Effect of dietary zinc oxide and phytase on the plasma metabolites and enzyme activities in aged broiler breeder hens

Sharideh, H.[1], Zhandi, M.[1*], Zaghari, M.[1], Akhlaghi, A.[2]

[1]*Department of Animal Sciences, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*

[2]*Department of Animal Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran*

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Correspondence
Zhandi, M.
Department of Animal Sciences, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
Tel: +98(26) 32248082
Fax: +98(26) 32246752
Email: mzhandi@ut.ac.ir

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Abstract:

BACKGROUND: It has been shown that zinc has an effect on physiological responses in animals and birds. On the other hand, dietary phytase in poultry results in increased availability of zinc. **OBJECTIVES:** This study was conducted to investigate the effects of zinc oxide (ZnO) and *Escherichia coli*-derived 6-phytase supplemented diets on the plasma metabolites and enzyme activities of broiler breeder hens from 60 to 72 weeks of age. **METHODS:** A total of 128 breeder hens were randomly assigned to eight dietary treatments, with four replicates of four hens each. Blood concentration of Zn, Ca, P, total protein, cholesterol, triglyceride (TG) and high density lipoprotein (HDL), and plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured. **RESULTS:** Results showed that supplementary ZnO increased plasma Zn, Ca, P, HDL, and total protein ($p<0.01$) concentrations, as well as enzyme activities of LDH, ALT and AST ($p<0.01$). Also, a ZnO-supplemented diet resulted in a decrease in plasma cholesterol and TG ($p<0.01$) levels. Adding phytase to the diet increased plasma (P) and HDL contents ($p<0.01$). The interactive effect of phytase × ZnO × period on the plasma levels of Zn, P, total protein, HDL, total cholesterol, and the enzymatic activity of LDH, ALT and ALP was significant. **CONCLUSIONS:** It is concluded that supplementary ZnO and phytase may improve metabolism and enzymatic activity of aged broiler breeder hens.

Introduction

Broiler breeder hens have been selected due to their maximum growth (Zaghari et al., 2013). Therefore, they are predisposed to metabolic abnormalities and impaired reproduction. In most domestic animals, reproductive performance is significantly influenced by nutrition (Armstrong and Benoit, 1996). It has been clarified that using suitable levels of nutrients in the diet may improve metabolism and reproductive function in broiler breeder hens.

The main role of zinc in the body seems to be associated with the enzymes and proteins both as of the molecule and as an activator. Zinc plays an important role in metabolic activities such as protein synthesis, carbohydrate metabolism, enzyme activation and growth. Zinc metalloenzymes are identified in all six

enzyme types, which include oxidoreductas, transferase, hydrolase, lyase, isomerase and ligase (Park et al., 2004). Zinc deficiency reduced protein in the liver, brain and testes of mice (Yousef et al., 2002). Zinc deficiency in the broiler breeder's diet results in decreased hatchability, hatching of weak chicks, and increased embryonic mortality (Wilson, 1997). It has been reported that zinc deficiency in rats increased the triglyceride (TG) level in liver (Eder and Kirchgessner, 1995), and zinc deficiency in rabbits increased the testicular cholesterol (Eltohamy and Younis, 1991). Also, it has been reported that supplementation of ZnSO4 in the diet reduced the plasma cholesterol level in broiler chickens (Herzig et al., 2009). In broiler breeder hens, TG accretion in non-adipose tissue results in obesity, lipotoxicity and ovarian dysfunction (Chen et al., 2006). Recent studies show that the ingestion of phytate can affect the excretion and digestibility of minerals and protein, and reduce availability of energy.

Phytate-P cannot be fully utilized by poultry due to a lack of effective endogenous phytase (Maenz and Classen, 1998). Phytate is a polyanionic molecule, and has some sites for chelating cation nutrients in the digestive tract, making them (Ca, Zn, Mg, and Fe) unavailable to the poultry (Cheryan, 1980). Phytase is an enzyme which is capable of degrading phytate in the digestive tract to yield myoinositol phosphates, inositol and inorganic phosphorus (P) (Liu et al., 1998). A number of studies have also shown that phytase supplementation improves the utilization of Zn and Ca in poultry diets (Mohanna and Nys, 1999; Viveros et al., 2002). Lim et al. (2003) showed that low Ca in the diet of laying hens caused decreases in the specific gravity of eggs, eggshell strength, and eggshell thickness. It has been reported that supplementation of microbial phytase (300 U per kg diet of a laying hen) can improve egg production, decrease broken and soft egg production rate, and P excretion (Lim et al., 2003). Phosphorus is necessary to support normal embryonic bone development and hatchability (Wilson, 1997). *Escherichia coli*-derived 6-phytase remains active in a very broad range of pH (2.5–6.0). In the present study, it seems that using *E. Coli*-derived 6-phytase in diet can improve bioavailability of nutrients such as Zn. Thus, the aim of this study was to evaluate the effects of dietary ZnO supplementation with and without phytase on some plasma metabolites and enzyme activities in aged broiler breeder hens.

Materials and Methods

Ethics: All procedures and experiments were approved by the local Scientific Ethical Committee, University of Tehran, Karaj, Iran.

Birds and experimental treatments: A total of 128 Cobb 500 broiler breeder hens (weighing 5200 ± 200 g) were selected at 60 weeks of age from a commercial flock, and were kept under a 16L:8D photoschedule at 22 ± 2°C environmental temperature. Hens were randomly assigned into 32 floor pens (1.25 m × 2.50 m), and each pen was equipped with a linear trough feeder and an automatic bell drinker. The birds were randomly assigned to the dietary treatments with four replicates of four hens each. In a 2 × 4 factorial arrangement, the birds were provided diets with two inclusion levels of *E. Coli*-derived 6-phytase (0 or 300 FTU/kg; AB Vista Feed Ingredients, Wiltshire, UK), and four inclusion levels of zinc oxide (ZnO; 30, 60, 90 and 120 mg/kg diet). Diets contained 2,750 kcal ME/kg and 14.5% CP (Table 1). Prior to the start of the experiment, all hens received a Zn-depleted diet with 2,800 kcal of AMEn/kg, 14.5% CP, and 9.5 mg ZnO/kg for 2 weeks.

Plasma metabolites and enzymatic activities: Plasma concentrations of minerals, lipids, total protein content and enzyme activities were determined in one hen per replicate. At 61, 66 and 72 weeks of age, the birds were bled

from the brachial vein (2 mL) into heparinized tubes. The blood samples were centrifuged for 12 min (1800 × g at 18°C), and plasma was collected and kept at −20ºC for later analysis. All the plasma samples were analyzed at the same time to avoid interassay variations. Plasma Zn was determined by the colorimetric enzymatic method using a commercial kit (Ziestchem Diagnostics, Tehran, Iran) with an intra-assay CV of 3.6%. Plasma Ca, P, total protein, TGs, total cholesterol, and HDL concentrations were determined by a colorimetric enzymatic method using a commercial kit (Parsazmun, Tehran, Iran) with an intra-assay CV of 4.8, 3.07, 3.4, 2.1, 2.6 and 3.8%, respectively. Enzymatic activities of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by the colorimetric enzymatic method using a commercial kit (Parsazmun, Tehran, Iran) with an intra-assay CV of 6.2, 3.2, and 1.5%, respectively.

Statistical Analysis: The data were analyzed by the mixed procedure of SAS 9.1 (SAS Institute, 2002). The results were represented as LS mean ± SEM and were compared by the Tukey's test at $p \leq 0.05$.

$$Y_{ijkl}=\mu+Z_i+P_j+ZP_{ij}+T_k+ZPT_{ijk}+e_{ijkl}$$

where Y_{ijkl} is the observed dependent variable (including level of Ca, P, total protein, TGs, total cholesterol and HDL concentrations, and activity of LDH, ALT, AST and ALP enzymes), μ is mean of population, Zi is the effect of ith level of Zn (i = 1, 2, 3 and 4), P_j is the effect of jth level of phytase (i = 1 and 2), ZP_{ij} is the interaction between Zn and phytase levels, Tk is the effect of kth period (k = 1, 2 and 3), ZPT_{ijk} is the interaction between Zn, phytase and period. Also, e_{ijkl} is a random residual error.

Results

Dietary supplementation of ZnO increased plasma levels of zinc, calcium, phosphorus and total protein ($p<0.01$; Table 2). However, added phytase had no effect on these traits.

Table 1. Ingredients and chemical composition of diets fed to aged Cobb 500 breeder hens (As fed basis). (1) Available potassium 44.6%. (2) Available phosphorus 27.5%. (3) Provides (per kg of diet): copper (CuSO4•5H2O), 10 mg; iodine (KI), 2 mg; iron (FeSO4•7H2O), 50 mg; manganese (MnSO4•H2O), 120 mg; selenium (Na2SeO3), 0.3 mg. (4) Provides (per kg of diet): vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 2.5 mg; vitamin K, 0.5 mg; thiamin, 2.0 mg; riboflavin, 10 mg; D-pantothenic acid, 25 mg; niacin, 40 mg; pyridoxine, 6 mg; biotin, 0.66 mg; folic acid, 4 mg; vitamin B12, 0.035 mg.

Ingredient (%)	Depletion diet	Experimental diet
Corn	0.00	64.61
Corn starch	46.1	0.35
Soybean meal, 42.6% CP	11.16	16.22
Corn gluten meal, 62% CP	14.7	0.27
Alfalfa meal, 24% CF	0.00	8.78
Cellulose, 89%CF	13.0	0.00
Corn oil	2.26	1.00
Sodium bicarbonate (NaHCO3)	0.28	0.22
Dicalcium phosphate	1.48	1.48
Calcium carbonate	7.78	6.52
Potassium sulfate1 (K2SO4)	0.94	0.00
Phosphoric acid2 (H3PO4)	1.09	0.00
Common salt	0.20	0.17
Mineral premixes3	0.13	0.17
Vitamin premix4	0.02	0.02
DL-Methionine, 99%	0.30	0.11
L-Thereonine	0.30	0.00
L-Lysine HCl, 78%	0.26	0.08
Calculated nutrient content		
AME (kcal/kg)	2750	2750
CP (%)	14.50	14.41
Calcium (%)	3.0	3.0
Available phosphorus (%)	0.35	0.38
Sodium (%)	0.16	0.15
Lys (%)	0.64	0.65
Met (%)	0.58	0.32
Met + Cys (%)	0.81	0.53
Thr (%)	0.78	0.46
Arg (%)	0.62	0.74
Zinc (mg/kg)	9.50	30.0

Table 2. Plasma concentration of total protein, Zn, Ca, and P in broiler breeder hens fed diets supplemented with the different levels of zinc oxide and phytase. [(a-c)] LSmeans within each column and for each effect without common superscript are significantly different * ($p< 0.05$), ** ($p< 0.01$).

Item	Total protein (g/dL)	Zn (µg/dL)	Ca (mg/dL)	P (mg/dL)
Zinc levels (mg/kg)				
30	6.05 ± 0.11^{c}	165.05 ± 5.75^{c}	9.20 ± 0.19^{b}	6.16 ± 0.18^{c}
60	6.61 ± 0.11^{b}	191.32 ± 5.75^{b}	10.16 ± 0.19^{a}	6.73 ± 0.17^{b}
90	7.02 ± 0.11^{a}	212.00 ± 5.75^{a}	10.35 ± 0.20^{a}	7.12 ± 0.17^{a}
120	7.05 ± 0.11^{a}	214.04 ± 5.75^{a}	10.16 ± 0.20^{a}	7.07 ± 0.17^{a}
Phytase levels (U/kg)				
0	6.57 ± 0.08	194.12 ± 4.13	9.98 ± 0.14	6.68 ± 0.12
300	6.79 ± 0.08	197.08 ± 4.13	9.94 ± 0.14	6.87 ± 0.12
Period (week)				
61	6.15 ± 0.11^{b}	169.02 ± 4.19^{c}	9.62 ± 0.15^{b}	5.49 ± 0.13^{b}
66	6.96 ± 0.11^{a}	204.61 ± 3.92^{b}	9.79 ± 0.14^{b}	7.25 ± 0.13^{a}
72	6.93 ± 0.11^{a}	213.18 ± 3.92^{a}	10.46 ± 0.14^{a}	7.56 ± 0.14^{a}
Effect (P value)				
Zinc levels	**	**	**	**
Phytase levels	NS	NS	NS	NS
Period	**	**	**	**
Zinc*Period	NS	NS	NS	NS
Zinc*Phytase*Period	*	*	*	**

Table 3. Plasma concentrations of LDL, HDL, cholesterol, VLDL, and triglyceride in broiler breeder hens fed diets supplemented with the different levels of zinc oxide and phytase. [(a-c)] Lsmeans within each column and for each effect without common superscript are significantly different *($p< 0.05$), **($p< 0.01$).

Item	HDL (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/10 mL)
Zinc levels (mg/kg)			
30	70.77 ± 2.31^{b}	174.94 ± 4.24	117.08 ± 3.84^{a}
60	87.17 ± 2.31^{a}	157.9 ± 4.24	97.69 ± 3.89^{b}
90	91.52 ± 2.31^{a}	166.36 ± 4.24	95.61 ± 3.87^{b}
120	86.79 ± 2.31^{a}	161.58 ± 4.24	97.8 ± 3.84^{b}
Phytase levels (U/kg)			
0	80.61 ± 1.67^{b}	168.55 ± 3.06	105.58 ± 2.82
300	87.52 ± 1.59^{a}	161.84 ± 3.00	98.5 ± 2.64
Period (week)			
61	71.95 ± 2.00^{b}	159.1 ± 3.60^{b}	113.85 ± 4.26^{a}
66	91.05 ± 1.85^{a}	173.29 ± 3.41^{a}	103.38 ± 2.35^{b}
72	89.19 ± 2.03^{a}	163.19 ± 3.41^{b}	88.89 ± 2.16^{c}
Effect (P value)			
Zinc levels	**	NS	**
Phytase levels	**	NS	NS
Period	**	*	**
Zinc*Period	NS	NS	NS
Zinc*Phytase*Period	*	*	NS

During the experiment, the level of plasma zinc, phosphorus, calcium and total protein were increased. Also, the effect of period resulted in increased plasma levels of zinc, calcium, phosphorus and total protein ($p<0.01$). The interactive effects of phytase × ZnO ×

Table 4. Plasma enzyme activities of lactate dehydrogenase (LDH), alanine amino transferase (ALT), alkaline phosphatase (ALP), and aspartate amino transferase (AST) in broiler breeder hens fed diets supplemented with the different levels of zinc oxide and phytase. [(a-c)] Lsmeans within each column and for each effect without common superscript are significantly different *(p< 0.05), **(p< 0.01).

Item	LDH (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)
Zinc levels (mg/kg)				
30	61.72 ± 1.33[b]	3.88 ± 0.19[c]	69.4 ± 3.99	25.69 ± 1.33[c]
60	63.42 ± 1.33[b]	4.67 ± 0.19[b]	56.19 ± 4.08	27.62 ± 1.33[bc]
90	67.48 ± 1.33[a]	5.46 ± 0.19[a]	63.99 ± 4.08	30.4 ± 1.33[b]
120	69.04 ± 1.33[a]	5.38 ± 0.19[a]	66.09 ± 3.99	35.61 ± 1.33[a]
Phytase levels (U/kg)				
0	65.07 ± 0.94	4.7 ± 0.13	61.14 ± 2.82	28.86 ± 0.94
300	65.77 ± 0.94	4.99 ± 0.13	66.69 ± 2.88	30.81 ± 0.94
Period (week)				
61	66.92 ± 1.23	3.59 ± 0.24[c]	87.35 ± 3.33[a]	31.99 ± 1.4
66	65.35 ± 1.24	5.14 ± 0.1[b]	66.35 ± 3.21[b]	27.95 ± 1.06
72	63.98 ± 0.79	5.81 ± 0.09[a]	38.05 ± 3.08[c]	29.56 ± 0.82
Effect (P value)				
Zinc levels	**	**	NS	**
Phytase levels	NS	NS	NS	NS
Period	NS	**	**	NS
Zinc*Period	NS	NS	NS	NS
Zinc*Phytase*Period	**	**	**	NS

period on the plasma levels of zinc, calcium, phosphorus and total protein were also significant (p<0.05).

Addition of ZnO to the experimental diets decreased the plasma level of low density lipoproteins (LDL) and TGs, and increased that of HDL (p<0.01; Table 3). Phytase supplementation increased HDL (p<0.05). The effect of period resulted in increased HDL, and decreased TG (p<0.05). The interactive effects of phytase × ZnO × period on plasma HDL and total cholesterol were significant (p<0.05).

Dietary supplementation of ZnO increased the enzymatic activity of LDH, ALT and AST (p<0.01; Table 4), but it had no effect on the activity of ALP. The effect of period resulted in decreased ALP activity, and increased ALT activity (p<0.01). However, phytase supplementation had no effect on the enzymes activities. The interactive effects of phytase × ZnO × period on the enzymatic activity of LDH, ALT and ALP were significant (p<0.01).

Discussion

The results of this study showed that dietary supplementation of ZnO increased the plasma levels of Zn, and the effect of period increased levels of Zn. A reason for significant effect of period may be due to feeding of depletion diet for two weeks, and this diet probably resulted in decreased Zn in the body compared to the later period of the balanced diet. The binding strength of minerals to phytate has been identified as Zn2+ > Cu2+ > Ni2+ > Co2+ > Mn2+ > Ca2+ > Fe2+ (Cheryan, 1980), and is in the inverse order when compared with the improvement in availability of minerals (Weaver and Kannan, 2002). The bioavailability of Zn was improved by adding microbial phytase to low-Zn diets fed to chicks (Mohanna and Nys, 1999). In accordance with previous reports (Mohanna and Nys, 1999; Jondreville et al., 2007), adding microbial phytase to the diet did not have any effect on the availability of Zn.

Dietary supplementation of ZnO increased

the plasma levels of Ca, P and total protein. The current results are in agreement with the finding of Feng et al. (2010) who demonstrated that addition of Zn-glycine to broiler diets increased serum total protein, P and Ca concentration. On the other hand, some studies showed that dietary phytase had an inverse (Broz et al., 1994) or no effect on plasma Ca concentration (Roberson and Edwards, 1994). It seems that the low level of Zn in diet can decrease blood Ca and P via reducing parathyroid hormone and calcitonin content of the blood (Zhang et al., 2003). Unlike our present study, one study showed that dietary supplementation of phytase had no effect on serum total protein (Viveros et al., 2002). It has been found that Zn deficiency has a negative effect on the synthesis of DNA, cell division and protein synthesis (Prasad, 1996). A reason for significant effect of period may be due to feeding of Zn-depleted diet for two weeks, and this diet probably resulted in decreased Zn and other parameters in the body compared to the later period of the balanced diet.

In the present study, addition of ZnO to the experimental diets decreased plasmaTG and increased HDL. In a study on the broiler chickens, it has been shown that feeding a mixture of organic supplements with Zn, copper and manganese decreased total cholesterol and LDL, and increased HDL in plasma (Aksu et al., 2010). On the other hand, Paul et al. (2001) found that Zn supplementation reduced plasma cholesterol and TG in mice. Eder and Kirchgessner (1995) reported that Zn deficiency in rats increased concentration of total lipids, cholesterol and LDL oxidation sensitivity.

In the present study, addition of phytase to diet increased HDL levels. The current results are consistent with the finding of Zyla et al. (2013) who found an increased serum TG and HDL, and the ratio of HDL to total cholesterol in broiler chickens that were fed corn-based diets along with phytase B and 6-phytase. Our present results confirm previous findings that Zn and phytase have an effect on fat metabolism (Aksu et al., 2010; Zyla et al., 2013). The effect of period on HDL, TG and cholesterol were significant. It has been found that the broiler breeder hen's body weight was positively correlated with plasma levels of TG, liver fat and liver cholesterol (Zaghari et al., 2013). In the current study, it seems that decreases in the hen's body weight during the period resulted in decreased plasma levels of TG.

Dietary supplementation of ZnO increased activity of LDH, ALT and AST, but had no effect on the activity of ALP. It has been found that Zn deficiency in pigs resulted in a decreased AST and ALT activity (Rupic et al., 1997). In a study on mice, it was found that Zn deficiency reduced LDH and ALP activity in serum (Yousef et al., 2002). Like the findings of Mohanna and Nys (1999), our results in this study showed that Zn supplementation had no effect on ALP activity. This study confirms that probably a severe Zn deficiency is necessary to affect the ALP activity (Mohanna and Nys, 1999). Also, the results showed that ALP activity was decreased from the 61st to 72nd week of experiment. This effect may be due to the increase in age (Viveros et al., 2002). We also observed that the effect of period on ALT activity was significant. It seems that period effect on ALT may be due to feeding of depletion diet for two weeks, and this diet decreased Zn in the plasma to the later period of the balanced diet.

In conclusion, the data obtained indicated that Zn supplementation can improve the concentration of calcium and phosphorus in plasma at examined levels in this study. Also, addition of Zn to diet has improved enzymes activity and total protein. Finally, ZnO and *E. Coli*-derived 6-phytase supplemented diet can increase the plasma level of HDL.

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اثر اکسید روی و فیتاز جیره بر متابولیت‌های پلاسما و فعالیت‌های آنزیمی در مرغ‌های مادر مسن

حسین شریده[۱] مهدی ژندی[۱*] مجتبی زاغری[۱] امیر اخلاقی[۲]

۱) گروه علوم دامی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران
۲) گروه علوم دامی، دانشکده کشاورزی، دانشگاه شیراز، شیراز، ایران

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چکیده

زمینه مطالعه: اثر روی بر پاسخ‌های فیزولوژیک در حیوانات و پرندگان نشان داده شده است. از طرف دیگر، فیتاز جیره در طیور منجر به افزایش دسترسی روی می‌شود. **هدف:** این مطالعه برای بررسی اثرات جیره‌های مکمل شده با اکسید روی و ۶-فیتاز مشتق شده از *E. Coli* بر متابولیت‌ها و فعالیت‌های آنزیمی پلاسما در مرغ‌های مادر گوشتی مسن از سن ۶۰ تا ۷۲ هفتگی انجام شد. **روش‌کار:** تعداد ۱۲۸ مرغ مادر به صورت تصادفی به هشت تیمار تغذیه‌ای با چهار تکرار و چهار مرغ در هر تکرار اختصاص پیدا کردند. غلظت خونی روی، کلسیم، پروتئین کل، کلسترول، تری‌گلیسرید و HDL و فعالیت پلاسمایی ALP، ALT، AST و LDH اندازه‌گیری شدند. **نتایج:** نتایج نشان داد که مکمل روی با افزایش پلاسمایی غلظت‌های روی، کلسیم، فسفر، HDL و پروتئین کل ($p>۰/۰۱$)، به علاوه با افزایش فعالیت آنزیم‌های LDH، ALT و AST ($p>۰/۰۱$) همراه بود. همچنین، جیره مکمل شده با روی منجر به کاهشی در سطوح کلسترول و تری‌گلیسرید شد ($p>۰/۰۱$). افزودن فیتاز به جیره مقدار فسفر و HDL را افزایش داد ($p>۰/۰۱$). اثر متقابل فیتاز، اکسید روی و دوره بر سطوح پلاسمایی روی، فسفر، پروتئین کل وHDL کلسترول کل و فعالیت آنزیمی LDH، ALT و ALP معنی‌دار بود. **نتیجه‌گیری‌نهایی:** نتیجه‌گیری می‌شود که مکمل اکسید روی و فیتاز ممکن است متابولیسم و فعالیت آنزیمی در مرغ‌های مادر گوشتی مسن را بهبود دهد.

واژه‌های کلیدی: آنزیم، مرغ، متابولیت، فیتاز، روی

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*) نویسنده مسؤول: تلفن: ۳۲۲۴۸۰۸۲ (۲۶)۹۸+ نمابر: ۳۲۲۴۶۷۵۲ (۲۶)۹۸+ Email: mzhandi@ut.ac.ir