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Evaluation of Trace Elements Serum Concentrations and Their Correlation Together, and with Thyroid Hormones in Water Buffalo (*Bulbalus bulbalis*)

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Abstract: To evaluate the serum concentration of manganese, copper, iron, selenium and zinc and their correlation together and with thyroid hormones in water buffalo (*Bulbalus bulbalis*), the serum concentrations of manganese, copper, iron, selenium and zinc and their correlation with triiodothyronine and thyroxine hormones were measured in 100 clinically healthy water buffaloes. The mean serum concentrations of manganese, copper, iron, selenium and zinc were 1.04 ± 0.09, 9.06± 0.79, 5.5 ± 0.34, 1.44 ± 0.13 and 46.78 ± 3.82 µmol/L, respectively. No significant differences were detected for measured microelements between the different age groups of buffaloes. Serum thyroxine had marginally significant correlations with manganese (r= 0.443, p= 0.065), selenium (r= -0.536, p= 0.072) and zinc (r= -0.358, p= 0.061). Also, Serum triiodothyronine had marginally significant correlations with zinc (r= -0.362, p= 0.058). Serum iron had significant correlations with copper (r= 0.21, p=0.045), zinc (r= 0.35, $p \leq 0.001$) and had a marginally significant correlation with selenium (r= -0.227, p=0.069). Also, serum zinc had significant correlations with copper (r= 0.248, p=0.016) and selenium (r= -0.251, p=0.042).

Key words: Thyroid hormones. Trace elements. Bulbalus bulbalis

INTRODUCTION

Trace elements such as manganese (Mn), copper (Cu), iron (Fe), selenium (Se) and zinc (Zn), are essential in animal nutrition and are needed in very small amounts for essential metabolic reactions in the body. Their deficiencies are often associated with alterations in many metabolic processes and cause various kinds of diseases. Deficiency of these trace elements causes severe economic loss due to increased susceptibility to oxidative stress, growth retardation in young animals, anemia (Bureau *et al*., 2008), decrease in feed efficiency and fertility (Grenier *et al*., 2003), enhance the virulence of the infectious agent (Failla, 2008) and decrease immune system function(Rink & Ibs, 2003 and Knutson & Wessling-Resnick, 2003).

On other hand, normal thyroid status is dependent on the presence of many trace elements such as Se, Fe, Cu and Zn, for both the synthesis and metabolism of thyroid hormones. Although the role of some of these elements such as iron, zinc and copper in the thyroid are less well defined, subor super optimal dietary intakes of these elements can adversely affect thyroid hormone metabolism (Nazifi *et al*., 2009).

To our knowledge, there is no previous report of the probable relationships between the serum profiles of trace elements and thyroid hormones status in water buffalo. Therefore, this study was undertaken to investigate the serum profiles and relationship between these parameters.

MATERIALS AND METHODS

The investigation was carried out on water buffaloes (*Bulbalus bulbalis*) which were slaughtered in a slaughter house reserved only for buffaloes in Ahvaz City, southwest of Iran, from July to September 2009.

After clinical examination, jugular blood samples in plane tubes, free from anticoagulant, were collected from 100 clinically healthy water buffaloes. Buffaloes were of both sexes, with different ages and were selected randomly. Age of animals was estimated using dental characteristics. All animals had grazed the previous summer on ranges around the city.

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The blood serum was separated after centrifugation at 750*g* for 10 min and the serum samples stored at -18 C until analysis. The samples with haemolysis were thrown away.

Digestion of serum was performed by a mixture of perchloric and nitric acid (3:7 ratios respectively). Manganese, copper, iron, selenium and zinc were measured using an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan). Serum triiodothyronine (T3) and thyroxine (T4) were measured using commercial ELISA detection kits (Pishtaz teb, Tehran, Iran).

The areas of validation for T3 and T4 assays included limits of detection and precision in standard curve following sample dilution, inter- and intra- assay coefficients of variation results were considered and Intra- and inter- assays for T4 and T3 were found to be below 5%, 4.6%, 4.7% and 5.4%, respectively.

Statistical analysis was performed using SPSS12 (Illinois, Chicago). Two sample t-tests were used to detect differences in the parameters between the two sexes. Correlations of each of the trace elements and thyroid hormones were analyzed by Pearson's correlation tests. Analysis of variance (ANOVA) tests were used to compare the trace elements between the different age groups of water buffaloes. Differences were considered significant at $p<0.05$.

Results:

Overall, 26 male buffaloes and 74 female buffaloes were sampled. The average ages (mean± SEM) of the male and female buffaloes were 3.135± 0.348 and 6.81± 0.414 years, respectively. The average age of female buffaloes was significantly more than male buffaloes. There were no significant differences between male and female buffaloes in serum concentrations of Mn, Cu, Fe, Se, Zn, T3 and T4. The results of the measurement of concentrations of serum Mn, Cu, Fe, Se, Zn, T3 and T4 in water buffaloes are shown in Table 1. Buffaloes were divided into three groups, according to their age as G1 ≤2 years, 2 years<G2 ≤5 years and, G3> 5 years.

Serum concentrations of T3 and T4 had no significant correlations with serum Mn, Cu, Fe, Se and Zn, but the correlation between serum T3 and T4 was significant ($r= 0.369$, $p<0.001$).

There were significant correlations between Cu and Zn ($r= 0.248$, $p=0.016$), Cu and Fe ($r= 0.21$, $p=0.045$), Zn and Fe ($r= 0.35$, $p\leq0.001$) and Se and Zn ($r= -0.251$, $p=0.042$). Also, the correlation between Se and Fe was marginally significant ($r= -0.227$, $p=0.069$).

There were no significant differences between the three age groups for serum concentrations of Mn, Fe, Se, Zn, T3 and T4. Because of the unequal variances, the Kruskal -Wallis test was used to compare Cu between the three age groups. This showed that the differences were marginally significant ($p=0.067$).

In the G1 group, serum T4 had marginally significant correlations with serum Mn ($r= 0.443$, $p= 0.065$) and Se ($r= -0.536$, $p= 0.072$). Also, serum Zn in the G2 group had marginally significant correlations with T4 ($r= -0.358$, $p= 0.061$) and T3 ($r= -0.362$, $p= 0.058$).

Discussion:

To the best of our knowledge, there is no previous research about the relationships between the serum profiles of trace elements and thyroid hormones status in water buffaloes, and the serum concentrations of Mn, Fe and Se, are reported for the first time for water buffalos.

The serum concentrations of measured microelements for water buffaloes in current study were somewhat different from the previously reported ranges for water buffalo (Akhtar *et al*., 2009) and other ruminants, including cattle and dromedary camel (Kaneko *et al*., 1997 and Nazifi *et al*., 2009). In comparison with the results of Akhtar *et al*'s (2009) study on Nilli-Ravi buffaloes during pregnancy and lactation, water buffaloes in current study had rather equal serum Cu and more serum Zn (about 5 times higher). Nazifi *et al*. (2009) measured same trace elements in the serum of dromedary camel in Iran. Serum concentrations of measured microelements were significantly different between water buffaloes and dromedary camels in Iran (Table 2).

According to our results, water buffaloes had less serum iron and more serum copper than the reference values of cow (Kaneko *et al*., 1997). The differences between water buffalo and other ruminants in the serum concentration of microelements may be regarded as physiological peculiarities due to their adaptation to environmental conditions and poor feeding resources.

Selenium, via the enzyme type 4 deiodinase, is required for conversion of T4 into the T3 (Awadeh *et al*., 1998). Although the ability of some tissues to maintain deiodinase activity and normal plasma T3 levels in the face of dietary selenium deprivation has been shown, it may be in association with a concomitant local preservation of selenium concentration (Nazifi *et al*., 2009).

The roles of iron, zinc and copper in the thyroid hormones synthesis and metabolism are less well defined, but their sub-or super optimal dietary intakes can adversely affect thyroid hormone metabolism (Aurthor & Beckett, 1999). In our study, in the G2 group, serum Zn had marginally significant negative correlations with

T3 and T4 hormones. A significant negative correlation between the zinc concentration of erythrocytes and serum thyroid hormones was shown in healthy male Herino lambs and Angora goats (Kececi & Keskin, 2002). Interrelationships among copper and iodine and thyroid hormones were studied in rats by Esipenko and Marsakova (1990) and Aurthor *et al*. (1996). Copper deficiency causes a decrease in the iodine metabolism in different organs and enhances the effect of hypothyroidism in rats (Aurthor *et al*., 1996).

An early study on humans with thyroid disease by Aihara *et al*. (1984) showed significant correlation between erythrocyte manganese concentrations and circulating T4 and T3 concentrations, suggesting that the thyroid hormone may affect manganese metabolism. On other hand, in agreement with other studies, some authors suggest that the role of manganese in thyroid hormone regulation and metabolism is not directly mediated by thyroid hormone synthesis (Soldin & Aschner, 2007).

We found a significant correlation between serum Fe and Cu. Some copper containing proteins, such as ceruloplasmin and hephaestin, are involved in iron transport (Latimer *et al*., 2003). Also, the correlation between serum Cu and Zn was significant. Zinc induces production proteins responsible for intestinal copper absorption (Reeves *et al*., 1998). Fe and Zn contents of the gastrointestinal tract affect the availability and absorption of Se in dairy cows (Harrison & Conrad, 1984). On the other hand, there might be other reasons for these changes.

In the current study no significant differences for trace elements were found between the sexes and age groups. Similar to our results, Zongping (2003) found no differences for trace elements between the sexes in Bactrian camels. In another study Badiei *et al*. (2006) found the same results in dromedary camels in Iran.

Essential trace elements are integral components of certain enzymes and of other biologically important compounds that have major physiological and biochemical roles (Zongping, 2003), and the incidence and importance of their deficiency are probably underestimated because subclinical forms of deficiency can go unnoticed for prolonged periods (Badiei *et al*., 2006). The serum concentrations of trace elements in domestic animals may vary according to geographic and dietary factors (Zongping, 2003) and further studies will be needed to evaluate the effects of diet, regional differences, season and physiological status of the animals on serum trace elements of water buffaloes in Iran.

Table 1: The concentrations (mean± SEM) of serum manganese, copper, iron, selenium, zinc (μmol/L), triiodothyronine and thyroxine (ng/mL) in Iranian water buffaloes

	Number of buffaloes	Manganese (μmol/L)	Copper (μmol/L)	Iron (μmol/L)	Selenium (μmol/L)	Zinc	Triiodothyronine (μmol/L)	Thyroxine (μmol/L)
All sampled buffaloes	100	1.04 ± 0.09	9.06± 0.79	5.5 ± 0.34	1.44 ± 0.13	46.78 ± 3.82	2.435 ± 0.11	6.297 ± 0.18
Male buffaloes	26	0.95 ± 0.11	11.02 ± 2.36	5.05 ± 0.64	1.27 ± 0.25	38.99 ±7.03	2.61 ± 0.25	6.47 ± 0.33
Female buffaloes	74	1.09 ± 0.11	8.185 ± 0.63	5.68 ± 0.37	1.52 ± 0.25	48.9 ± 4.59	2.36 ± 0.125	6.2 ± 0.22
G1 (≤2 years)	20	1 ± 0.14	12.8 ± 3.15	6.28 ± 1.02	1.14 ± 0.25	40.7 ± 8.7	2.7 ± 0.25	6.18 ± 0.48
G2(2 years < and ≤5 years)	30	1.3 ± 0.18	9.224± 0.94	5.34 ± 0.48	1.27 ± 0.25	42.9 ± 6.6	2.48 ± 0.24	6.44 ± 0.27
G3.(5 years<)	50	0.91 ± 0.09	7.24 ± 0.63	5.32 ± 0.43	1.64 ± 0.25	50.9 ± 5.5	2.28 ± 0.14	6.22 ± 0.27

Table 2: The concentrations (Mean± SEM) of serum manganese, copper, iron, selenium, zinc (μmol/L) in Iranian water buffalo, Iranian dromedary camel, Nilli-Ravi buffalo and cattle

	Iranian water buffaloes (Current study)	Iranian dromedary camel (Nazifi *et al*., 2009)	Nilli-Ravi buffaloes (Akhtar *et al*.,2009)(range)	Cow (Kaneko *et al*., 1997)
Manganese(μmol/L)	1.04 ± 0.09	0.025±0.005	------------------	-------------------
Copper(μmol/L)	9.06± 0.79	13.694±1.398	9.9±0.74-13.9±0.89	5.16-5.54
Iron(μmol/L)	5.5 ± 0.34	28.520±1.58	-------------------	10.2-29
Selenium(μmol/L)	1.44 ± 0.13	0.16±0.004	------------------------	-----------------
Zinc(μmol/L)	46.78 ± 3.82	10.429±0.975	9.51±0.72-11.66±0.91	------------------

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