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Serum Concentrations of Lipids and Lipoproteins and Their Correlations together and with Thyroid Hormones in Iranian Water Buffalo (*Bulbalus bulbalis*)

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ABSTRACT

To evaluate the concentrations of serum lipids and lipoproteins and their correlations together and with thyroid hormones in Iranian water buffalo (*Bulbalus bulbalis*), the serum concentrations of cholesterol, triglyceride, total lipids, very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol) and high density lipoproteins (HDL-cholesterol) and their correlations with triiodothyronine and thyroxine hormones were measured in 100 clinically healthy water buffaloes. The mean serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol were 4.15 ± 0.028 , 0.215 ± 0.005 , 2.06 ± 0.015 , 2.08 ± 0.025 and 0.43 ± 0.001 mmol L⁻¹, respectively. Also, the mean serum concentration of the total lipid was 2.9 ± 0.016 g L⁻¹. No significant differences were detected for the measured lipids and lipoproteins between the different age groups of buffaloes. Serum thyroxine had a significant correlation with cholesterol ($r = 0.239$, $p = 0.023$). Also, serum triiodothyronine had a significant correlation with HDL-cholesterol ($r = 0.52$, $p = 0.039$) in buffaloes up to 2 years of age, serum cholesterol had significant correlation with LDL-cholesterol ($r = 0.205$, $p = 0.05$) and a marginally significant correlation with HDL-cholesterol ($r = 0.187$, $p = 0.07$) and serum HDL-cholesterol and LDL-cholesterol had significant correlation ($r = -0.312$, $p = 0.03$). There is no previous research regarding the correlation of the thyroid hormones with the serum lipids and lipoproteins in water buffalo.

Key words: Thyroid hormones, lipids, lipoproteins, Iranian, *Bulbalus bulbalis*

INTRODUCTION

Studies on cholesterol, triglyceride and lipoproteins in domestic animals have made it clear that species variations exist and that, even within species, significant differences occur. The normal concentrations of serum lipids and lipoproteins of the cat, dog, sheep, cow, horse, pony, reindeer calf, cheetah and camel in various physiological conditions have been reported (Nazifi *et al.*, 2003; Kaneko *et al.*, 2008).

Thyroid hormones effect lipid metabolism by increasing lipolysis in adipose tissue and stimulating lipogenesis by increasing the activities of some enzymes (Eshratkhah *et al.*, 2010). The serum cholesterol level generally varies inversely with thyroid activity (Bruss, 2008; Gueorguieva and Gueorguiev, 1997), but there are some contradictory findings regarding the relation between serum thyroid hormones and cholesterol and triglycerides and in camels and goats the concentrations of thyroid hormones were not correlated with cholesterol levels (Wasfi *et al.*, 1987; Nazifi *et al.*, 2002).

There is little information about the serum lipids in water buffaloes and there is no information about the relation of serum thyroid hormones with lipids and lipoproteins. Although, the relation of serum thyroid hormones with lipids and lipoproteins have been evaluated in some species such as sheep, camel, goat and horse (Eshratkhah *et al.*, 2010; Wasfi *et al.*, 1987; Nazifi *et al.*, 2002, 2003, 2007, 2009), to the best of our knowledge, there is no previous report on the correlation of thyroid hormones with serum lipids and lipoproteins (cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in water buffalo. Therefore, this study was undertaken to investigate the serum profiles and the relationship between these parameters.

MATERIALS AND METHODS

The investigation was carried out on Iranian water buffaloes (*Bulbalus bulbalis*) which were slaughtered in a slaughter house reserved only for buffaloes in Ahvaz City, southwestern 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. The age of the animals was estimated using dental characteristics. All animals had grazed the previous summer on ranges around the city.

The blood serum was separated after centrifugation at 750 g for 15 min and the serum samples stored at -20°C until analysis. The samples with haemolysis were thrown away.

The serum was analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood, 1994), triglyceride by the enzymatic procedure of McGowan *et al.* (1983) and total lipids by the method described by Zollner and Kirsch (1962). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation and HDL-cholesterol was measured by the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate the non-HDL lipoproteins that had been sedimented by centrifugation (10000 g for 5 min). The residual cholesterol was then measured by the enzymatic method (Burtis and Ashwood, 1994). LDL-cholesterol was calculated as the difference between the cholesterol measured in the precipitate and in the HDL fraction. VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedwald *et al.*, 1972). Serum triiodothyronine (T₃) and thyroxine (T₄) were measured by radioimmunoassay (RIA) method (kits available from Immunotech Company, Immunotech-Radiova, Prague, Czech Republic) in the Jihad-Daneshghahi Research Center, Shiraz, Iran. The areas of validation for the T₃ and T₄ assays included the limits of detection and precision in the standard curve following sample dilution and inter- and intra-assay coefficients of the variation results were considered. Intra- and inter-assays for the T₄ and T₃ were found to be below 6.2, 8.6, 3.3 and 8.6%, 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 serum lipids and lipoproteins with the thyroid hormones were analyzed by Pearson's correlation tests. Analysis of Variance (ANOVA) tests were used to compare the serum lipids and lipoproteins 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,

Table 1: The concentrations (Mean±SEM) of serum cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in Iranian water buffaloes

	No. of buffaloes	Cholesterol (mmol L ⁻¹)	Triglyceride (mmol L ⁻¹)	Total lipids (g L ⁻¹)	HDL-cholesterol (mmol L ⁻¹)	LDL-cholesterol (mmol L ⁻¹)	VLDL-cholesterol (mmol L ⁻¹)	Triiodothyronine (µmol L ⁻¹)	Thyroxine (µmol L ⁻¹)
All sampled buffaloes	100	4.15±0.028	0.215±0.005	2.90±0.016	2.06±0.015	2.08±0.025	0.043±0.001	2.435±0.11	6.30±0.18
Male buffaloes	26	4.17±0.05	0.220±0.01	2.88±0.03	2.10±0.05	2.05±0.05	0.044±0.002	2.610±0.25	6.47±0.33
Female buffaloes	74	4.13±0.03	0.210±0.006	2.90±0.02	2.04±0.01	2.08±0.03	0.040±0.001	2.360±0.125	6.20±0.22
G ₁ (≤2 years)	20	4.14±0.08	0.240±0.01	2.90±0.03	2.10±0.06	2.06±0.06	0.050±0.002	2.700±0.25	6.18±0.48
G ₂ (2 years < and ≤5 years)	30	4.11±0.04	0.210±0.01	2.90±0.03	2.04±0.02	2.06±0.04	0.040±0.002	2.480±0.24	6.44±0.27
G ₃ (5 years <)	50	4.16±0.04	0.200±0.007	2.88±0.02	2.04±0.01	2.09±0.04	0.040±0.001	2.280±0.14	6.22±0.27

respectively. The average age of the female buffaloes was significantly more than that of the male buffaloes. There were no significant differences between the male and female buffaloes in the serum concentrations of cholesterol, triglyceride, total lipids, LDL-cholesterol and VLDL-cholesterol. The difference in the serum concentration of the HDL-cholesterol between the sexes was marginally significant ($p = 0.061$). The results of the measurement of concentrations of the serum cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in water buffaloes are shown in Table 1. The buffaloes were divided into three groups, according to their age as $G_1 \leq 2$ years, $2 \text{ years} < G_2 \leq 5$ years and, $G_3 > 5$ years.

The serum concentrations of T_3 and T_4 had no significant correlations with serum lipids and lipoproteins, but the serum T_4 and cholesterol had a significant correlation ($r = 0.239$, $p < 0.023$). There were significant correlations between the HDL-cholesterol and the LDL-cholesterol ($r = -0.312$, $p = 0.03$) and LDL-cholesterol and cholesterol ($r = 0.205$, $p = 0.05$). Also, the correlation between the HDL-cholesterol and cholesterol was marginally significant ($r = -0.187$, $p = 0.07$).

There were no significant differences between the three age groups for the serum concentrations of cholesterol, triglyceride, total lipid, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol ($p > 0.05$). Because of the unequal variances, the Kruskal-Wallis test was used to compare the HDL-cholesterol between the three age groups. This showed that the differences were not significant ($p > 0.05$).

Both sexes were evaluated separately. In the male buffaloes there was a significant correlation between the HDL-cholesterol and cholesterol ($r = 0.604$, $p = 0.003$). Also, the age had significant correlations with triglyceride ($r = -0.497$, $p = 0.019$) and VLDL-cholesterol ($r = -0.497205$, $p = 0.019$). In the female buffaloes cholesterol had a significant correlation with T_4 ($r = 0.258$, $p = 0.032$) and had a marginally significant correlation with T_3 ($r = 0.212$, $p = 0.08$). Also, the LDL-cholesterol had a significant correlation with the HDL-cholesterol ($r = -0.551$, $p < 0.01$) and had a marginally significant correlation with cholesterol ($r = 0.224$, $p = 0.06$). There was a significant correlation between the total lipid and age ($r = -0.265$, $p = 0.028$) and the correlation between the total lipid and the HDL-cholesterol was marginally significant ($r = -0.225$, $p = 0.06$).

In the G_1 group, serum T_4 had a significant correlation with the serum cholesterol ($r = 0.531$, $p = 0.034$) and serum T_3 had a significant correlation with the LDL-cholesterol ($r = 0.52$, $p = 0.039$).

DISCUSSION

To the best of our knowledge, there is no previous research regarding the correlation of the thyroid hormones with the serum lipids and lipoproteins (cholesterol, triglyceride, total lipids,

HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in water buffalo. Also, the serum concentrations of cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol are reported for the first time for water buffalos in Iran.

The serum concentrations of the measured serum lipids and lipoproteins for Iranian water buffaloes were somewhat different from the previously reported ranges for buffalo calves (Sivakanesan and Mariathan, 1996) and other ruminants, including cattle, goat and Iranian dromedary camel (Kaneko *et al.*, 2008; Nazifi *et al.*, 2002, 2009). In comparison to the results of Sivakanesan and Mariathan (1996) on buffalo calves, up to 6 months of age, in the current study water buffaloes had a higher serum cholesterol but a lesser serum triglyceride. Nazifi *et al.* (2009) measured the same lipids and lipoproteins in the serum of dromedary camel in Iran. Serum concentrations of the total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol were significantly different between Iranian water buffaloes and dromedary camels. Water buffaloes had higher serum cholesterol and triglyceride than the reference values of dromedary camels (Nazifi *et al.*, 2009). Water buffaloes had a higher serum total cholesterol and rather equal triglyceride than the reference values of cow (Kaneko *et al.*, 2008).

As expected, the serum cholesterol had significant correlations with the HDL- and LDL-cholesterol and there was a significant correlation between HDL- and LDL-cholesterol. Also, the correlation between the serum HDL cholesterol and the total lipids was significant. Nazifi *et al.* (2009) found the same correlations and results in camels. In water buffalo, the serum lipid profiles were also related with the thyroid hormones. According to our results, serum T_4 had a significant correlation with the total cholesterol and the serum LDL-cholesterol had a significant correlation with T_3 . There are some contradictory findings regarding the relation of the serum concentrations thyroid hormones with the cholesterol and triglyceride concentrations. The serum cholesterol level generally varies inversely with thyroid activity. The net effect of thyroid hormones on the cholesterol metabolism is to increase the rate of cholesterol catabolism by liver (Bruss, 2008; Gueorguieva and Gueorguiev, 1997; Mansourian, 2010). However, in camels, male goats and fat tailed sheep the concentrations of thyroid hormones were not correlated with cholesterol levels (Wasfi *et al.*, 1987; Nazifi *et al.*, 2002, 2007). In another studies Nazifi *et al.* (2007, 2009) found significant correlations between serum thyroid hormones and cholesterol levels and believe that, these discrepancies may be due to the hydration or health status of the animals. On the other hand, no correlation was found between serum concentrations of thyroid hormone and serum lipids, lipoproteins or triglycerides in goats (Nazifi *et al.*, 2007).

In the current study no significant differences for serum lipids and lipoproteins were found between the sexes. However, Sivakanesan and Mariathan (1996) found that female buffalo calves had higher blood total cholesterol than male calves from 14-18 weeks of age, but the HDL-cholesterol and triglyceride had no significant differences between either of the sexes until 7 months of age. Similar to our results, sex had no significant effect on the concentrations of cholesterol, triglyceride, total lipid, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in Turkman horses (Nazifi *et al.*, 2003).

According to our results, serum lipids and lipoproteins had no significant differences between the different age groups. In opposite to our results, Sivakanesan and Mariathan (1996) reported that in buffalo calves, up to seven months of age, the blood concentration of total cholesterol and HDL-cholesterol gradually increased until 22 weeks of age and then declined. But, they found that blood triglyceride had no significant differences between different ages. Also, age had a significant effect on the serum concentration of cholesterol, triglyceride, total lipid, the HDL cholesterol, LDL

cholesterol and VLDL cholesterol of Turkman horses, with the values being higher in older animals (Nazifi *et al.*, 2003). In another study, age had a significant effect on the serum triglyceride and VLDL-cholesterol of the male goats and the values were lower in older animals (Nazifi *et al.*, 2002). Same to our results, Bennis *et al.* (1992) reported that in kids, the concentration of all lipids was similar to mature goats. In calves, the cholesterol concentration increased transiently with age, but triglycerides showed no consistent change (Hugi and Blum, 1997). In the current study, separate evaluation of both sexes showed significant correlations of age with triglyceride and VLDL-cholesterol in male animals. Also, in the female buffaloes a significant correlation between the total lipid and age was found.

The cause of these findings and some contradictory findings regarding the relation between serum thyroid hormones and lipids and lipoproteins are not clear and may be due to the effect of some factors such as age, sex, health status, breed and pregnancy on serum lipids and lipoproteins profile. Also, geographic and dietary factors may affect the serum concentrations of lipids and lipoproteins in domestic animals and more work is required on a larger number of animals before the importance of these findings can be assessed.

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