

Serum Biochemistry and Haematology of Iranian Red Sheep (*Ovis orientalis gmelini*) in Sorkhabad Protected Area, Zanjan, Iran: Comparison with Age and Sex

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Abstract

BACKGROUND: Comprehensive studies in blood values have been somewhat reported in wildlife animals but this study is the first one in Iranian red sheep.

OBJECTIVES: The present study was aimed to reveal alterations of some biochemical and hematological parameters in Iranian red sheep (*Ovis. Orientalis gmelini*) in the Zanjan province (Sorkhabad protected area), Iran.

METHODS: Blood samples were obtained from 52 clinically and para-clinically healthy Iranian red sheep with different age (under 2 years, 31 sheep and over 2 years, 21 sheep) and sex (37 male and 15 female). Thereafter, some hematological parameters such as RBC, PCV, Hb, MCH, MCV, MCHC, WBC, Differential leukocyte count along with biochemical parameters (T3, T4, glucose, total protein, albumin, cholesterol, HDL, LDL, triglyceride, creatinine, urea, calcium, phosphorus, magnesium, ALT, AST, GGT, ALP, CK) were analyzed.

RESULTS: The results indicated significant alterations ($p < 0.01$) of T3, T4, phosphorous, magnesium, GGT and ALP in both sex and age groups, significant alterations in glucose and creatinine only in sex group and significant changes based on age in cholesterol, HDL and LDL. No significant changes were observed in the other parameters.

CONCLUSIONS: In Iranian red sheep, some parameters of serum biochemistry indicate significant changes based on gender and age that should be considered in laboratory tests of animal health. Meanwhile, further research should be performed in this regard.

KEYWORDS: hematology, *Ovis orientalis gmelini*, serum biochemistry

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Introduction

Four breeds of Iranian wild sheep include (*Ovis orientalis argali*, *Ovis Orientalis gmelini*, *Ovis orientalis esphahanica* and *Ovis orientalis laristanica*) as well as *Ovis Orientalis gmelini* are the predominant breed in northwest of Iran. *Ovis orientalis gmelini* (Iranian red sheep and/or Armenian mouflon) is an endangered species in Iran and it is better that more attention be given to it (Mostaghni et al., 2005). The blood analysis may be applied to evaluate the physiological and health conditions of domestic or wild animals, and may provide habitat quality, nutrition, disease, and other environmental conditions (Perez et al. 2003). For this purpose, it should be compared with normal values or reference ones (Kaneko et al., 2008). Different factors such as age, sex, nutrition conditions, stress, blood sampling method, season and physical activity can influence on those intervals in many species (Ozarda 2016). In this issue, determination of precise biochemical and haematological values of healthy wildlife animals is shown to be difficult in those populations.

Since the wild animals possess importance in veterinary medicine, more data from hematology and biochemistry of wildlife animals such as mouflons (for example *ovis orientallis gmelini*) are needed to prepare recommendations and interpretations for management and control of disease in the suffered populations (Volmer and Hecht 2006). In general, several studies have reported the haematological and biochemical parameters of captive wild sheep (Pošiváková et al., 2019; McDonald et al. 1981; Rathwa et al. 2017; Marco et al. 1998).

To our knowledge, this study is the first one to prepare some of haematological and serum biochemistry parameters in the Irani-

an red sheep (*Ovis orientalis gmelini*) which may be utilized for monitoring the health conditions and evaluation of metabolic status of Iranian red sheep. Furthermore, comparison of parameters with different age and sex may assist us in better interpretation of blood parameters. This study was aimed to evaluate blood parameters of Iranian red sheep (*Ovis orientalis gmelini*) in Sorkhabad protected area with use of uniform capture method in those ones with sex and age variations.

Materials and Methods

Study area and animals

This study was conducted in Zanjan province (northwest of Iran) during May 2014. The captive Iranian red sheep were in a limited hunting region, located in the Sorkhabad protected area, Zanjan province, Iran. The nutrition conditions including, freely available grass hay and occasionally, beans along with water. Animal health status was evaluated through clinical and para-clinical examination such as assessment of presence or absence of any blood parasite and egg parasites in feces in 76 mouflons and finally 52 cases (37 males and 15 females) were selected at the age of under 2 years and over 2 years.

Blood sampling

Before sampling, all animals were manually restrained with relatively little stress and no tranquilizer was applied. The needed raw materials and devices for preparing blood samples such as centrifuge instrument were used in this study. Blood samples (ten milliliters) were collected via the jugular vein and then eight milliliters of blood was transferred to plain tubes for sera preparation and after standing for 20 min, were centrifuged 3000 rpm for 10 min in that location with the help

of power supply. Remained two milliliters of whole fresh blood was anticoagulated with ethylene diamine tetra-acetic acid (EDTA) for hematology determination. Thereafter, all samples (sera and EDTA-contained blood samples) were transferred (with ice) to the laboratory during a one-hour period. All 52 cases were sampled during one week and obtained daily blood samples for haematological parameters were analyzed in the same day and all of sera were kept at -20 °C and were determined in the one of medical laboratories of Zanjan. Haematological parameters analysis was manually performed by standard methods (Jain, 1993). Differential counts of leukocyte were carried out through Giemsa (5%)-stained blood films. Packed cell volume (PCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were determined in all samples. Haemoglobin (Hb) was determined by a cyanmethaemoglobin method.

The sera parameters included T3, T4, calcium (Ca), phosphorous (P), magnesium (Mg), urea, creatinine, glucose, total protein (TP), albumin (Alb), cholesterol (Chol), triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and creatine-kinase (CK). Serum T3 and T4 were measured using a Roche Co. Elecsys 2010 in accordance with the electrochemiluminescence (ECL) method and other selected parameters were detected by (Pars Azmoon Co. kits, Tehran, Iran) using a Hitachi-917 Auto analyzer (Japan). It is worth mentioning that, serum HDL and LDL were detected based on precipitation method.

Data statistical analysis

Statistical analysis was accomplished in all analyses. The Mean \pm SD was determined and for comparing male Iranian red sheep with female ones and to compare those under 2 years of age with over 2 year ones, data results were analyzed with Wilcoxon rank-sum test through SAS v9.1 (SAS Institute Inc., Cary, NC, USA). In addition, the significance level was specified at ($p < 0.01$) (Didara et al., 2011).

Results

All data as hematological and biochemical parameters in the healthy Iranian red sheep are revealed in Tables 1 and 2. As regards hematological alterations (Table 1), no significant changes ($p < 0.01$) were obtained in those parameters based on sex and age. In terms of serum biochemistry, remarkable changes ($p < 0.01$) were observed. Thyroid hormones (T3 and T4) were significantly higher ($p < 0.01$) in male group than female ones and over 2 years' group compared with under 2 years. In both sex and age, notable alterations ($p < 0.01$) were shown in phosphorous, magnesium, GGT and ALP. Based on sex category, significant changes ($p < 0.01$) were observed only in glucose and creatinine. According to age category, alterations of cholesterol, HDL and LDL were considerable. Finally, in the other parameters, no significant changes were indicated in all groups.

Discussion

In present study, in parameters based on age and sex, no significant changes were revealed in all haematological parameters. Rathwa et al. (2017) reported no difference in the sex in MCH and WBC in indigenous sheep which is consistent with our study based on sex. In terms of leukocytes, no sta-

Table 1. Mean \pm SD of Haematological parameters of wild sheep (*O. orientalis gmelini*) in Zanjan, Iran (PCV, packed cell volume, Hb haemoglobin, RBCs red blood cells, WBCs white blood cells, Neutrophils, Lym lymphocytes, Mono monocytes, Eos eosinophils, Baso basophils)

| Gender and age | Number of animals | PCV (l/l) | Hb (g/dl) | RBC ($\times 10^{12}/l$) | MCV (fl) | MCH (pg) | MCHC (g/dl) | WBC ($\times 10^9/l$) | Neutrophil (%) | Lymphocyte (%) | Monocyte (%) | Eosinophil (%) | Basophil (%) |
|----------------|-------------------|-----------------|-----------------|----------------------------|------------------|------------------|------------------|-------------------------|------------------|------------------|-----------------|-----------------|-----------------|
| Male | 37 | 0.44 \pm 0.01 | 15.81 \pm 5.8 | 11.1 \pm 0.5 | 38.25 \pm 3.50 | 14.2 \pm 1.26 | 39.5 \pm 7.8 | 7.32 \pm 1.12 | 26.12 \pm 2.11 | 70.56 \pm 4.12 | 2.19 \pm 0.42 | 1.55 \pm 0.12 | 0.12 \pm 0.02 |
| Female | 15 | 0.43 \pm 0.02 | 13.61 \pm 5.1 | 11.4 \pm 0.7 | 36.41 \pm 2.5 | 13.68 \pm 0.87 | 38.95 \pm 9.1 | 8.15 \pm 1.49 | 27.64 \pm 1.2 | 68.84 \pm 3.18 | 2.52 \pm 0.68 | 1.41 \pm 0.10 | 0.13 \pm 0.01 |
| p \leq 0.01 | 0.941 | 0.047 | 0.0854 | 0.0854 | 0.048 | 0.0671 | 0.0643 | 0.0649 | 0.0629 | 0.0511 | 0.0883 | 0.0819 | 0.0765 |
| Under 2 years | 31 | 0.44 \pm 0.03 | 14.1 \pm 11.5 | 11.2 \pm 0.65 | 34.56 \pm 3.27 | 13.49 \pm 1.55 | 39.73 \pm 11.6 | 8.22 \pm 1.81 | 25.81 \pm 2.12 | 69.72 \pm 3.42 | 3.13 \pm 0.11 | 1.68 \pm 0.18 | 0.14 \pm 0.01 |
| Over 2 years | 21 | 0.39 \pm 0.01 | 13.76 \pm 4.2 | 10.5 \pm 0.9 | 36.81 \pm 1.82 | 14.32 \pm 0.71 | 38.54 \pm 12.3 | 7.33 \pm 1.42 | 26.59 \pm 2.24 | 71.19 \pm 2.19 | 2.28 \pm 0.33 | 1.52 \pm 0.09 | 0.12 \pm 0.04 |
| p \leq 0.01 | 0.0341 | 0.0854 | 0.0873 | 0.0873 | 0.0489 | 0.0763 | 0.0753 | 0.0789 | 0.0873 | 0.0517 | 0.0809 | 0.0854 | 0.0805 |

Table 2. Mean \pm SD of serum biochemical factors of captive wild sheep (*Ovis orientalis gmelini*) in Zanjan, Iran.

| Gender and age | Number of animals | T3 (ng/ml) | T4 (μ g/dl) | Glucose (mg/dl) | Total Protein (g/dl) | Albumin (g/dl) | Cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | Triglyceride (mg/dl) |
|----------------|-------------------|----------------|------------------|-------------------|----------------------|-----------------|---------------------|------------------|------------------|----------------------|
| Male | 37 | 1.23 \pm 0.3 | 4.87 \pm 0.9 | 108.25 \pm 7.26 | 7.24 \pm 1.02 | 4.03 \pm 0.2 | 139.62 \pm 11.8 | 27.22 \pm 6.51 | 38.82 \pm 3.15 | 53.46 \pm 3.52 |
| Female | 15 | 2.69 \pm 0.8 | 2.27 \pm 0.3 | 72.12 \pm 3.52 | 7.39 \pm 0.6 | 3.86 \pm 0.3 | 136 \pm 5.1 | 29.15 \pm 4.82 | 36 \pm 4.39 | 57.71 \pm 4.68 |
| p \leq 0.01 | 0.034 | 0.038 | 0.003 | 0.078 | 0.066 | 0.051 | 0.054 | 0.055 | 0.047 | 0.047 |
| Under 2 years | 31 | 0.71 \pm 0.3 | 1.15 \pm 0.2 | 76.32 \pm 3.59 | 6.92 \pm 0.53 | 4.19 \pm 0.31 | 93.63 \pm 4.6 | 16.23 \pm 2.11 | 17 \pm 2.86 | 48.94 \pm 3.79 |
| Over 2 years | 21 | 1.89 \pm 0.6 | 4.58 \pm 0.5 | 73.15 \pm 4.28 | 7.39 \pm 0.43 | 4.35 \pm 0.23 | 126.4 \pm 10.3 | 24.62 \pm 3.41 | 29 \pm 3.14 | 52.47 \pm 2.81 |
| p \leq 0.01 | 0.039 | 0.014 | 0.049 | 0.068 | 0.066 | 0.024 | 0.017 | 0.028 | 0.041 | 0.041 |

Table 2 (continued). Mean \pm SD of serum biochemical factors of captive wild sheep (*Ovis orientalis gmelini*) in Zanjan, Iran.

| Gender and age | Number of animals | Creatinine (mg/dl) | Urea (mg/dl) | Calcium (mg/dl) | Phosphorus (mg/dl) | Magnesium (mg/dl) | AST (U/L) | ALT (U/L) | GGT (U/L) | ALP (U/L) | CK (U/L) |
|----------------|-------------------|--------------------|----------------|-----------------|--------------------|-------------------|--------------------|-------------------|------------------|--------------------|--------------------|
| Male | 37 | 1.31 \pm 0.56 | 17.6 \pm 3.8 | 4.21 \pm 0.15 | 2.25 \pm 0.16 | 4.81 \pm 0.08 | 219.23 \pm 39.28 | 38.32 \pm 11.10 | 69.86 \pm 9.25 | 171.64 \pm 23.41 | 356.21 \pm 41.02 |
| Female | 15 | 0.54 \pm 0.12 | 16.1 \pm 2.1 | 3.82 \pm 0.11 | 4.53 \pm 0.42 | 2.73 \pm 0.06 | 212.52 \pm 30.21 | 35.29 \pm 9.37 | 32.39 \pm 5.08 | 61.52 \pm 0.18 | 371.39 \pm 23.81 |
| p \leq 0.01 | 0.038 | 0.077 | 0.055 | 0.055 | 0.043 | 0.046 | 0.049 | 0.001 | 0.001 | 0.043 | 0.043 |
| Under 2 years | 31 | 0.85 \pm 0.03 | 16.3 \pm 2.4 | 4.29 \pm 0.65 | 4.12 \pm 0.17 | 4.49 \pm 0.05 | 234.36 \pm 29.25 | 36.62 \pm 9.03 | 72.33 \pm 7.12 | 162.69 \pm 10.32 | 299.45 \pm 23.81 |
| Over 2 years | 21 | 0.79 \pm 0.04 | 18.6 \pm 2.2 | 4.58 \pm 0.9 | 2.09 \pm 0.02 | 2.63 \pm 0.07 | 241.28 \pm 41.8 | 40.38 \pm 4.31 | 29.41 \pm 3.44 | 57.44 \pm 7.59 | 309.60 \pm 13.29 |
| p \leq 0.01 | 0.076 | 0.051 | 0.076 | 0.035 | 0.029 | 0.041 | 0.044 | 0.002 | 0.003 | 0.029 | 0.029 |

tistically significant alterations were denoted between sex and age.

Comprehensive reference values were detected for extensive biochemical parameters in Armenian mouflon. For calculation of reference values, parametric and non-parametric procedures were used in biology science (Borjesson et al., 2000). In present study, nonparametric method was selected to avoid making presumptions with respect to patterns dispensation of each parameter (Solberg 1999).

According to serum biochemistry, glucose concentration as the main analyte of carbohydrate metabolism accompanied with significant increase which is in agreement with the glucose concentration in the Iranian wild sheep (*O. orientalis esphahanica*) (Mostaghni et al., 2005) and (*O. orientalis musimun*) (Masek et al., 2009; Didara et al., 2011). In addition, no significant alterations were observed between age, but in terms of sex, high level of glucose was revealed in male than female. The level of glucose in domestic sheep (*O. aries*) and etorphine immobilized Nelson's desert bighorn sheep was relatively low (98 ± 28.5) (McDonald et al. 1981). The glucose levels in the captured mouflons and after repeated handling are known to be high (191.18 ± 69.97 , 145.08 ± 1.93 respectively) (Marco et al. 1997 ; Marco et al. 1998). Following capture stress in mouflon and other sheep breeds, catecholamine and glucocorticoid-induced hyperglycemia appears (Masek et al., 2009). Hence, this may have been the case in our study as glucose elevation (108.25 ± 7.26) can be attributed to glucocorticoids and catecholamines effects.

Based on sex and age, TP level in our study was found to be similar to several studies, Dall's sheep, Nelson's desert bighorn sheep, Rocky mountain bighorn sheep

and domestic sheep (Pošiváková et al., 2019; McDonald et al. 1981), which is consistent with other ones in wild sheep (*O. orientalis esphahanica*) (Mostaghni et al., 2005) and (*O. orientalis musimun*) (Masek et al., 2009). According to age and sex, Alb level is in accordance with McDonald et al.'s (1981) study in Nelson's desert bighorn, wild sheep (*O. orientalis isphahanica*) (Mostaghni et al., 2005) and (*O. orientalis musimun*) (Didara et al., 2011).

In present study significant increase was shown in creatinine levels in male mouflons than female ones and no significant changes in urea concentration were revealed either in sex or age. Low concentration of urea was reported by Pošiváková et al. (2019) and McDonald et al. (1981) for *O. canadensis* and Nelson's bighorn sheep respectively. It is well known that, creatinine elevation occurs during high muscular activity after capture. In addition, high level of urea and creatinine occur during capture due to catecholamines-induced kidney vasospasm and muscular activity (Masek et al., 2009). On the other hand, high creatinine value, likely without renal damage, has been reported by Cattet et al. (2003). In our study, creatinine elevation in male mouflons can be attributed either to muscular mass or breed characteristic, peculiar to this wild sheep in Iran which needs more research.

In the case of thyroid hormones, low levels of ($P < 0.01$) T3, T4 along with lipid profiles (Chol, HDL and LDL) were observed between under 2 years than over 2 years ones. We could not find any information about T3 and T4 in *O. orientalis gmelini* but the significant increase in T3 and T4 compared with adult ones has been reported in Tsigai lambs (Novoselec et al., 2009). Colavita et al., in 1983 demonstrated elevated T3 and

T4 levels in growing goats than adults and Todini (2007) reported increased levels of T4 in lactating goats in association with late pregnancy which is not in agreement with our study. In other words, Karapehliyan in 2007 determined low levels of T3 and T4 in Tuj ewes during lactation and dry period. It is postulated that certain factors such as low concentration of iodine in the soil of region, trace elements deficiencies like selenium and/or seasonal alterations cause low levels of T3 and T4 in Iranian red sheep (*O. orientalis.gmellini*). Lipid and lipoprotein metabolism are affected by thyroid hormones. T3 and T4 stimulate lipogenesis and elevate lipolysis in adipose tissue. Chol decrease due to catabolic effect of liver induced by T3 and T4 has been reported by Bartly (1994). Moreover, the reverse association between hypothyroidism and total cholesterol and LDL-cholesterol has been pointed out by Santi in 2010. Different factors may have influenced this decrease, such as seasonal effects and/or hypothyroidism.

The AST and ALT alterations were similar to those demonstrated for domestic sheep (Kaneko 1989), *O. orientalis esfahanica* (Mostaghni et al., 2005), *O. Canadensis* with different capture methods (Michael et al. 1987) and *O. musimon* (Didara et al., 2011).

There were not significant alterations in Ca level between all cases which is in accordance with Mostaghni et al., 2005 in *O. esfahanica*. The CK level in our study was found to be similar with the Masek et al in 2009 and Didara et al., 2011 studies in *O. musimon*.

Concerning ALP and GGT, their increase was observed in male sheep than female and under 2 years than over 2 years. Masek et al., in 2009 determined high level of ALP in male mouflon than female ones and linked its elevation to various osteoblast activity

and bone growth. Borjesson et al., 2000 and Perez et al., 2003 reported increased levels of ALP in male desert bighorn sheep and Spanish ibex respectively, whereas, GGT activity level in both studies did not alter between males and females. Perhaps, low levels of T3 and T4 and/or peculiar conditions involve in ALP and GGT enhancement. Higher mean values of Mg were determined than the age and sex. It was also higher than *Ovis ammon* (Marco et al. 1998). Furthermore, increased concentration of Mg has been reported by Masek et al., in 2009 in European mouflon and Spanish ibex (Perez et al. 2003).

In this study, it is possible that stress impresses on many of the obtained results of blood parameters in *O. orientalis.gmellini*. In line with this, stress and handling interfere in the demonstration of reference values in wild species. In a study, effects of repetitious handling upon mouflon blood parameters was performed by Marco et al. (1998) and it was resulted that acclimatization had not been achieved. Hence, despite all efforts to alleviate stress, apparent deviations of those results were determined from the accurate value. Generally, the results demonstrated that significant differences were observed in some blood values in *O. orientalis.gmellini*. The most likely explanation may be that Iranian red sheep are more prone to handling stress. It is worth mentioning that the evaluated mouflons number was approximately sufficient to authorize an acceptable definition for biochemical values and the fact that published literature in relation to alterations of biochemical parameters in *O. orientalis gmellini* is of no consequence, then it is possible the results of this study can be used as guidance for diagnosis and research. However, more detailed clarification is needed for identification of other variables effects

on those parameters.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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بیوشیمی سرم و هماتولوژی گوسفند قرمز ایرانی (*Ovis orientalis gmelini*) در منطقه حفاظت شده سرخ آباد، زنجان، ایران: مقایسه بر اساس سن و جنس

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چکیده

زمینه مطالعه: تا حدودی مطالعات جامعی در رابطه با مقادیر پارامترهای خونی در حیوانات وحش گزارش شده است، اما این مطالعه اولین بار است که در گوسفند قرمز ایرانی انجام می گیرد.

هدف: هدف از این مطالعه بررسی تغییرات برخی از پارامترهای بیوشیمیایی و هماتولوژیک در گوسفند قرمز ایرانی (*Ovis orientalis gmelini*) در منطقه حفاظت شده سرخ آباد زنجان می باشد.

روش کار: در این مطالعه نمونه خون از ۵۲ گوسفند قرمز ایرانی با سن (زیر ۲ سال ۳۱ راس و بالای ۲ سال ۲۱ راس) و جنس (۳۷ نر و ۱۵ ماده) که از نظر بالینی و آزمایشگاهی سالم بودند اخذ شد. در ادامه تعدادی از پارامترهای هماتولوژیک اعم از RBC, PCV, Hb, MCH, MCV, MCHC, WBC و شمارش تفریقی لکوسیت ها همراه با پارامتر بیوشیمیایی (T^4 , T^3 ، گلوکز، توتال پروتئین، آلومین، کلسترول، HDL، LDL، تری گلیسرید، کراتینین، کلسیم، فسفر، منیزیم، CK, ALP, GGT, AST, ALT) مورد تجزیه و تحلیل قرار گرفتند.

نتایج: نتایج به دست آمده حاکی از تغییرات معنی دار ($p > 0.01$) در T^4 , T^3 ، فسفر، منیزیم، GGT, ALP در هر دو گروه جنس و سن، تغییرات معنی دار در گلوکز و کراتینین فقط در گروه جنس و تغییرات معنی دار فقط در گروه سن در کلسترول، HDL و LDL بود.

نتیجه گیری نهایی: در گوسفند قرمز ایرانی برخی پارامترهای بیوشیمی سرم بر اساس جنس و سن تغییرات معنی داری را نشان می دهد که می بایست در بررسی های آزمایشگاهی وضعیت سلامتی حیوان مورد توجه قرار گیرد. البته در این ارتباط تحقیقات بیشتری نیز می بایست صورت گیرد.

واژه‌های کلیدی:

Ovis orientalis gmelini، بیوشیمی سرم، هماتولوژی