}
	200	256.12^{a}	186.29^{a}	1.38^{a}	321.12^{a}	426.83^{a}	235.12^{a}	1.81^{b}	556.24^{a}
	100	250.58^{a}	173.08^{a}	1.44^{ab}	314.15^{a}	357.79^{a}	201.37^{a}	1.77^{b}	515.52^{a}
Oxide FG B	150	255.79^{a}	175.58^{a}	1.45^{ab}	315.16^{a}	400.45^{a}	230.66^{a}	1.73^{b}	545.82^{a}
	200	264.79^{a}	182.29^{a}	1.45^{ab}	312.57^{a}	420.03^{a}	231.40^{a}	1.81^{b}	543.98^{a}
	100	266.29^{a}	187.25^{a}	1.42^{a}	319.37^{a}	418.99^{a}	232.37^{a}	1.80^{b}	551.74^{a}
Bioplex	150	254.00^{a}	173.08^{a}	1.46^{ab}	279.58^{a}	378.62^{a}	221.87^{a}	1.70^{b}	501.45^{a}
	200	246.16^{a}	170.12	1.44^{ab}	302.08^{a}	381.29^{a}	219.33^{a}	1.74^{b}	521.41^{a}
P-value									
Source		0.38	0.65	0.0.27	0.54	0.46	0.39	0.35	0.53
Level		0.93	0.99	0.75	0.94	0.75	0.92	0.002	0.95
Source × level		0.45	0.44	0.68	0.74	0.31	0.43	0.40	0.75
SE		2.71	2.10	0.007	4.70	21.88	3.35	0.011	7.02

FCR: feed conversion ratio; BW: body weight; FI: average daily feed intake; BWG: body weight gain; SE: standard error.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).

Table 4 Effects of different zinc sources and levels on growth performance of broilers fed with conventional corn-soybean meal diets

Zinc source	Zinc added level mg/kg	22-28 d			8-28 d			BW 28d
		FI	BWG	FCR	FI	BWG	FCR	
Control (basal diet)	0	580.25^{abc}	311.50^{ab}	1.86^{c}	976.24^{ab}	694.28^{a}	1.40^{c}	833.61^{a}
	100	571.74^{abc}	331.70^{abc}	1.72^{ab}	998.08^{ab}	749.82^{ab}	1.33^{a}	885.57^{a}
Sulfate FG	150	526.29^{a}	316.49^{ab}	1.66^{a}	945.32^{a}	708.28^{ab}	1.33^{ab}	855.31^{a}
	200	605.64^{ab}	354.28^{c}	1.70^{ab}	1013.91^{ab}	760.49^{ab}	1.33^{a}	913.49^{a}
	100	585.20^{abc}	343.33^{bc}	1.70^{ab}	1006.48^{ab}	749.15^{ab}	1.34^{ab}	887.26^{a}
Oxide FG A	150	613.45^{c}	354.87^{c}	1.72^{ab}	1060.45^{b}	784.78^{b}	1.35^{ab}	927.56^{a}
	200	562.46^{abc}	323.66^{abc}	1.73^{ab}	1006.62^{ab}	745.07^{ab}	1.35^{ab}	879.90^{a}
	100	589.95^{bc}	342.37^{bc}	1.72^{ab}	950.74^{ab}	716.82^{ab}	1.32^{a}	857.89^{a}
Oxide FG B	150	604.66^{bc}	358.41^{c}	1.68^{a}	1014.66^{ab}	764.66^{ab}	1.32^{a}	904.24^{a}
	200	548.25^{ab}	303.50^{a}	1.80^{bc}	988.32^{ab}	717.36^{ab}	1.37^{bc}	847.48^{a}
	100	560.75^{abc}	333.25^{abc}	1.68^{a}	1018.53^{ab}	752.87^{ab}	1.35^{ab}	884.99^{a}
Bioplex	150	546.12^{ab}	327.75^{abc}	1.66^{a}	960.37^{ab}	772.99^{ab}	1.32^{a}	829.20^{a}
	200	567.75^{abc}	326.58^{abc}	1.73^{ab}	954.03^{ab}	716.03^{ab}	1.33^{a}	847.99^{a}
P-value								
Source		0.36	0.16	0.003	0.043	0.20	0.0002	0.32
Level		0.89	0.19	0.06	0.98	0.018	0.19	0.93
Source × level		0.014	0	0.62	0.24	0.24	0.06	0.41
SE		5.67	3.42	0.011	9.20	6.67	0.004	8.75

FG: feed grade; FCR: feed conversion ratio; BW: body weight; FI: average daily feed intake and BWG: body weight gain.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SE: standard error.

In the second weeks, all of zinc sources in different levels had significant effects on FCR, but the lowest FCR was observed in 150 mg/kg levels of Bioplex Zn in diet. In the third week, the highest feed intakes were assigned to chicks fed on zinc oxide FG B supplemented diets with 150 mg/kg, the least feed intakes were attributed to Bioplex Zn supplemented with 150 mg/kg. The highest body weight gain or BWG were shown in the 200 mg/kg of zinc sulfate, 150 mg/kg of zinc oxide FG A and 100 mg/kg of zinc oxide FG B (P<0.05). FCR in different zinc sources except 200 mg/kg of zinc oxide FG B had significant difference with basal unsupplemented diet (P<0.05).

In the entire experimental periods, the lowest and the highest FI were observed in the 150 mg/kg of zinc sulfate and 150 mg/kg of zinc oxide FG A, respectively (P<0.05). Regardless WG only 150 mg/kg of zinc oxide FG A had significant difference with basal unsupplemented diet (P<0.05). The better FCR was observed in all experimental treatments except 200 mg/kg levels of zinc oxide FG B in comparing with basal unsupplemented diet (P<0.05). In this experiment not interactions effects between zinc sources and added levels of zinc on growth performance were observed (Table 3 and 4). Supplementation of zinc to the basal diet at graded levels had no significant influence on body weight gain and feed intake at 3 wk of age (Table 3 and 4). This was in agreement with the findings of Burrell *et al.* (2004) who reported that a practical diet of maize-soybean meal containing 30 mg/kg of zinc was adequate to support optimum performance during the initial 3 wk of age. Similarly, others (Stahl *et al.* 1986) reported that a basal diet containing 37 mg/kg of zinc was optimum for realizing good growth in chicks and additional supplementation had no further advantage.

In another study with male broiler chicks, little effect was found on body weight, feed efficiency or livability with supplementation of zinc up to 6 wk of age because the basal diet contained 44 mg/kg of zinc. The present study also indicated that the zinc content available in corn soybean diet (25.50 mg/kg) was adequate to sustain growth and other related parameters at par with those supplemented with zinc up to 4 wk of age. Obviously, this level was lower than that recommended by NRC (1994) (40 mg/kg) for broiler chicks. The absence of any difference in performance between groups fed diets with or without supplemental zinc could be due to slower rate of zinc utilization, necessitating no further replenishment in diets (Collins *et al.* 1999).

The lack of increase in feed intake or body weight gain with added zinc in this phase indicates that the amount of the element in the nonsupplemented basal diet was adequate for growth specially until 21 day of life despite of the fact that NRC (1994) suggested that 40 mg/kg as the requirement for broiler chicks. The contribution of zinc from the remaining yolk sac during the 1st week of age and easier permeability of the still developing gastrointestinal tract may have contributed to this observation (Cao *et al.* 2002). Also, the lack of responses in feed intake and weight gain to added zinc levels up to 100 mg/kg it was probably due to increased synthesis of intestinal metallothionein. Zinc intake has been shown to induce intestinal metallothionein synthesis (Sandoval *et al.* 1997). Increased synthesis of this zinc binding protein is associated with reduced zinc absorption. This protein will influence the regulation of zinc absorption and possibly the response of broiler chicks to supplemental levels of zinc from different sources. But the improvements from adding different zinc sources on FCR in week 3 and total experimental periods indicated that the using of these supplements might be suitable in practical diet of broilers in the grower phase.

The average zinc stored by the experimental birds in the body tissues, such as tibia bone, liver and pancreases are presented in Table 5. There was a significant effect of zinc source on ash content of tibia bone (P<0.05). It seems that supplementation basal diet by organic and inorganic zinc sources has led to greater calcium and phosphorus retention in tibia bone and finally bone ash percent increased, but higher levels of zinc due to establish interaction effects by calcium and phosphorus can be adverse effects on mineralization and tibia bone ash (Underwood, 1981). However, the results of present study are match with Sunder *et al.* (2008) but were opposed of other authors (Pimentel *et al.* 1991; Mohanna and Nys, 1999). Zinc storages in tibia bone of birds fed by basal diet was 285 mg/kg of ash and was lower than others (P<0.05). Also, basal diet supplemented with 100, 150 mg zinc sulfate, 100, 200 zinc oxide FG A and 100 mg zinc oxide FG B / kg of diet were statistically lower than others (P<0.05).

Similarly, Sandoval *et al.* (1998) observed a linearly increase in zinc storages of broiler chickens tibia bone, liver or kidney when it was used 0-1500 mg zinc in kg of basal diets.

Therefore, for zinc assay trials in broiler chickens diets, it is essential to use high level of zinc to establish sufficient potential growth (Emmert and Baker, 1995). Pimentel *et al.* (1991) did not report differences in zinc concentrations of broiler chickens tibia bone when fed by semi-purified and corn-soybean meal diets supplemented by 8-88 mg of zinc oxide and zinc-methionine for 49 days. The highest (P<0.05) zinc concentration in the liver was obtained in the birds feeding 200 mg sulphate, oxide FG B, 100 and 200 mg Bioplex Zn/kg of basal diet.

In general, except zinc oxide B, there was a linear increase in liver storage zinc by using 100 to 200 mg of other zinc sources / kg of basal diet. Pancreas zinc concentration only in basal diet supplemented with 200 mg of zinc oxide B / kg of diet was significant (P<0.05) different of others.

These results indicate that pancreatic reserves of zinc are less sensitive to the addition of different zinc to the diet and not appropriate parameter for evaluating the added zinc to the diet of broiler chickens, these findings are however in contrast with other results (Huang *et al.* 2007), but it is consistent with other study results (Pimentel *et al.* 1991; Wedekind *et al.* 1992).

Inconsistency in the results of various studies may be due to the experimental periods, techniques used, species of animals, composition of basal diets and different physiological conditions of animals. The bioavailability value of different zinc sources based on tibia bone zinc storage adding of zinc levels up to 100 mg/kg of basal diets resulted in a linear increase but higher supplementation levels were reduced this value and from the point of view of bioavailability in the same adding level, Bioplex Zn was better than others (Table 6).

A higher relative bioavailability of different Zn sources according to Zn levels in the liver was observed for 150 mg Bioplex, 200 mg zinc oxide B (Table 7).

Table 5 Effects of different zinc sources and levels on tibia ash, tibia zinc, liver zinc and pancreas zinc

Zinc sources	Zinc added level mg/kg	Ash mg/kg	Tibia bone mg/kg	Liver mg/kg	Pancreas mg/kg
Control (basal diet)	0	47.25[a]	285[a]	181[a]	248[a]
Sulfate FG	100	59.48[b]	393[b]	186[a]	355[a]
	150	57.68[b]	502[bf]	214[a]	453[ab]
	200	56.92[b]	785[ceh]	267[b]	445[ab]
Oxide FG A	100	55.83[b]	349[b]	215[a]	342[ab]
	150	60.01[b]	638[dh]	213[a]	346[ab]
	200	58.96[b]	461[b]	200[a]	340[ab]
Oxide FG B	100	56.04[b]	460[b]	177[a]	326[a]
	150	57.61[b]	773[eh]	168[a]	328[a]
	200	60.16[b]	789[eh]	278[b]	507[b]
Bioplex Zn	100	57.96[b]	616[df]	290[b]	392[ab]
	150	59.91[b]	664[deh]	196[a]	353[a]
	200	60.42[b]	763[h]	293[b]	439[ab]
P-value					
Source		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
Level		0.284	≤ 0.001	≤ 0.001	≤ 0.001
Source × level		0.407	≤ 0.001	≤ 0.001	≤ 0.001
SE		1.66	25.37	8.42	34.78

FG: feed grade.

The means within the same column with at least one common letter, do not have significant difference (P<0.05).

SE: standard error.

Table 6 Estimated relative Zn bioavailability values of tibia zinc concentration (mg/kg) on dietary zinc sources intake (mg/21d) (Zn from $ZnSO_4$ H_2O set at 100%)

Zinc sources	Zinc added level mg/kg	Regression equation	Relative Bioavailability (%)	R^2
Sulfate	100	Y= 153 + 6.54 X	100	96.2
	150	Y= 121 + 4.64 X	100	98.8
	200	Y= 2 + 8.45 X	100	98.4
Oxide A	100	Y= 149 + 3.09 X	47.24	98
	150	Y= 206 + 3.35 X	72.19	89.5
	200	Y= 1 + 2.89 X	34.20	96
Oxide B	100	Y= 309 + 2.37 X	36.23	71.6
	150	Y= 150 + 4.97 X	107.11	81.4
	200	Y= 527 + 2.04 X	24.14	94.8
Bioplex Zn	100	Y= 620 + 2.94 X	44.95	71.8
	150	Y= 167 + 10.7 X	239.91	82.7
	200	Y= 392 + 8.32 X	98.46	52

[a] Data are means of four pens of six male chicks fed the experimental diets for 21d (d 8 to d 28 post hatching).

[b] Standard curve for tibia zinc concentration (Y, in mg/kg) regressed on supplemental Zn intake (X, in mg/21 d) from different zinc sources.

[c] Relative bioavailability (RBV) was calculated from the standard curve regressions, setting RBV of Zn in the $ZnSO_4.H_2O$ standard at 100%.

Table 7 Estimated relative Zn bioavailability values of liver zinc concentration (mg/kg) on dietary zinc sources intake (mg/21d) (Zn from $ZnSO_4$ H_2O set at 100%)

Zinc sources	Zinc added level mg/kg	Regression equation	Relative bioavailability (%)	R^2
Sulfate	100	Y= 11.4 + 2.92X	100	98.9
	150	Y= 12.4 + 2.12X	100	96.8
	200	Y= 33.8 + 2.25X	100	92
Oxide A	100	Y= 5 + 1.9X	65	76.5
	150	Y= 13 + 1.25X	58.96	62
	200	Y= 4.7 + 1.01X	44.88	82.3
Oxide B	100	Y= 36 + 1.33X	45.54	45
	150	Y= 16.8 + 1.04X	49	97
	200	Y= -346 + 3.52X	156.44	81
Bioplex Zn	100	Y= 167 + 2.52X	132.63	74.6
	150	Y= 2.9 + 3.78X	182.54	98.5
	200	Y= 153 + 2.48X	110.22	97.8

[a] Data are means of four pens of six male chicks fed the experimental diets for 21d (d 8 to d 28 post hatching).

[b] Standard curve for liver zinc concentration (Y, in mg/kg) regressed on supplemental Zn intake (X, in mg/21 d) from different zinc sources.

[c] Relative bioavailability (RBV) was calculated from the standard curve regressions, setting RBV of Zn in the $ZnSO_4.H_2O$ standard at 100%.

Oxide A had a lower average percentage of relative bioavailability than the other supplements and its highest value was reached at 100 mg/kg diet. Also, for the relative bioavailability estimated from pancreas level of Zn, the highest value was recorded for Bioplex, in this case at 200 mg/kg diet. Also, for the other Zn supplements, the best value of bioavailability was recorded for 200 mg/kg of integration (Table 8).

None linear response, in relative bioavailability of different level of zinc sources, to tissue storages may be related to presence effective homeostatic mechanisms on zinc absorption in the gut (Weigand and Kirchgessner, 1980). Metallothionein is zinc binding proteins involved in the regulation of zinc homeostasis, detoxification of some heavy metals and short-lived storage of zinc for metabolic processes in the body (Karin, 1985). Increasing zinc intake has been shown to leads to intestinal metallothionein synthesis. In present study, chicks feeding by high level of zinc in dietary might be have high amounts of intestinal metallothionein production, which are associated with reduced zinc absorption, that result in tissue zinc storage decline, which is consistent with the results of Sandoval *et. al.* (1997). According to Kratzer and Vohra (1986) suggestions complexes and chelates have the ability to compete with phytic acid for its Zn-binding capacity. The zinc within organic Zn chelates forms a soluble complex with zinc and it is therefore unavailable to bind with the phytate, but is available to the animal. The zinc contained in inorganic sources is not as strongly associated and thus can be bound to phytic acid, which makes it less available to the animal. One of the possible reasons might explain the Bioplex Zn higher bioavailability than others. Also nutritional study done by Leeson (2003) showed that biological value of proteinated minerals complex in the diets of broiler chickens at least 30% is more than inorganic sources.

It seems that the main differences in zinc bioavailability among different zinc sources such as zinc-methionine, zinc-lysine and zinc oxide can be related to differences in absorption and net utilization. These differences in utilization may be due to differences in endogenous zinc loss, which has been shown to increase due to homeostatic mechanisms with increasing zinc absorption (Weigand and Kirchgessner, 1980). In monogastric animal's nutrition, it is difficult to avoid the presence of phytates as they are the main storage forms of phosphorus in seeds (Attia *et al.* 2010a; Attia *et al.* 2010b; Attia *et al.* 2011; Attia *et al.* 2013). Diets based on corn and soybean meal generally contain between 2.0 and 2.5 g phytic P / kg. Zinc content in feed components from plant origin is positively correlated to the phytic P content, with nearly 10 mg of Zn to 1 g phytic P (Revy *et al.* 2003). It is proposed that zinc from organic sources is protected by the ligand from reacting with feed antagonists, such as phytate to form insoluble complexes.

Zinc from organic sources is absorbed by the intestinal cells as an ion. Also, a review of Zn bio-availability data (Wedekind *et al.* 1992; Ledoux, 2005) indicates that in most studies, organic mineral sources were at least as available as the standard inorganic sources, and in some cases were more available (Attia *et al.* 2010a; Attia *et al.* 2010b; Attia *et al.* 2011; Attia *et al.* 2013). One of the hypothesized reasons for increased bioavailability of organic minerals is that this form is protected form unwanted interactions in the gastrointestinal tract. Conversely, some reports have shown no influences of complexing with an organic ligand (protein, methionine or lysine) on mineral (Zn and Mn) bioavailability (Pimentel *et. al.* 1991; Aoyagi and Baker, 1993). The discrepancy of the results in different experiments might relate to the difference in the age of the chicks when feeding high Zn commenced, duration of feeding, dietary Zn concentrations,

Table 8 Estimated relative Zn bioavailability values of pancreas zinc concentration (mg/kg) on dietary zinc sources intake (mg/21d) (Zn from $ZnSO_4$ H_2O set at 100%)

Zinc sources	Zinc added level mg/kg	Regression equation	Relative bioavailability (%)	R^2
	100	Y= 71.3 + 5.57 X	100	94
Sulfate	150	Y= 59.4 + 3.68 X	100	92.6
	200	Y= 297 + 1.42 X	100	94.2
	100	Y= 25.7 + 2.87 X	51.52	99.6
Oxide A	150	Y= 128 + 1.37 X	37.22	86.4
	200	Y= 6.2 + 1.72 X	121.12	95.7
	100	Y= 165 +1.52 X	27.28	82.9
Oxide B	150	Y= 91.8 + 1.63 X	44.29	97.2
	200	Y= 150 + 2.01 X	141.54	70.6
	100	Y= 267 +2.58 X	46.31	72.5
Bioplex Zn	150	Y= 29.3 + 6.35 X	149.77	98.5
	200	Y=263 + 3.58 X	252.11	78

[a] Data are means of four pens of six male chicks fed the experimental diets for 21d (d 8 to d 28 post hatching).

[b] Standard curve for pancreas zinc concentration (Y, in mg/kg) regressed on supplemental Zn intake (X, in mg/21 d) from different zinc sources.

[c] Relative bioavailability (RBV) was calculated from the standard curve regressions, setting RBV of Zn in the $ZnSO_4.H_2O$ standard at 100%.

differences in criteria chosen, differing type of chicks and previous body stores.

CONCLUSION

In conclusion, Bioplex Zn in the same level was more bioavailable than others. Tissues zinc concentration in chicks fed diets supplemented by zinc seem to be a useful criterion for estimating bioavailability from inorganic or organic zinc sources for chicks under practical conditions. Bioavailability estimation in broiler chickens fed in conventional feeding practices can be available for solving numerous problems associated with purified and semi purified diets, such as feed and sample contamination and low palatability of diets. Also, the results obtained from this method are usefulness and applicable for feed formulation in the commercial conditions.

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