

Evaluation of serum lipids and lipoproteins and their correlations together, and with thyroid hormones in gray necked ostrich

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Summary

To evaluate the concentrations of serum lipids and lipoproteins and their correlations together and with thyroid hormones in gray necked ostrich (*Struthio camelus*), the serum levels 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 tri-iodothyronine and thyroxine hormones were measured in 50 clinically healthy gray necked ostriches (30 male and 20 female) which were divided into three age groups. The mean serum concentrations \pm SE of cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol were 102.62 ± 30.23 , 120.22 ± 54.25 , 458.96 ± 106.37 , 29.96 ± 6.737 , 48.61 ± 23.11 and 24.04 ± 10.85 mgr/dL, respectively. Also, the mean serum concentrations \pm SE of thyroxine and tri-iodothyronine hormones were 7.89 ± 1.52 μ g/dL and 2.54 ± 1.31 ngr/mL, respectively. The mean serum concentration of the cholesterol, total lipids and T₃ had a negative significant correlation and age ($r = -0.340$, $P=0.016$), ($r = -0.335$, $P=0.017$), ($r = -0.282$, $P=0.047$), and a marginally negative significant correlation was detected between HDL-cholesterol with age ($r = -0.271$, $P=0.056$). Serum thyroxine and tri-iodothyronine had no significant correlation with the lipids and lipoprotein concentrations ($r = -0.115$, $P=0.083$), ($r = -0.187$, $P=0.070$).

Key words: Thyroid hormones, Lipids, Lipoproteins, Gray necked ostrich (*Struthio camelus*)

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).

The serum cholesterol level generally varies inversely with thyroid activity (Gueorguieva and Gueorguiev, 1997; Bruss, 2008), 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 gray neck ostriches and there is no information about the concentration of thyroid hormones and the relation of serum thyroid hormones with lipids and lipoproteins. 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 gray neck ostrich. 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 gray necked ostrich which were kept in an ostrich farm around Shiraz in the southwest of Iran. These ostriches diet included corn, hay, barley Calcium phosphate, lysine, methionine, NaCl, vitamins and minerals. The samples were collected in spring. Ostriches were of both sexes (30 male and 20 female), with different ages and were selected randomly. The ostriches were divided into three groups, according to their age as $G_1 \leq 12$ months (n:15), $12 < G_2 < 23$ months (n:15) and, $G_3 \geq 24$ months (n:20). After clinical examination, jugular blood samples were collected from 50 clinically healthy gray necked ostriches into plane tubes, free from anticoagulant. 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 hemolysis were thrown away.

The serum was analysed 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 ultra-centrifugation, 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 precipitated 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 (Friedewald *et al.*, 1972). Serum tri-iodothyronine (T_3) and thyroxine (T_4) were measured by radioimmunoassay (RIA) method (kits available from Immunotech Company, Immunotech-Radiova, Prague, Czech Republic) in the Jahad-Daneshgahi Research Center, Shiraz, Iran. The areas of validation for the T_3 and

T_4 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 T_4 and T_3 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 analysed by Pearson's correlation test. One-way analysis of variance (ANOVA) tests were used to compare the serum lipids and lipoproteins between the different age groups of gray neck ostriches. Differences were considered significant at $P < 0.05$.

Results

Overall, 30 male ostriches and 20 female ostriches were sampled. There were no significant differences between the male and female ostriches in the total serum parameters measured in this study. The results of the measurement of concentrations of the serum cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, thyroxine (T_4) and tri-iodothyronine (T_3) in gray neck ostriches are shown in Table 1. The serum concentrations of T_3 and T_4 had no significant correlations with the serum lipids and lipoproteins ($r = -0.187$, $P=0.070$) ($r = -0.115$, $P=0.083$).

There were significant differences between the three age groups for the serum concentrations of cholesterol, total lipid, LDL-cholesterol and T_3 ($P < 0.05$).

The mean serum concentration of the cholesterol, total lipids and T_3 had a negative significant correlation with age ($r = -0.340$, $P=0.016$), ($r = -0.335$, $P=0.017$), ($r = -0.282$, $P=0.047$) and a marginally negative significant correlation was detected between HDL-cholesterol with age ($r = -0.271$, $P=0.056$). But no significant correlations were seen between lipids, lipoproteins, T_3 and T_4 with sex. There was a severe positive significant correlation between the VLDL-cholesterol and total lipids ($r = 0.866$, $P=0.000$), and the correlation between the

Table 1: The concentrations (mean±SEM) of serum cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol T₃ and T₄ in gray necked ostriches

| | Number of ostriches | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Total lipids (mg/dL) | HDL-cholesterol (mg/dL) | LDL-cholesterol (mg/dL) | VLDL-cholesterol (mg/dL) | Tri-iodothyronine (ng/mL) | Thyroxine (µg/dL) |
|-----------------------|---------------------|---------------------|----------------------|----------------------|-------------------------|-------------------------|--------------------------|---------------------------|-------------------|
| All sampled ostriches | 50 | 102.62 ±30.23 | 120.22 ±54.25 | 458.96 ±106.37 | 29.96 ±6.737 | 48.61 ±23.11 | 24.04 ±10.85 | 2.54 ±1.31 | 7.89 ±1.52 |
| Male ostriches | 30 | 106 ±35.05 | 114 ±22.43 | 460 ±113.67 | 30.7 ±8.358 | 52.93 ±18.05 | 22.87 ±9.002 | 2.66 ±1.25 | 7.61 ±1.33 |
| Female ostriches | 20 | 96.8 ±30.03 | 129 ±27.006 | 457 ±103.02 | 28.85 ±6.01 | 42.14 ±15.03 | 25.81 ±14.001 | 2.37 ±1.125 | 8.31 ±1.22 |
| G1 (<12 months) | 15 | 85.11 ±23.08 | 134.33 ±25.01 | 440.56 ±100.03 | 31.56 ±6.06 | 26.69 ±9.06 | 26.87 ±13.002 | 3.16 ±1.25 | 7.67 ±1.48 |
| G2 (12<age<23 months) | 15 | 112.97 ±33.04 | 120.66 ±51.01 | 489.81 ±120.03 | 30.69 ±6.02 | 58.15 ±34.04 | 24.13 ±10.002 | 2.6 ±1.24 | 8.18 ±1.27 |
| G3.(≥24 months) | 20 | 83.33 ±21.04 | 104.56 ±17.007 | 403.22 ±90.02 | 25.78 ±4.01 | 36.64 ±20.04 | 20.91 ±6.001 | 1.71 ±0.94 | 7.11 ±1.27 |

VLDL-cholesterol and the triglyceride ($r = 1.000$, $P=0.000$), as well as the correlation between the VLDL-cholesterol and cholesterol ($r = 0.524$, $P=0.000$). There was a severe positive significant correlation between the LDL-cholesterol and total lipids ($r = 0.657$, $P=0.000$), and a severe positive correlation between the VLDL-cholesterol and cholesterol ($r = 0.919$, $P=0.000$). Also, there was a positive significant correlation between the LDL-cholesterol with HDL-cholesterol ($r = 0.308$, $P=0.000$). Positive significant correlations between the HDL-cholesterol and total lipids ($r = 0.370$, $P=0.008$) and HDL-cholesterol and cholesterol ($r = 0.498$, $P=0.000$) were also seen, and positive significant correlations between total lipids and cholesterol ($r = 0.880$, $P=0.000$), total lipids and triglyceride ($r = 0.866$, $P=0.000$), and cholesterol and triglyceride ($r = 0.524$, $P=0.000$) were found.

Discussion

To the best of our knowledge, no previous research exists 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 ostrich. Also, the serum concentrations of T₃ and T₄ are reported for the first time for ostriches worldwide.

The serum concentrations of the measured serum lipids and lipoproteins for gray neck ostriches were somewhat different from the previously reported ranges for ostriches. In comparison to the results of

Renata *et al.* (2008), on 30-day-old ostriches, and Chegeni *et al.* (2008), on 15 male and 15 female ostriches, in the current study gray neck ostriches had lesser serum lipids and lipoproteins. Palemeque *et al.* (1991) measured the serum cholesterol in adult and young ostriches (ostriches with 5 months of age). The serum concentration of the cholesterol had no significant difference between the adult and the young ostriches (Palemeque *et al.*, 1991). The serum cholesterol of gray neck ostriches in the current study was the same as the Okotie-Eboh *et al.* (1992) report. In comparison to the results of Khazrainia *et al.* (2006) on ostriches, in the current study gray necked ostriches had higher serum cholesterol but a lesser serum triglyceride. These differences may be due different nutrition.

The serum cholesterol had significant correlations with the HDL- and VLDL-cholesterol, and there was a significant correlation between HDL- and LDL-cholesterol. There were significant correlations between the HDL-, LDL-, VLDL-cholesterol and total lipids. Nazifi *et al.* (2009) found the same correlations and results in camels, but the correlation between the serum VLDL-cholesterol and total lipids was severe. In gray neck ostriches the serum lipid profiles were not related to the thyroid hormones. Also, 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; Nazifi *et al.*, 2007). In another study, 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 the serum concentrations of thyroid hormone and serum lipids, lipoproteins or triglycerides in goats (Nazifi *et al.*, 2007). However, in other animals 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 (Gueorguieva and Gueorguiev, 1997; Bruss, 2008).

In the current study, no significant differences for serum lipids and lipoproteins were found between the sexes. 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 Turkmen horses (Nazifi *et al.*, 2003).

According to our results, serum total lipids, triglyceride, HDL-, VLDL-cholesterol T_3 and T_4 had no significant differences between the different age groups, but cholesterol and LDL-cholesterol showed significant differences between the different age groups. There were significant differences between serum cholesterol and LDL-cholesterol in the second age group with the first age group, but there were no significant differences between serum cholesterol and LDL-cholesterol in the first and thirtieth age groups. 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 the blood triglyceride had no significant differences between different ages. 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). Age had a significant effect on the serum concentration of cholesterol, triglyceride, total lipid, the HDL cholesterol, LDL cholesterol and VLDL cholesterol of Turkmen horses, with the values being higher in older animals (Nazifi *et al.*, 2003). Age also 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). Also, in the gray neck ostriches negative significant correlations between the cholesterol, HDL-cholesterol, T_3 , total lipid and age were found ($P < 0.05$).

In comparison, the results of the current study to the results of other researches indicate that serum cholesterol concentration is related to age, but serum triglyceride concentration is not under age effects. In this study, there was a negative correlation between cholesterol and age ($r = 0.794$, $P = 0.004$).

In the present study in gray neck ostriches, the T_4 concentration was higher than the T_3 concentration. The mean concentration of T_3 in gray neck ostriches had negative significant differences between the different age groups, but there was no correlation between the T_3 concentration and age. According to our results, triiodothyronine had significant differences between the different age groups. The higher T_3 concentration was in the first age group (under 12 months of age) and the lesser T_3 concentration was in the third age group (up to 23 months of age). The differences can be due to different metabolic conditions in different ages. T_3 is an active thyroid hormone that is very effective in body metabolism. Cebulj-Kadunc *et al.* (2003) reported that in the Lippizan horse, age, sex and pregnancy have significant effects on the serum concentrations of T_3 and T_4 hormones. The lesser serum concentration of T_4 was observed in ≥ 16 -year-old horses and the lesser serum concentration of T_3 was observed in 1-3-year-old horses. In this study, the serum concentration of T_3 in male was higher than in female ostriches and the serum concentration of T_4 in female was

higher than in male, but a significant correlation between T₃ and T₄ with sex was not observed ($P \geq 0.05$). Cebulj-Kadunc *et al.* (2003) reported that T₄ concentration in male Lippizan horses is significantly higher than in females.

The cause of these findings is not clear and may be due to the effect of some factors such as age, sex, health status and breed on serum lipids and lipoproteins profile. Also, geographic and dietary factors may affect the serum concentrations of lipids and lipoproteins in ostriches and more work is required on a larger number of birds before the importance of these findings can be assessed.

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