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Full Length Research Paper

The effects of thyme (*Thymus vulgaris*) extract supplementation in drinking water on iron metabolism in broiler chickens

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The effects of 0 (ZT), 0.2 (LT), 0.4 (MT) and 0.6% (HT) thyme extract in drinking water on the amounts of blood iron, hemoglobin (Hb), red blood cell (RBC), hematocrit (Hct) and total iron binding capacity (TIBC) on days 21 and 42 broilers were investigated. No significant differences were observed between the treatments for the amounts of blood Hb, RBC and Hct, but blood iron concentration of birds that received the thyme extract was lower as compared to that of control birds (ZT) on both ages (P<0.05). Blood iron concentration of LT and MT birds was lower than that of ZT birds and higher than that of HT birds (P<0.05) on days 21 and 42 of age. Blood TIBC of birds that received the thyme extract was higher (P<0.05) as compared to that of ZT birds on both determinations. HT birds had a higher blood TIBC amount (P<0.05) as compared to MT birds on day 42 of age. Negative and positive regression models (linear and quadratic) (P<0.0001) existed respectively between the thyme extract supplementation with the amounts of blood iron and TIBC on both ages. Inhibited iron absorption by thyme polyphenols or saponins is the possible reason.

Key words: Broiler chickens, *thymus vulgaris*, extract, iron, total iron binding capacity.

INTRODUCTION

Iron is a chemical element that plays an essential role in several biologically important processes including oxygen transport, electron transfer, and enzymatic reactions (Quintana et al*.,* 2006). It is the most abundant metal in the brain and participates in the main neuronal processes, including neurotransmitter synthesis and myelination of axons. Iron is important for all forms of life and considered to be a principal oligo-element of the human organism. It well assumes a metabolic action at the cellular level through the activation of catalase and peroxydase (Bothwell, 1968; Khemiss et al., 2006; Bothwell and Charlton, 1970). It also is the metal which carries oxygen to all the cells and present about 65% at the hemoglobin level, 5% at the myoglobin level, 0.3% in enzymes and cytochrome and 30% in ferritin and hemosiderine form.

Dietary iron deficiency is the most common nutritional deficiency in the world and can ultimately result in anaemia (Cook, 1990). Iron deficiency affects approximately 2 billion people worldwide (Stoltzfus, 2001) and is the most prevalent nutritional deficiency in the world and affects up to two-thirds of children in most developing countries (World Health Organization, 2000). Typical homemade complementary foods used in developing countries are poor sources of bioavailable iron and thus are inadequate to meet infants' high iron requirements for rapid growth and blood volume expansion after 6 months of age (Gibson et al., 1998; Dallman, 1986).

Iron level in an organism is regulated by absorption, excretion and storage mechanisms (Bothwell, 1968; Khemiss et al., 2006; Bothwell and Charlton, 1970). Iron forms derived from foods can be divided into heme and non heme iron. Heme iron exists in meat and highly

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bioavailable. The absorption of this form is not affected by other factors presented in foods. Non heme iron is the other form of iron present in vegetable, cereals and dairy product (Saltzman and Russell, 1998). Adversely with heme iron, this form have a low absorption and markedly affected by gastro intestinal acidity, tannins, polyphenols, phytates, calcium and phosphate (Kaltwasser et al., 1998; Hurrell et al., 1999; Davidsson et al., 2001; Cook et al., 1991). The presence of absorption inhibitors such as phytic acid or polyphenol compounds in the plant foods is a major cause of iron deficiency (Fairweather and Hurrell, 1996). Polyphenol compounds are widely present in the human diets as components of fruits, vegetable, spices, cereals, tea, coffee, red wine, cacao and different herb teas (Hurrell et al., 1999). The phenolic compounds are released from the foods and beverage during digestion process and can complex with iron and making it unavailable for absorption (Hurrell et al., 1999).

Negative effects of phenolic compounds in black tea (Thankschan et al., 2008), grape seed extract (King et al., 2008), spinach and aubergine (Gillooly et al., 1983) on iron absorption has earlier been reported. However no information is published regarding the thyme plant or its extract effects on iron absorption. *Thymus vulgaris* is a perennial medicinal herb in the *Lamiaceae* family and cultivated throughout the world for culinary, cosmetic and medical purposes. This species has special activities such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant (Abudarwish et al., 2009; Hertrampf, 2001). Herbal thyme contains about 1 to 2.5% essential oils (Franz et al., 2005) such as thymol (44.4 to 58%), carvacrol (2.4 to 4.2%) and γ-terpipen (6.9 to 18.9%) that have been identified as the phenolic compounds (Sengul et al., 2008).

Since thyme extract does have essential oil, tannins, glycosides, saponins and other components (Escop, 2003) and inhibitory effects of these components were indicated previously, it was hypothesized that thyme extract may changes the iron metabolism. So thyme extract supplementation in drinking water effects were evaluated on the amounts of blood iron, Hemoglobin (Hb), red blood cell (RBC), Hematocrit (Hct) and total iron binding capacity (TIBC) in broiler chickens. According to previous suggestions of Conway et al. (2006), plasma iron concentration was used as an indicator of iron absorption in recent experiment. These authors provided some beneficial information regarding the use of serum iron concentration in individuals as a good estimate of relative bioavailability and iron absorption in foods or meals.

MATERIALS AND METHODS

One hundred and sixty one-day-old broiler chicks (Ross 308) were provided from a local hatchery, weighed on arrival and randomly divided between 16 pens (1×1 m) of 10 birds each and each four were assigned to each treatment. Water and feed were provided for *ad libitum* consumption. All the chickens were fed the same starter (from day one to 21 of age) and grower (from day 22 to 42 of age) diets in pellet form (Table 1) but received 0.0% (ZT), 0.2% (LT), 0.4% (MT) and 0.6% (HT) *T vulgaris* alcoholic extract (having 0.06 thymol and pH=5) in drinking water from day one to day 42 of age. *T. vulgaris* alcoholic extract was prepared using the standard maceration method of Zhang et al. (2005). Vegetative parts of the shade dried *T. vulgaris* full bloom stage were crushed and soaked in ethanol 80% in 1:5 ratios (w/v) for 72 h on a shaker. The extract strained and its thymol content pH were determined by TLC method (Thin Layer Chromatography) and a pH meter instrument (HACH, HQ40D, USA), respectively.

At days 21 and 42 of age, two birds per pen (eight per dietary treatment) were randomly selected and killed by decapitation to obtain the blood samples. Blood in microcapillary tubes were used for blood hematocrit (Hct) measurements after centrifugation (5000 rpm) for 7 min. Blood samples were collected in anticoagulant tubes (citrate sodium 3.6% solution). A commercial kit (Zist-Shimi Company, Iran) was used for hemoglobin determination. In this method, ferrous ions of hemoglobin were oxidized to the ferric state by potassium ferricyanide to form hemiglobin (methemoglobin). Hemiglobin reacts with cyanide to form hemiglobincyanide (cyanmethemoglobin) that can be measured spectrophotometry. The amounts of blood iron and TIBC were determined colorimetrically with a commercial kit (Zist-Shimi Company, Iran) using a spectrophotometer (Unico 2100, Japon). Red blood cells concentration was determined by a hemocytometer manually. The data were subjected to one way analyses of variance using SAS statistical package, version 9.1 (SAS Institute, Cary, NC, USA) and analyzed based on a completely randomized design using the General Linear Model procedure. Turkey–Kramer Multiple Comparison Test at significance level of 0.05 was used to compare the mean values. In addition, regression models (linear and quadratic) were performed to show the changes in the amounts of blood iron and TIBC by thyme extract supplementation at days 21 and 42 of the experiment.

RESULTS

The amounts of blood iron, Hb, and RBC, Hct and TIBC of the birds in different treatments are presented in Table 2. There was no significant differences between the treatments for the amounts of blood Hb, and RBC and Hct at days 21 and 42 of age ($P>0.05$) but blood iron concentration of all the thyme extract received birds was lower as compared to that of control birds (ZT). Blood iron concentration of LT and MT birds was lower than that of ZT birds but higher than that of HT birds ($P<0.05$) at days 21 and 42 of age. HT birds had the lowest ($P<0.05$) blood iron concentration between the treatments at both ages (day 21 and 42). The amount of blood TIBC in all the thyme extract received birds was higher ($P<0.05$) as compared to that of ZT birds at day 21 and 42 of age. Furthermore, there was no significant difference between the HT, MT and LT birds for blood TIBC amount at day 21 of age. At day 42 of age, HT birds had the higher TIBC amount ($P<0.05$) as compared to MT birds.

Thyme extract consumption significantly affected the blood iron concentration and additional negative response was observed at greater thyme levels (Figure 1). Negative linear and quadratic regressions existed between the thyme extract supplementation with blood iron concentration at days 21 (quadratic regression,

Table 1. Composition of experimental diets.

Ingredients (%)	Starter (0-21 d)	Grower (21-42 d)
Corn	54.87	61.78
Soybean meal (44% protein)	36.72	26.36
Fish meal	1.31	4.50
Vegetable oil	3.00	4.00
Limestone	1.15	1.05
Dicalcium phosphate	1.94	1.49
Vitamin and mineral premix[1]	0.50	0.50
Salt	0.30	0.30
DL-methionine	0.21	0.02
Total	100.00	100.00
Calculated analysis		
ME (kcal/kg)	2937	3100
CP (%)	21.44	19.37
Calcium (%)	1.05	1.00
A. Phosphorus (%)	0.51	0.50
Sodium (%)	0.16	0.14
Arginine (%)	1.41	1.23
Methionine + Cystine (%)	0.91	0.69
Lysine (%)	1.20	1.10
Tryptophan (%)	0.31	0.26

[1] provide per kilogram of diet: retinol, 15000 IU; cholecalciferol,8000 IU; vitamin K3, 3 mg; B_{12}, 15 µg; niacin, 32 mg; choline, 840 mg; biotin, 40 µg; thiamine, 4 mg; riboflavin, 6.6 mg; pyridoxine, 5 mg; folic acid, 1 mg; zinc, 80 mg; manganese, 100 mg; selenium, 200 mg; iron, 80 mg; magnesium, 12; copper, 10 mg; calcium, 15 mg; iodeine,1 mg.

Table 2. blood Hematocrit (Hct) and total iron binding capacity (TIBC) percents, and Hemoglobin (Hb), Red blood cell (RBC), iron concentrations of broiler chickens[1] received free thyme extract water (ZT) or 0.2 (LT), 0.4 (MT) and 0.6% (HT) of thyme extract in drinking water.

Treatment	Hct (%)		Hb (g/dl)		RBC ($\times 10^6$ cells/mm3)		iron (mg/dl)		TIBC (%)	
	d 21	d 42	d 21	d 42	d 21	d 42	d 21	d 42	d 21	d 42
ZT	26.75	28.5	8.9	9.5	2.97	3.17	15.88^{a}	16.88^{a}	41.63^{b}	35.50^{c}
LT	27.75	25.75	9.24	8.58	3.21	2.86	12.88^{b}	15.00^{b}	46.50^{a}	41.38^{ab}
MT	26.88	26.38	8.96	8.79	2.99	2.93	13^{b}	14.63^{b}	47.13^{a}	40.88^{b}
HT	26	26.13	8.67	8.71	2.89	2.9	11.13^{c}	12.62^{c}	48.13^{a}	42.75^{a}
P value	0.48	0.33	0.48	0.33	0.14	0.33	0.0001	0.0001	0.0001	0.0001
SEM	0.38	0.57	0.13	0.19	0.05	0.06	0.3	0.34	0.51	0.54

[a-b] Means with no common superscript letter in each columns differ significantly (P<0.05). [1]blood samples of eight chickens per treatment were used for these determinations.

$y = 13.6-1.68x + 0.281x^2$, P<0001, R2 = 0.59; liner regression, y = 0.0671 + 0.411, P<0001, R2 = 0.58) and 42 of age (quadratic regression, $y = 6.65 + 1.65x-0.0312x^2$, P<0001, R2 = 0.59; liner regression, y = -1.22+ 0.454x, P<0001. R2 = 0.59) (Figure 1). Moreover positive linear and quadratic regressions existed between the thyme extract supplementation and blood TIBC amount at days 21 (quadratic regression, $y = 28.8+8.64x- 0.068x^2$, P<0001, R2 = 0.73; liner regression, y = 19.68 - 0.309X, P<0001, R2 = 0.62) and 42 of age (quadratic regression, $y = 22.8 + 8.86x - 1x^2$, P<0001, R2 = 0.71; liner regression, y = 17.04 - 0.287x, P<0001. R2 = 0.61) (Figure 2).

DISCUSSION

So far, some animal models have been used for the studies of anemia or iron absorption. Furugouri (1972)

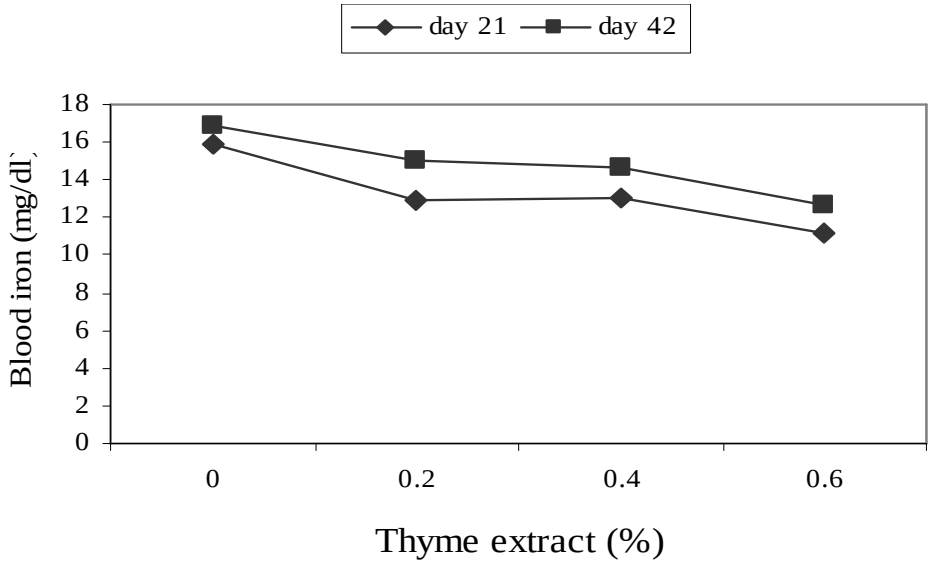

Figure 1. The relationship between the level (%) of *Thymus vulgaris* supplementation in drinking water and the blood iron concentration at day 21(quadratic regression, $y = 13.6 - 1.68x + 0.281x^2$, P<0001, R2 = 0.59; liner regression, y = 0.0671 + 0.411,P<0001, R2 = 0.58) and day 42 of age (quadratic regression, $y = 6.65 + 1.65x - 0.0312x^2$, P<0001, R2 = 0.59; liner regression, y = -1.22 + 0.454x, P<0001. R2 = 0.59) in broiler chickens.

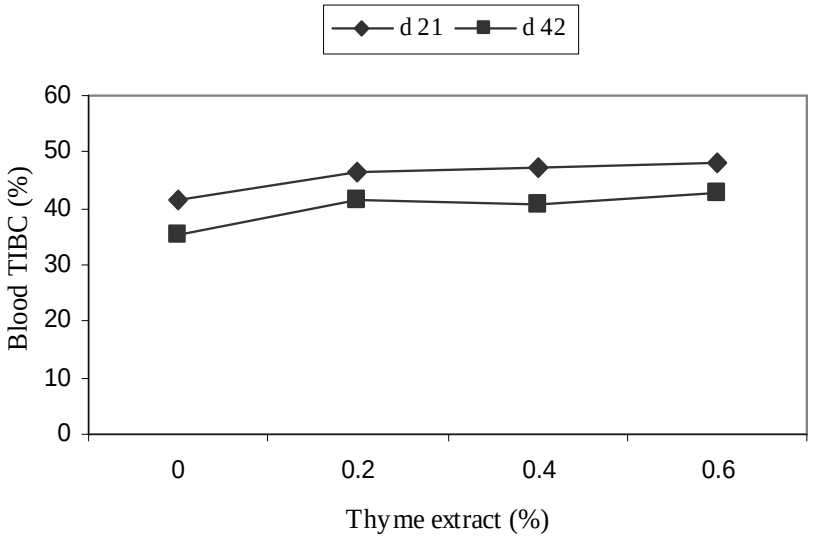

Figure 2. The relationship between the level (%) of *Thymus vulgaris* supplementation in drinking water and blood TIBC percent at day 21(quadratic regression, $y = 28.8 + 8.64x - 0.068x^2$, P<0001, R2 = 0.73; liner regression, y = 19.68 - 0.309X, P<0001, R2 = 0.62) and day 42 of age (quadratic regression, $y = 22.8 + 8.86x - 1x^2$, P<0001, R2 = 0.71; liner regression, y = 17.04 - 0.287x, P<0001. R2 = 0.61) in broiler chickens.

used the piglets in an anemia study. Zhang et al. (1989) investigated the iron bioavailability from meat, spinach and their mixtures in anaemic and non anaemic rats. Zeyuan et al. (1998) examined the effects of green tea and black tea on metabolisms of mineral elements in old rats. Greger and Lyle (1988) evaluated the iron, copper and zinc metabolism in rats that were fed the various levels and types of tea. The chicken model was used to study the thyme effects on iron metabolism. Nevertheless chicken has not been used for this purpose but it was

used as a model to study the cystic fibrosis (Craig-Schmidt et al., 1986) and proposed to study the cause of Kashin-Beck disease (Cook, 2000) in human.

No effects of thyme extract on the amounts of blood Hct and Hb and RBC were observed in present study. Consistently results have been published regarding the nonexistence effects of phenolic compounds on these indices. No association was found between the black tea consumption and either of blood hemoglobin or ferritin concentrations in adult South African (Hogenkamp et al., 2008). Siegenberg et al. (1991) did not indicate any changes of blood hemoglobin concentration in human subjects that fed from a bread meal containing different proportions of phytate-containing maize bran (14 to 58 mg) or from a bread meal with increasing doses of tannic acid (12 to 833 mg) in his attempt to explain the inhibitory effects of polyphenols and phytates on iron absorption by ascorbic acid,. In other consistent results, Wren et al (2002) observed no changes of blood hemoglobin, RBC or MCV in Dawley rats fed 0, 0.5, 1.0, or 2.0% grape seed extract for a period of 90 days but show decreased serum iron levels in rats fed the high-dose extract.

As expected thyme extract supplementation decreased the blood iron concentration in broiler chickens. There is no information regarding the inhibitory effects of thyme on iron absorption but in a contradictory research, no effects of red (high polyphenol contain) and white (low polyphenol contain) beans (*Pha seolus vulgaris L*) was indicated on iron bioavailibilities in pigs (Tako et al., 2009). However, the inhibited absorption of iron by phenolic compounds from other plants or their extracts is in agreement with the results of our experiment. Drinking of 200 ml tea (prepared from 5 g dry tea) by individuals inhibited the iron absorption from solutions of $FeCl_3$ and $FeSO_4$, bread, a meal of rice with potato and onion soup. The authors connected this inhibition effects to the tannin contents of tea and the formation of insoluble iron-tannin complex in the intestinal lumen (Disler et al., 1975). Hurnell et al. (1999) observed the inhibited iron absorption by tea from a bread meal by 50-90% depending on the kind of the tea and the concentration of polyphenols in the brewed tea.

Thankschan et al. (2008) investigated the effects of drinking tea (1 or 2 cup black tea) on iron absorption in iron deficient and iron adequate foods with rice meal in women and observed an inhibited iron absorption by a similar amount in both groups and mentioned the low bioavailability of iron from plant-based diet containing mineral absorption inhibitors such as polyphenols and phytates as the reason. Moreover a negative correlation was observed between the amount of spinach and aubergine (rich in polyphenols) with iron absorption in men (Gillooly et al., 1983). As same as tea, spinach, aubergine and coffee, thyme extract have polyphenolic components such as essential oils (Escop, 2003). Thymol and carvacrol phenols are the main constituent volatile oils of thyme extract (Massada, 1976). Phenolic compounds influence the iron absorption by complexing iron in the intestinal lumen. The functional group of polyphenol compounds is an aromatic ring structure with one or more hydroxyl groups and combines with iron and causes destruction of iron absorption (Harborne, 1986). Moreover, phenolic compounds could inhibit the iron absorption by lowering the intestinal permeability (Harborne, 1986). King et al. (2008) examined the influence of the dietary polyphenols epigallocatechin-3-gallate (EGCG) and grape seed extract (GSE) on transepithelial iron transport in $CaCo^{-2}$ intestinal cells and reported the inhibited non heme iron absorption by polyphenol compounds from apical iron import in intestinal cells. Besides thyme contains the components such as tannins, glycosides and saponins (Escop, 2003) that affect the iron absorption. It was suggested that saponins may interfere with iron metabolism either by forming complexes with the dietary iron thereby unavailable for absorption or by producing changes in mucosal function with long-term consumption thus reducing the efficiency nutrients absorption (Southon et al., 1988a, b). Siddiqi (1994) reported the reduced iron absorption by *Cicer arietinum* consumption (rich in saponins) and the insoluble iron-saponin complex formation at the stomach pH. Thyme extract saponins and its low pH may be other reason of low iron absorption in our study. We used a *T. vulgaris* extract with low pH (pH = 5) that possibly has caused the greater decrease in gastrointestinal pH and consequently the easier formation of complex between saponin and iron in the intestinal lumen.

An opposite relations for blood TIBC and iron with the thyme extract supplementation were found in our experiment. This shows that thyme supplementation decreases the blood iron concentration and increases the blood iron requirements for maximum saturation of blood transferring since TIBC is the blood capacity to bind iron with transferrin or the amount of iron needed to 100% saturation of transferrin. Blood TIBC often increases in iron deficiency and decreases in choronic inflammatory disorders and hematochromatosis (Tietz, 1999). In the same way, Furugouri (1972) observed higher blood TIBC at 10 and 20 days of age in the pigs with anemia and unsupplemented ferrous fumarate diets than in pigs receiving ferrous fumarate.

In conclusion, supplementation of thyme extract in drinking water decreases the blood iron concentration in broiler chickens and possibly in human. Inhibited absorption of iron by phenolic compounds or the saponins in thyme extract is the possible reason.

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