IJAS Iranian Journal of Applied Animal Science

The Investigation on Interaction between Two Hepatic Enzymes and some Minerals in Broiler Chickens

Research Article

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Received on: 26 Sep 2013
Revised on: 15 Feb 2014
Accepted on: 15 Mar 2014
Online Published on: Dec 2014

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Online version is available on: www.ijas.ir

ABSTRACT

The objectives of this study were to determine the interactions between two hepatic enzymes and some minerals in the liver of broiler chickens. The study was performed with male and female from 1 to 56 days of age of broiler chickens. Malic acid was added to the water and offered to chickens freely from the start to the end of the experiment with constant concentration. The treatments consisted on zero (as a control), 0.05, 0.10 and 0.15% of malic acid which dissolved in water and given to them in waterer pan. The chicks were slaughter on 56 days old and liver enzymes including malate dehydrogenase (MDH) and isocitrate dehydrogenase (IDH) were measured on liver extract along with some mineral in dried liver. No significant difference ($P>0.05$) was observed between treatments for weight gain. Liver MDH activity did not show any significant difference, but IDH activity was increased ($P<0.05$) by malic acid consumption. Male chicks showed a 28% higher IDH-NADP activity in their liver compared to female chicks ($P<0.05$). Zinc and iron showed significant correlation with MDH and IDH, respectively. No significant negative correlation ($P>0.05$) was observed among mineral concentration in the chick liver and selenium concentration in the liver. However, it was found a significant positive correlation with concentration of lead, magnesium, nickel, manganese, mercury and cobalt in the chicken liver ($P<0.05$). In conclusion, malic acid administration to the water of chickens resulted in a linear accumulation of iron into the liver of them, but there was not found other mineral accumulation in this organ.

KEY WORDS broiler, enzyme interaction, IDH, MDH, mineral.

INTRODUCTION

In avian species, the liver is the main site for the de novo synthesis of fatty acids (Goodridge and Ball, 1967; Goodridge, 1968; Leveille *et al.* 1968). Malic acid plays an important role in generating mitochondrial ATP both under aerobic (Cheeseman and Clark, 1988) and hypoxic (Wiesner *et al.* 1988; Hoehl *et al.* 1987) conditions. Most of the NADPH necessary for the synthesis of fatty acids in birds is believed to be derived from the activity of malate dehydrogenase (MDH) enzyme (Tanaka *et al.* 1983) because the hepatic monophosphate-shunt dehydrogenases appear not to play an important role in chick hepatic lipogenesis (Romsos and Leveille, 1974).

Isocitrate dehydrogenase (IDH) is an oxidoreductase that catalyzes an oxidation reduction in which isocitrate is converted to alpha-ketoglutarate and CO_2. It is the first of four oxidative steps within the TCA cycle and is the key rate-limiting step of the TCA cycle. On the other hand, activator or inhibitory role of some mineral on enzyme activity has been reported by researchers. For example, IDH has an absolute requirement for divalent metal ions, using Mn^{++} about 1.5 times as efficiently as Mg^{++} but cannot use Cd, Ca, Cu, Ni, Hg, Sr or Zn ions (Gordon *et al.* 2000). In this

manner, several iodinated agents, iodine, cyanide and molecular iodine also inactivate the MDH by oxidizing the -SH groups (Varrone *et al.* 1970). While traditional reductionism approaches have revealed many aspects of mineral metabolism and function, significant gaps still exist. For example, we don't know how mineral interactions influence the absorption, excretion, storage and utilization of chemically similar elements (Fleet and Salt, 2009). Environmental factors such as water, equipment and / or soil conditions for crops may also contribute to a bird's exposure to excessive trace minerals. The importance of considering mineral availability in the poultry metabolism and enzyme function that is related to animal performance was illustrated by many researchers such as Engle *et al.* (1997). Therefore, the current study was conducted to determine the interaction between two hepatic enzyme activities and some mineral concentration in the liver of broiler chickens.

MATERIALS AND METHODS

Animals and treatments

Two hundred and fifty 1-d-old commercial broiler chickens (Ross) were housed in floor pens containing litter composed of wood shaving and received a corn-based starter diet. Four or five days after hatching, the chicks were sorted and those with extreme weights discarded. After sorting, the chicks were randomly assigned to 16 pens each consisting of 12 birds (both sex) means four pens for each treatment. The room temperature was gradually decreased from 32 ˚C at d1 to 24 ˚C at d 22. The chicks were fed with three type diets consisted starter, grower and finisher (Table 1).

Table 1 Composition of the experimental diets (g/kg)

Ingredients and analysis	Experimental period		
	Starter	Grower	Finisher
Ground yellow corn	618	485	615
Soybean meal (44% CP)	280	330	190
Fish meal	49.5	27	20
Plant oil	19	19	25
Wheat bran	-	84	95
Dicalcium phosphate	12	28	28
Oyster shell	13	10	10
Sodium chloride	1	4.5	4.5
DL-methionine	0.5	0.25	0.25
Vitamin / mineral premix[1]	7	10	10
Chemical composition (calculated)[2]			
ME kcal/kg	3002	2721	2890
Crude protein (%)	20.8	21.9	16.6
Methionine (%)	0.44	0.6	0.53
Lysine (%)	1.21	1.26	0.86
Methionine + cysteine (%)	0.75	0.94	0.79

[1] The premix supplied the following (mg/kg diet): Retinol 3.6; Cholecalciferol 0.075; Biotin 1; DL-α-tocopherylacetate 10; Riboflavin 10; Pantothenate 20; Choline 2000; Niacin 100; Thiamine 10; Pyridoxine 10; Menadin sodium bisulphate 1.5; Cyanocobalamin 0.1; Folic acid 2; Ethoxyquin 150; Mn 100; Fe 100; Cu 10; Co 1; I 1 and Zn 100.
[2] Calculated from NRC (1994) composition tables.
ME: metabolisable energy.

The light was continuous during the experiment. The corn-based diet was formulated according to the nutritional requirements for chickens (NRC, 1994). Diet was fed in mesh form and contained no growth factors, coccidiostats, exogenous enzymes or antibiotics. Malic acid was added to the water and offered to chicken freely from the start to the end of the experiment with constant concentration through the entire experiment. The treatments were zero (as a control), 0.05, 0.10 and 0.15% of malic acid which dissolved in water and given to them in waterer pan. Feed and water were supplied *ad libitum* throughout the entire experiment.

Sample collection and preparation

Feed samples in each period dried in oven at 70 ˚C, grinded and passed through 0.9 mm mesh sieve and stored in air locked bag and kept in dry and cool place.

Two birds (one male and another female which phenotypically selected) from each pen were killed at 06.00 hours on d 56, by cutting the carotid artery. Livers were excised rapidly, washed with 155 m*M* NaCl to remove exterior blood and debris and then divided into two parts. One part of the liver was dried in oven at 70 ˚C, grinded and passed through 0.9 mm mesh sieve, then stored in air locked bag and finally kept in a dry and cool place. Another portion of the liver was immediately chilled and homogenized (1, 10 wt/vol) in 100 m*M* HEPES (pH 7.5), 3.3 m*M* ß-mercaptoethanol and centrifuged at 14000 × g for 30 min (Rosebrough *et al.* 1999). The supernatant fractions were kept in liquid nitrogen (-186 ˚C) and analyzed within three days for enzyme activity.

Mineral determination

Mineral concentrations in feed and liver samples were determined by inductively coupled argon plasma mass spectrometry (ICP-MS). Briefly, approximately 1 g of dried samples (liver, feed or water) were weight accurately into a 150 mL capacity flask and added 5 mL HNO_3 (Suprapure, 65%, MerckR, Germany) and then digested at 120 ˚C. During digestion a 2 mL H_2O_2 (Suprapure, 35%, Merck R, Germany) was added to each flask to enhance dissolution of organic matter. After completing the digestion, the flask content filtered through acid washed filter paper in 100 mL capacity flask using deionized water. At the end, digested material transferred into polyethylene bottles and kept in a dust prof chamber until analyzed (AOAC, 2000).

Enzyme assays

The activities of malate, nicotinamide adenine dinucleotide phosphate oxidoreductase-[decarboxylating] (MDH) and isocitrate, nicotinamide adenine dinucleotide phosphate oxidoreductase [decarboxylating] (IDH) were estimated. The activity of MDH was determined by the method of Rosebrough *et al.* (1999). Reactions contained 50 m*M*

HEPES (pH 7.5), 1 m*M* NADP, 10 m*M* $MgCl_2$ and the substrate, 2.2 m*M* L-malate (disodium salt) in a total volume of 1 mL. Portions (50 µL) of the 14000 × g supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 ˚C. The activity of IDH activity was determined by the method of Rosebrough *et al.* (1999). Reactions contained 50 m*M* HEPES (pH 7.5), 1 m*M* NADP, 10 m*M* $MgCl_2$ and the substrate, 4.4 m*M* DL-isocitrate in a total volume of 1 mL. Portions (50 µL) of the 14000 × g supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 ˚C. The amount of activity was corrected for the amount of protein in the sample. Enzyme activities are expressed in unit of nanomoles of NADP reduced per minute per mg protein in the extract, under the assay conditions.

Chemical measurements

The determination of nitrogen in the liver was performed with the macro-kjeldahl method. The glycogen content in the liver samples was measured as described by Djawdan *et al.* (1998). Protein concentration in the liver extract sample was determined using the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Statistical analysis

The complete randomised model was used to analyse data. The experimental design was a completely randomized one with a 4 × 2 factorial arrangement of treatments. Each of the four treatments was replicated four times per sex (n=4). The data were analysed using general linear model procedure of SAS (2009). Duncan's multiple range test (SAS, 2009) (P<0.05) was used to test the significance of difference between means. A correlation among parameters was determined and correlation coefficients were tested using a *t*-test (SAS, 2009). Values are given as means SEM and the homogeneity of variance was checked.

RESULTS AND DISCUSSION

The mineral concentration in three type diets consisted starter, grower and finisher along with water consumption has been shown in Table 2. Table 3 summarizes the effects of different levels of malic acid on final live weight at 56-day old and liver composition of chickens. Supplemental malic acid in different levels had no effect (P>0.05) on final body weight and liver percentage, but liver composition has been affected by malic acid supplementation (P<0.05). Highest dry matter content along with lower protein content in the liver occurred in birds on control treatment (P<0.05). The lowest (P<0.05) dry matter and glycogen content and the highest (P<0.05) protein content in the liver have been shown in birds on 0.15% malate concentration. Effect of sex, only was observed on body weights (P=0.0110) and protein concentration (P=0.0336). Supplemental of malic acid in different levels did not show any significant effect on mineral concentration in the liver dry matter content with the exception of the iron (P>0.05). Significant difference (P<0.05) between the two sexes was observed for iron concentration in the liver. Female chicks showed 37% higher iron concentration in their liver compared to male chicks (P<0.05). Interaction of sex in treatment was not significant (P>0.05) for all above parameters.

Table 2 Feed (dry weight basis) and water mineral composition

Ingredients and analysis	Experimental diets			
	Starter	Grower	Finisher	Water
	µg/g			
Pb	0.107	0.248	0.412	0.010
Mg	630	580	624	14
Fe	74	83	88	0.25
Zn	54	58	67	1.0
Se	0.37	0.39	0.44	0.02
Ni	18	22	20	12
Mn	74	63	59	0.04
Hg	0.137	0.545	0.471	0.120
Co	0.44	0.57	0.63	0.029

Pb: lead; Mg: magnesium; Fe: iron; Zn: zinc; Se: selenium; Ni: nickel; Mn: manganese; Hg: mercury and Co: cobalt.

The incorporation of malic acid in the water significantly affected (Table 4) IDH activity (P<0.05) and had no effect (P>0.05) on MDH activity. However, at the highest dosage of malic acid supplementation (0.15%), a decrease in IDH activity was shown. It means that no significant difference (P>0.05) was detected between control group and the highest malic acid concentration was found for IDH activity. There was found a significant effect (P=0.0015) of sex on liver IDH activity and a significant interaction (P=0.0017) between sex and treatment was also observed. Male chicks showed 28% higher IDH activity in their liver compared to female chicks (P<0.05). However, male chicks showed different pattern response to malic acid levels compared to female chicks. The male chicks at the level of 0.05% of malic acid administration showed the highest activity for IDH activity and then with increasing another five percent of malic acid they showed a reduction in IDH activity and then remain constant in chicks at the level of 0.15% malic acid treatment.

In the female chicks, IDH activity increased along with increasing of malic acid administration till to 0.10% and then it was reduced sharply in chicks on 0.15% malic acid treatment.

Table 3 Liver composition in birds (n=32; 16 male and 16 female)

Composition	Malic acid concentration (%)				SEM	Sex		
	0	0.05	0.10	0.15		Male	Female	P-value
Body weight[1] (g)	2600	2530	2525	2470	54.4	2606	2456	0.0110
Liver (%)	1.89	1.95	1.81	1.89	.080	1.89	1.88	0.8965
Dry matter (%)	29.0^{a}	28.6^{ab}	28.2^{ab}	27.5^{b}	0.42	27.9	28.7	0.0936
Glycogen (%)	11.2^{ab}	12.5^{a}	10.0^{ab}	8.4^{b}	1.29	9.9	11.2	0.1645
Crude protein (%)	65.8^{b}	65.8^{b}	70.3^{ab}	73.1^{a}	1.68	66.9	70.6	0.0336
Liver mineral composition (µg/g DM)								
Pb	0.962	1.763	0.613	1.360	0.468	1.505	0.844	0.1703
Mg	73.61	73.95	86.22	82.93	6.402	75.90	82.45	0.3165
Fe	507.9^{b}	475.6^{b}	675.8^{ab}	910.7^{a}	89.98	541.1	743.8	0.0337
Zn	92.11	104.5	136.5	145.6	23.04	111.0	128.4	0.4577
Se	303.9	388.5	365.6	344.5	38.89	356.6	344.7	0.7630
Ni	0.038	0.100	Ud	0.063	0.055	0.063	0.038	0.6547
Mn	10.90	11.48	13.32	12.17	1.289	12.28	11.65	0.6320
Hg	19.18	58.49	35.85	13.53	35.84	24.69	38.83	0.6966
Co	0.988	0.763	0.804	0.712	0.270	0.881	0.752	0.6375

[1] Final body weight at 56 d old broiler chicken.
The means within the same row with at least one common letter, do not have significant difference (P<0.05).
Pb: lead; Mg: magnesium; Fe: iron; Zn: zinc; Se: selenium; Ni: nickel; Mn: manganese; Hg: mercury and Co: cobalt.
Ud: undetectable.
SEM: standard error of means.

Table 4 Specific activities of hepatic enzyme for chicken given malic acid in different concentration (n=32; 16 male and 16 female)

Enzyme	Malic acid concentration (%)				SEM	Sex		
	0	0.05	0.10	0.15		Male	Female	P-value
MDH[1]	130.8	141.7	139.4	130.5	8.37	137.9	133.3	0.5924
IDH[2]	128.4^{b}	153.3^{ab}	181.0^{a}	127.0^{b}	10.16	165.6	129.2	0.0015

MDH: malate dehydrogenase and IDH: isocitrate dehydrogenase.
[1, 2] Enzyme activity is noted nanomoles NADP reduced / min per mg protein in the extract.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of means.

The data from all treatments were pooled for each parameter and correlation coefficients with levels of significant probability related to these data are also shown in Table 5. With the exception of the positive correlation coefficient between MDH with zinc concentration, no significant correlation was observed between MDH activity and the all other parameters (P>0.05).

In this manner, with the exception of the negative correlation coefficient between IDH with iron concentration (P=0.053), no significant correlation was observed between IDH activity and the all other parameters (P>0.05). However, between MDH and IDH activities no significant correlation was observed (P=0.471), which cannot explain the close relationship between two enzymes. Zinc and iron showed either significant correlation with MDH and IDH, respectively or positive correlation together (r=0.562; P=0.0008). Selenium showed a positive significant correlation with all the other minerals with the exception of iron and zinc concentrations. Magnesium also showed a positive significant correlation with four (Fe, Zn, Se and Mn) out of seven others minerals in the liver of chicks (Table 5). Mercury as a toxic mineral showed a significant correlation with nickel (r=0.688; P<0.0001).

Trace minerals function primarily as catalysts in enzyme systems within cells. Deficiencies and / or imbalances of trace minerals can alter the activities of certain enzymes and functions of specific organs thus impairing specific metabolic pathways as well as overall immune response (Spears, 2000).

Chicken feed in the present study was the combination of foodstuff and pre-mixers (Table 1). Administration of malic acids may be attributed to the lowering of gastro intestinal tract pH by using this acid, which increases the absorption of minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus due to provision of organic acids was approved by Boling Frankenbach *et al.* (2001). In this respect, Edwards and Baker (1999) found that the acidic anion has been shown to complex with Ca, P, Mg and Zn, which results in an improved digestibility of these minerals. However, the use of higher contents of heavy metals are introduced for getting maximum weight within six to seven weeks (Khan and Meijer, 2005; Abdullah *et al.* 2010; Rehman *et al.* 2012). It may cause the health hazardous effects on humans taking broilers as meat (Khan and Meijer, 2005). Rehman *et al.* (2012) showed that the trend of heavy metals accumulation, mostly occur in metabolic organs such as liver. These heavy metals perform many functions in body such as alter the activities of certain enzymes in the liver. In this regard, the use of organic acid treatments in broiler chickens may enhance higher bioavail-

ability of minerals due to better ionization of the elements in the alimentary tract. Skinner *et al.* (1991) reported that intestinal pH was reduced by level of organic acid mix. In the present study, iron accumulation in the liver of chickens showed linearity increase due to malic acid concentration increase. But this trend for iron accumulation was not showed in other minerals. Iron must be in ferrous form to be absorbed and the malic acid as an acidifier converts ferric iron to ferrous iron. Iron concentration in the female liver showed 37% higher concentration than liver in male chicks. It may refer to higher requirements of iron in female for egg production in the nature. The body of female chicks has been adapted to provide iron for egg production by a higher iron deposition in soft tissues and metabolic organs such as liver. Because, the iron content of the egg is relatively high (1.9 mg per egg of 57 g), and consequently the requirement of the laying hen is large compared with the requirement for maintenance (Mc Donald *et al.* 2010) or male. Administration of malic acid in the level of 0.15% showed no effect on IDH activities compared to the lower dosages (0.05% and 0.10%).

The reason for this is not clear but in the metabolic control theory there are some assumptions that might have to be met. One possibility is diminishing returns of enzyme activity meaning that the rate at which a substrate is processed can no longer be approximated as a linear function of substrate concentration at higher substrate concentrations. This is because collisions between substrate molecules and enzymes ready to accept substrate no longer occur with a likelihood approaching. Another eventuality is under such conditions where pH or salt concentrations differ from those in which the enzyme function evolved. Enzyme activity, minimally, can be expected to reversibly decline. These are the same things that are known to disrupt protein structure (denaturation). However, it requires special attention to characterize the enzyme activity patterns of birds under different substrate concentration.

The values for MDH activity were in agreement with the findings of Balnave (1975) who has worked on laying hens, but the values of the IDH activity which were reported by him are about two times more than whatever funded in the present study. Reduction in IDH activity has been observed, leading to an accumulation of citric acid, which is a positive effector of acetyl Co A carboxylase, key enzyme in fatty acid biosynthesis (Dousset *et al.* 1987).

In this regard, Rikans *et al.* (1991) reported that cytosolic superoxide dismutase and glutathione peroxidase activities in rat liver displayed sex-dependent variations in activity but were unaffected by aging. The present study has been showed that the IDH activity is partly controlled by sex characteristics.

MDH is found in all eukaryotic cells and zinc ion is a stimulatory element for this enzyme activity (Blonde *et al.* 1967). In the present study, the coefficient of determination (r^2) showed about 12.7% cooperative action between MDH activity and zinc concentration. However, Yamaguchi *et al.* (1982) reported that administration of zinc did not cause significant changes in malate dehydrogenase activity.

Table 5 Correlation coefficient among some of enzymes and minerals (n=32; 16 male and 16 female)

Item	MDH	IDH	Pb	Mg	Fe	Zn	Se	Ni	Mn	Hg	Co
MDH	1.0000[a]	0.1320	0.0581	-0.0724	0.2520	0.3570	-0.1040	-0.0108	-0.0665	0.0816	-0.0739
	(0.0000[b])	0.471	0.752	0.694	0.165	0.045	0.570	0.953	0.718	0.657	0.692
IDH	-	1.0000	0.0375	-0.1720	-0.3330	-0.2940	0.2200	-0.0726	0.0867	0.2130	0.2430
		(0.0000)	0.839	0.346	0.053	0.102	0.227	0.693	0.637	0.243	0.180
Pb	-	-	1.0000	0.1390	-0.0059	0.1720	0.4260	0.0305	0.4220	-0.0280	0.2890
			(0.0000)	0.449	0.974	0.348	0.015	0.869	0.016	0.879	0.108
Mg	-	-	-	1.0000	0.4990	0.3940	0.3530	-0.0113	0.5810	-0.0627	0.0957
				(0.0000)	0.004	0.025	0.047	0.951	0.0005	0.733	0.602
Fe	-	-	-	-	1.0000	0.5620	0.0611	-0.0457	0.2960	0.0179	-0.0305
					(0.0000)	0.0008	0.740	0.804	0.100	0.922	0.868
Zn	-	-	-	-	-	1.0000	0.0687	0.1090	0.1840	-0.0963	-0.0550
						(0.0000)	0.709	0.554	0.315	0.600	0.765
Se	-	-	-	-	-	-	1.0000	0.6060	0.3960	0.5400	0.3710
							(0.0000)	0.0002	0.025	0.001	0.037
Ni	-	-	-	-	-	-	-	1.0000	-0.0837	0.6880	0.1380
								(0.0000)	0.649	0.000	0.453
Mn	-	-	-	-	-	-	-	-	1.0000	-0.1730	0.3290
									(0.0000)	0.343	0.066
Hg	-	-	-	-	-	-	-	-	-	1.0000	0.0605
										(0.0000)	0.742

MDH: liver malate dehydrogenase-NADP activity; IDH: liver isocitrate dehydrogenase-NADP activity; Pb: lead; Mg: magnesium; Fe: iron; Zn: zinc; Se: selenium; Ni: nickel; Mn: manganese; Hg: mercury and Co: cobalt.

[a] Coefficient of correlation.

[b] Level of probability.

Negative correlation between IDH activity and iron concentration may explain the inhibition of this enzyme by various metal ions such as iron. Such inhibition has been reported in bacteria (Murakami *et al.* 2006; Ogawa *et al.* 2007) and in porcine heart (Murakami *et al.* 1997). In this manner, citrate production in roots of Fe-deficient plants has been proposed to be the source of reducing equivalents through an increase in IDH (Bienfait, 1996). In the present study, the coefficient of determination (r^2) showed about 11.1% cooperative action between IDH activity and iron concentration in the liver.

The interaction of minerals with each other is an important factor in animal nutrition, and an imbalance of mineral elements as distinct from a simple deficiency is important in the etiology of certain nutritional disorders of farm animals (McDonald *et al.* 2010). The liver is known to deposit many trace elements and it is expected that the quantity of the elements in the liver will depend on the chicken's nutrition (Ekmekci *et al.* 2003).

Selenium as a trace mineral after absorption is cleared by the liver and then transported to peripheral tissues by a specific transporter, selenoprotein P. In this way, selenium is distributed to all organs, with the highest concentrations occurring in kidney, liver, spleen and skeletal muscle (Brown and Burk, 1973; Thomassen and Aaseth, 1986). Selenium also protects the body from heavy metals by forming complexes to render them harmless (Nix, 2002). Because of the positive interactions that occur between various minerals such as manganese and magnesium, selenium and mercury, manganese and zinc, selenium and manganese, excessive concentrations of one element may result in an extravagancy in the amount available to the bird of some other elements (NRC, 1994).

However, mineral to mineral interactions such as Fe and Zn are affected by source, species of the element, dietary concentrations and which element is in excess (Hill and Link, 2009).

CONCLUSION

Trace mineral nutrition has a rich history of discovery and research in the field of poultry nutrition. Malic acid administration to the water of chickens resulted in a linear accumulation of iron into the liver of them, but had no effect on other minerals in this organ. Female chicks showed 37% higher iron concentration in their liver compared to male chicks, but no interaction of sex in treatment was observed. In this way, iron concentration in the liver showed negative significant correlation with IDH activity and zinc concentration in the liver showed a positive significant correlation with MDH activity. Selenium showed a positive significant correlation with all the other minerals with the exception of iron and zinc.

ACKNOWLEDGMENTS

I am grateful to all the staff from the Section of Research Department in the Shahrekord University.

REFERENCES

Abdullah N., Osman A.K. and Salaman K.A. (2010). Monitoring of aflatoxins and heavy metals in some poultry feeds. *AJFS.* **4,** 192-199.

AOAC. (2000). Official Methods of Analysis. 17th Ed. Association of Official Agricultural Chemists International. Gaithersburg, MD, USA.

Balnave D. (1975). The influence of essential fatty acids and food restriction on the specific activities of hepatic lipogenic and glutamate-metabolizing enzymes in the lying hen. *Br. J. Nutr.* **33,** 439-445.

Bienfait H.F. (1996). Is there a metabolic link between H+ excretion and ferric reduction by roots of Fe-deficient plants – A viewpoint? *J. Plant. Nutr.* **19,** 1211-1222.

Blonde D., Kresack E. and Kosicki G. (1967). The effects of ions and freeze-thawing on the supernatant and mitochondrial malate dehydrogenase. *Can. J. Biochem.* **45,** 641-646.

Boling-Frankenbach S.D., Snow J.L., Parsons C.M. and Baker D.H. (2001). The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. *Poult. Sci.* **80,** 783-788.

Brown D.G. and Burk R.F. (1973). Selenium retention in tissues and sperm of rats fed a torula yeast diet. *J. Nutr.* **103,** 102-108.

Cheeseman A.J. and Clark J.B. (1988). Influence of the malate-aspartate shuttle on oxidative metabolism is a synaptosomes. *J. Neurochem.* **50,** 1559-1565.

Djawdan M., Chippindale A.K., Rose M.R. and Bradley T.J. (1998). Metabolic reserves and evolved stress resistance in *Derosophila melanogaster*. *Physiol. Zool.* **71,** 584-594.

Dousset J.C., Rioufol C., Philibert C. and P. Bourbon P. (1987). Effects of inhaled HF on cholesterol, carbohydrate and tricarboxylic acid metabolism in guinea pigs. *Fluoride.* **20,** 137-141.

Edwards H.M. and Baker D.H. (1999). Effect of dietary citric acid on zinc bioavailability from soy products using an egg white diets with zinc sulfate hepatahydrate as the stander. *Poult. Sci.* **78,** 113.

Ekmekci G., Somer G. and Sendil O. (2003). Simultaneous determination of copper, zinc and selenium in chicken liver by differential pulse polarography. *Turk. J. Chem.* **27,** 347- 355.

Engle T.E., Nockels C.F., Kimberling C.V., Weaber D.L. and Johnson A.B. (1997). Zinc repletion with organic or inorganic forms of zinc and protein turnover in marginally zinc deficient calves. *J. Anim. Sci.* **75,** 3074-3081.

Fleet J. and Salt D. (2009). Ionomics: mineral nutrition, physiology, and interactions as a biological system. *J. Anim. Sci.* **87,** 370.

Goodridge A.G. and Ball O. (1967). Lipogenesis in the pigeon: *in vivo* studies. *Am. J. Physiol.* **213,** 245-249.

Goodridge A.G. (1968). Conversion of [U14-C] glucose into carbon dioxide, glycogen, cholesterol and fatty acids in liver

slices from embryonic and growing chicks. *Biochem. J.* **108,** 655-661.

Gordon E., Newman C., Campbell A.M. and Williamson J.H. (2000). Purification and characterization of isocitrate dehydrogenase from Chlamydomonas reinhardtii. Department of Biology, Davidson College, Davidson.

Hill M. and Link J.E. (2009). Trace mineral interactions, known, unknown and not used. *J. Anim. Sci.* **87,** 370.

Hoehl C., Oestreich R., Rosen P., Wiesner R. and Grieshaber M. (1987). Evidence for succinate production by reduction of fumarate during hypoxia in isolated adult rat heart cells. *Arch. Biochem. Biophys.* **259,** 527-535.

Khan C.A. and Meijer G.A.L. (2005). The risk of contamination of food with toxic substances present in animal feed. *Anim. Feed Sci. Technol.* **133,** 84-108.

Leveille G.A., O'Hea E.K. and Chakrabarty K. (1968). *In vivo* lipogenesis in the domestic chicken. *Proc. Soc. Exp. Biol. Med.* **128,** 398-401.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265-275.

Mc Donald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A. and Wilkinson R.G. (2010). Animal Nutrition. Prentice Hall, Publisher.

Murakami K., Haneda M., Iwata S. and Yoshino M. (1997). Role of metal cations in the regulation of NADP-linked isocitrate dehydrogenase from porcine heart. *Biometals.* **10,** 169-174.

Murakami K., Tsubouchi R., Fukayama M., Ogawa T. and Yoshino M. (2006). Oxidative inactivation of reduced NADP-generating enzymes in *E. coli*: iron-dependent inactivation with affinity cleavage of NADP-isocitrate dehydrogenase. *Arch. Microbiol.* **186,** 385-392.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC.

Nix J. (2002). Trace minerals important for cattle reproduction. http://www.sweetlix.com.

Ogawa T., Murakami K., Mori H., Ishii N., Tomita M. and Yoshin M. (2007). Role of phosphoenolpyruvate in the NADP-isocitrate dehydrogenase and isocitrate lyase reaction in *E. coli*. *J. Bacteriol.* **189,** 1176-1178.

Rehman K., Andleeb S., Mahmood A., Bukhar S.M., Naeem M.M. and Yousaf K. (2012). Translocation of zinc and nickel from poultry feed to broilers and their excretion through litters. *Global Vet.* **8,** 660-664.

Rikans L.E., Moore D.R. and Snowden C.D. (1991). Sex-dependent differences in the effects of aging on antioxidant defense mechanisms of rat liver. *Biochim. Biophys. Acta.* **1074,** 195-200.

Romsos D.R. and Leveille G.A. (1974). Effect of diet on activity of enzymes involved in fatty acid and cholesterol synthesis. *Adv. Lipid Res.* **12,** 97-146.

Rosebrough R.W., Mcmurtry J.P. and Vasilatos-Younken R. (1999). Dietary fat and protein interactions in the broiler. *Poult. Sci.* **78,** 992-998.

SAS Institute. (2009). SAS®/STAT Software, Release 9.2. SAS Institute, Inc., Cary, NC.

Skinner J.T., Izat A.L. and Waldroup P.W. (1991). Fumaric acid enhances performance of broiler chickens. *Poult. Sci.* **70,** 1444-1447.

Spears J.W. (2000). Micronutrients and immune function in cattle. *Proc. Nutr. Soc*. **59,** 1-8.

Tanaka K., Ohtani S. and Shigeno K. (1983). Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. I. Increasing energy by carbohydrate supplementation. *Poult. Sci*. **62,** 445-451.

Thomassen Y. and Aaseth J. (1986). Selenium in human tissues. Pp. 33-105 in Occurrence and Distribution of Selenium. M. Ihnat, Ed. Boca. Raton, FL, CRC Press.

Varrone S., Consiglio E. and Covelli I. (1970). The nature of inhibition of mitochondrial malate dehydrogenase by thyroxine, iodine, cyanide, and molecular iodine. *Eur. J. Biochem*. **13,** 305-310.

Wiesner R.J., Kreutzer U., Rosen P. and Grieshaber M.K. (1988). Subcellular distribution of malate-aspartate cycle intermediates during normoxia and anoxia in the heart. *Biochim. Biophys. Acta.* **936,** 114-123.

Yamaguchi M., Kura M. and Okada S. (1982). Role of zinc as an activator of mitochondrial function in rat liver. *Biochem. Pharmacol.* **31,** 1289-1293.