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Serum Protein Profile of Lori-Bakhtiari Ewes in Relation to Age, Body Weight, Birth Type and Birth Season

Research Article

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ABSTRACT

Serum protein profile could be considered as a useful indicator of physiological and pathological conditions, but several factors may interfere with the interpretation. This study was conducted to evaluate serum protein fractions in association with age, body weight, birth type and birth season in a meat-type sheep. Blood samples were randomly taken from 96 healthy Lori-Bakhtiari ewes in the Shooli breeding station in Iran. The sampled animals randomly belonged to different age, birth type, birth season and body weight groups. Total protein and other fractions were determined by Biuret and electrophoresis methods, respectively. IgG levels were measured by ELISA method. Total protein, albumin, total globulins, albumin/globulins ratio, α1, α2, β and γ globulins and IgG levels averages were 5.82, 2.35, 3.47, 0.69, 0.06, 0.42, 0.15, 2.82 and 1.56 g/dL, respectively. Total protein had high correlations with total and γ globulins, while albumin and IgG were almost independent from other fractions. Levels of serum protein fractions in adults were also independent from body weights at different ages. Serum protein profile was significantly affected by age and birth season ($P<0.05$), where by older ewes had lower levels of α2, γ and total globulins and higher albumin/globulin ratios. The winter-born ewes significantly had higher γ globulins levels and probably more immune system activities, compared to the spring-born ewes. Body weight and birth type did not have any significant effect on the studied serum protein fractions. This study suggested that the age and birth season should be considered for interpretation of the serum protein profiles.

KEY WORDS body weight, breeding value, health, immunology, sheep.

INTRODUCTION

Sheep is a multipurpose farm animal, with an important role in food supply and economic situation of many countries. Therefore, any production depression or mortality due to physiological disorders or disease outbreaks in sheep flocks may endanger economic status and food security of a large population all around the world. Therefore, regular monitoring of physiological and health indicators is necessary for economically viable farming. Managerial decisions for control of physiological and health conditions may be facilitated by use of phenotypic or genetic markers. Serum protein pattern can be used as a useful indicator of physiological status and an effective diagnostic aid for a wide range of infectious and inflammatory diseases, gastrointestinal disorders, immunodeficiency and paraproteinemia caused by plasma cell or lymphoid neoplasia (Tothova *et al*. 2016a). In other words, laboratory assessment of serum proteins concentrations is a useful diagnostic aid for clinicians and a valuable tool for construction of reference intervals for specific animal groups (Alberghina *et al*. 2010). Serum proteins are involved in several functions. Albumin is a multi-functional protein which can bind and transport many endogenous and exogenous substances (Merlot *et al*.

2014). Globulins area heterogeneous group of proteins, which based on of their electrophoretic mobility are classified as α, β and γ globulins, and include antibodies and other inflammatory molecules, hemostatic and fibrinolytic proteins, and carriers of various components such as lipids, vitamins, and hormones (Alberghina *et al*. 2010).

Serum protein profile is closely regulated, but could be altered by a wide spectrum of diseases and health disorders (Tothova *et al*. 2016a; Tothova *et al*. 2017). Moreover, serum proteins are involved in several physiological processes, such as immune responses, iron metabolism, haemostasis and apoptosis (Ignjatovic *et al*. 2011), thus their concentration abnormalities are related to pathological and physiological conditions.

In addition to physiological and health conditions, serum proteins profile is also affected by animal species and breed (Tschuor *et al*. 2008; Murariu *et al*. 2014; Njidda *et al*. 2014; Nagy *et al*. 2015). Reference values for ovine serum albumin and total globulins have been reported frequently, but only a few studies have been conducted on more detailed fractions, including α1, α2, β and γ globulins in sheep. Moreover, serum protein profile has been frequently studied in relation to age (Piccione *et al*. 2013; Tothova *et al*. 2016b; Da Cruz *et al*. 2017 and so on), but a few studies have been conducted on more factors such as body weight, birth type and birth season. A hypothesis is that all serum protein fractions are affected by various factors, and these factors should be considered for interpretation of serum protein profiles. The aim of this study was to assess the effects of body weight, birth type, birth season and age on different serum protein fractions, including albumin, total globulins, albumin/globulins ratio, and α1, α2, β and γ globulins in a native meat type breed of sheep and describe their variations in relation to age, birth type and birth season.

MATERIALS AND METHODS

Studied population

This study was performed on Lori-Bakhtiari flock of Shooli Sheep Breeding Station (32.31362˚ N, 51.05340˚ E), in Shahrekord, Chaharmahal va Bakhtiari province, Iran. The Lori-Bakhtiari sheep is one of the heaviest meat-type breeds in Middle East, reared in western and south-western areas of Iran (Almasi *et al*. 2020). This region is located in Zagros region which is the first historical place of sheep domestication (Zeder, 1999). The studied flock was located in a region, with annual temperature of -45.4 ˚C to 42 ˚C (average 11.8 ˚C), average precipitation of 323.3 mm, average humidity of 46% and 3144 sunny hours per year. The studied flock is managed in a semi-extensive system, where the animals graze on pasture during May to December, while in remaining months they are manually fed by the diets, formulated based on nutrient requirements of sheep (NRC, 2007), using common feed ingredients including alfalfa hay, wheat straw and barley grain. Breeding season in the studied population starts in late August and ends in late October and lambing begins in late January (Ghasemi *et al*. 2019). The studied flock is regularly treated against endoparasites, twice a year. A total of 96 healthy ewes with different ages (14, 22, 23, 20 and 17ewes in 2-3, 4, 5. 6 and+7 years of age, respectively) were randomly selected from the population. Male sheep were not included in this study, because male lambs are commonly sold before yearling age, thus only adult female animals were available in the population. Health status of the sampled animals was investigated based on different criteria, including rectal temperature, heart rate, appetite, fecal consistency and laboratory tests for parasites. All sampled animals were healthy and free of internal or external parasites.

Sampling and measurements

In September, blood samples (10 mL per animal) were collected from jugular vein, using vacutainer tubes and then centrifuged at 2100 g for5 minutes to separate serum fraction. Total serum proteins were determined by the Biuret method (Burtis and Bruns, 2014), using the TruCal U standard solutions (Pars Azmoon Co., Iran), by a Biochemistry Auto Analyzer (SinnowaD280, China). Different fractions of serum proteins were measured by electrophoresis on cellulose acetate strips (Helena Laboratories, Helena Biosciences, Gateshead, UK). The samples were layered on the strip and electrophoresed in voltage of 180V for 20 min. The strips were coloured by Ponceau colorant 4R for 15 min, discoloured by a destaining solution (100 mL distilled water+100 mL pure methanol+100 mL glacial acetic acid) and then immersed in 95% methanol for 30 s to dehydrate the materials. Finally, the strips were cleared by a clearing solution (70 mL methanol+30 mL glacial acetic acid+4 mL clearing aid) for 10 min and then were dried in70 ˚C for 10 min. The strips were scanned and the bands were read by the Helena electrophoresis interpretation software (Helena Laboratories, Helena Biosciences, UK). Concentrations of different serum protein fractions, including albumin and α1, α2, β and γ globulins (g/dL) were determined by multiplying of each fraction percentage by total protein level. IgG levels were measured by an Awareness ELISA reader (Stat Fax 3200, Awareness Technology, Inc., USA), using a sheep IgG ELISA kit, with intra and inter-assay CVs less than 8% and 10%, respectively (MyBioSource, USA).

Statistical analysis

Reference interval was defined as the range containing 95% of total observations (Geffré *et al*. 2009). The data were

subjected to three types of analysis. Descriptive statistics and pairwise rank correlation coefficients for the studied variables were estimated using the Proc MEANS and Proc CORR of the SAS software (SAS, 2013), respectively. Effects of different factors on the studied serum protein fractions were investigated using a general linear model, as follows:

$$Y_{ijkl} = \mu + A_i + B_j + S_k + \beta (EBV_{ijkl}) + e_{ijkl}$$

Where:
Y_{ijkl}: observation.
μ: overall mean.
A_i: age class (2-3, 4, 5, 6 and+7 years).
B_j: birth type (singleton and twin).
S_k: birth season (winter and spring).
β: regression coefficient of the studied parameter on EBV.
EBV_{ijkl}: estimated breeding value for body weight.
e_{ijkl}: residual effects.

No interaction was fitted in the model, because many subclasses did not have any observation. Least square means were compared based on Tukey-Kramer test at 0.05 level. Proc GLM of SAS (SAS, 2013) was used for general linear model analysis and least square means comparisons. Body weight could not be fitted in the model directly, because body weight was highly correlated to age and birth type, other independent variables in the model. Therefore, estimates of breeding values (EBVs) were fitted in the model, to include genetic potential of body weight. The EBVs were obtained for all population (15859 test-day birth to yearling body weight records from 4402 animals), using a fixed regression animal mixed model, containing fixed effects of sex, birth type, birth year, birth month, quadratic regression of body weight on age (day) and random effects of permanent environmental, direct additive genetic and maternal additive genetic effects. The EBVs were obtained based on Average Information algorithm of Restricted Maximum Likelihood (AI-REML), using Wombat software (Meyer, 2007).

RESULTS AND DISCUSSION

Five distinct bands, including albumin, α1-globulin, α2-globulin, β-globulin and γ-globulin were observed in serum protein electrophoresis. However, in half of animals the γ-globulin fraction was observed as a two-peak surge (Figure 1). Descriptive statistics for the studied serum proteins are presented in Table 1. Based on the obtained results, average of albumin level (2.346 g/dL) was lower than total globulins level (3.465 g/dL), whereby albumin/globulin ratio was 0.692. The γ globulin had the highest level (2.818 g/dL) among different serum globulin fractions. Based on coefficient of variation (CV), β and α1 globulins had the highest variations (90.89 and 85.20%, respectively) among the studied serum protein fractions (Table 1).

Correlation coefficients between different protein fractions are presented in Table 2. Total protein had significant correlations ($P<0.01$) with all fractions, other than α1 globulin and IgG. Total protein had high correlations with total and γ globulins (0.84 and 0.78, respectively). The highest correlation coefficient was observed between total and γ globulins (0.94). Serum albumin level did not have significant correlations with most of other protein fractions. Low correlation between albumin and β globulin levels (0.20) was an exception. There was no significant correlation between IgG and any other protein fraction. In other words, serum levels of albumin and IgG were almost independent from other protein fractions. Moreover, all serum protein fractions were also independent from body weights at birth and 3, 6, 9 and 12 months of age (Table 2).

In the general linear model analysis, serum protein profile was significantly affected by the age ($P<0.05$), whereby older ewes had lower levels of α2, γ and total globulins and higher albumin/globulin ratios. Birth season had a significant effect on γ globulin level, whereby winter-born ewes significantly had higher γ globulin levels, compared to the spring-born ewes ($P<0.05$). Other factors, including birth type and estimated breeding value for body weight, did not have any significant effect on the studied serum protein fractions. More detailed information on the generalized linear model (GLM) analysis and least square means comparisons are presented in Table 3. Number of the separated ovine protein fractions in electrophoresis, varies in different studies. Five serum protein fractions, observed in the present study (albumin and α1, α2, β and γ-globulins) agreed with Esmaeilnejad *et al*. (2014) and Zamani *et al*. (2016). In the present study, the γ-globulins fraction migrated as two fractions in half of the studied animals (Figure 1), which agreed with Nagy *et al*. (2015), who observed γ1 and γ2 globulins in Merino breed of sheep. Generally, it has been found that the γ-globulins in cattle and goats is observed as a single fraction, while they may form two sub-fractions in sheep (Tothova *et al*. 2016a). The observed averages of total protein, albumin, total globulins, albumin/globulins and γ globulin levels in this study were in the range, reported in literature, but α1, α2 and β globulins were to some extent different from most of the reported values in literature (Table 4). Possible differences between the observed levels of α1, α2 and β globulins in the present study and those in literature could be attributed to possible differences in breed properties, environmental factors and health conditions in the studied populations.

The globulins are a heterogonous group of blood serum proteins, which can be classified as α, β and γ globulins.

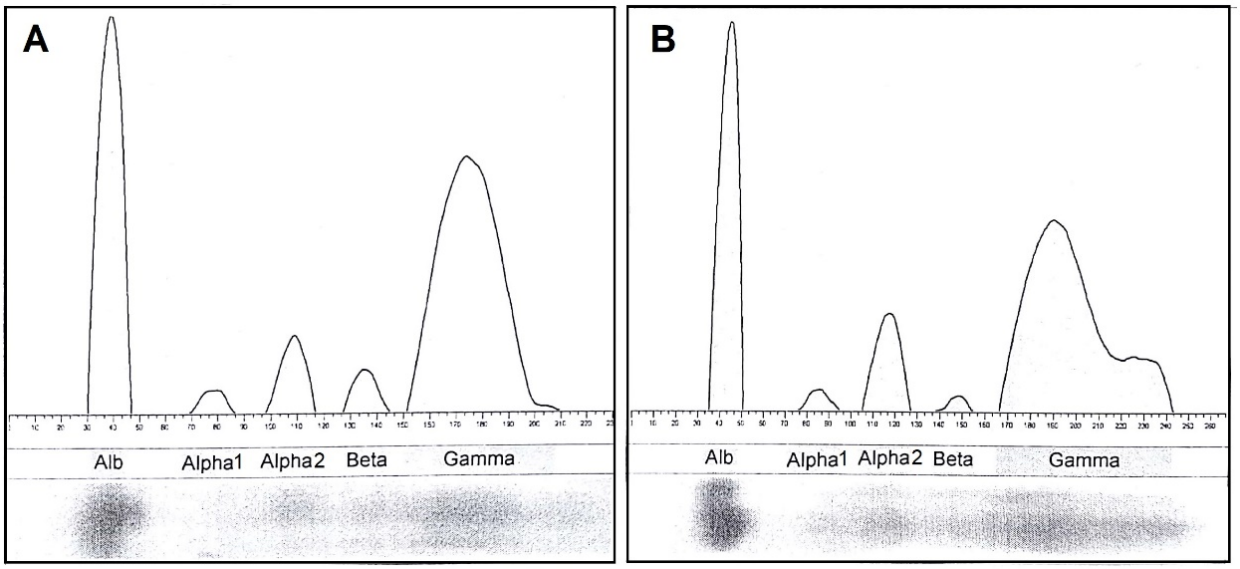

Figure 1 Two electrophoretograms, representing five distinct bands for albumin and α1, α2, β and γ-globulins
The γ-globulins, as a single (A) or two-peak (B) fractions

Table 1 Descriptive statistics for the studied serum protein variables and body weight traits (n=96)

Variable	Average	SD	Min	Max	CV (%)	Reference interval
Total protein (g/dL)	5.817	0.967	3.000	7.900	16.627	4.100-7.300
Albumin (g/dL)	2.346	0.509	1.229	3.727	21.693	1.493-3.500
Globulins (g/dL)	3.465	0.824	1.518	5.491	23.782	1.785-4.807
Albumin/globulins	0.692	0.216	0.311	1.353	31.274	0.375-1.296
α1 Globulin (g/dL)	0.062	0.053	0.001	0.248	85.200	0.001-0.198
α2 Globulin (g/dL)	0.416	0.174	0.017	0.951	41.831	0.152-0.780
β Globulin (g/dL)	0.151	0.137	0.001	0.738	90.891	0.001-0.637
γ Globulin (g/dL)	2.818	0.764	0.624	4.519	27.092	1.344-4.277
IgG (g/dL)	1.564	0.406	1.080	2.640	25.942	1.130-2.500
BW0 (kg)	5.286	0.778	3.100	7.000	14.711	-
BW3 (kg)	31.680	4.193	21.700	43.800	13.235	-
BW6 (kg)	39.292	4.229	30.000	51.500	10.763	-
BW9 (kg)	46.479	5.526	34.000	60.000	11.889	-
BW12 (kg)	50.467	4.955	37.900	61.547	9.819	-

SD: standard deviation; Min and Max: minimum and maximum observations, respectively; CV: coefficient of variation; IgG: immunoglobulin G and BW0, BW3, BW6, BW9 and BW12: birth, 3, 6, 9 and 12-month body weights, respectively.

Table 2 Pairwise rank correlation coefficients between the studied serum protein variables and body weight traits

Variable	TP	A	G	A/G	α1	α2	β	γ	IgG
A	0.53^{**}	-	-	-	-	-	-	-	-
G	0.84^{**}	0.05^{ns}	-	-	-	-	-	-	-
A/G	-0.29^{**}	0.54^{**}	-0.71^{**}	-	-	-	-	-	-
α1	0.19^{ns}	-0.02^{ns}	0.20^{*}	-0.19^{ns}	-	-	-	-	-
α2	0.51^{**}	0.12^{ns}	0.54^{**}	-0.38^{**}	0.22^{*}	-	-	-	-
β	0.29^{**}	0.20^{*}	0.19^{ns}	0.04^{ns}	0.30^{**}	0.38^{**}	-	-	-
γ	0.78^{**}	0.02^{ns}	0.94^{**}	-0.68^{**}	0.07^{ns}	0.33^{**}	-0.04^{ns}	-	-
IgG	-0.01^{ns}	0.07^{ns}	-0.12^{ns}	0.04^{ns}	0.11^{ns}	0.03^{ns}	-0.12^{ns}	-0.14^{ns}	-
BW0	0.12^{ns}	0.04^{ns}	0.16^{ns}	-0.15^{ns}	-0.01^{ns}	0.19^{ns}	0.03^{ns}	0.10^{ns}	0.12^{ns}
BW3	0.01^{ns}	-0.18^{ns}	0.12^{ns}	-0.18^{ns}	0.02^{ns}	0.08^{ns}	-0.05^{ns}	0.08^{ns}	0.10^{ns}
BW6	0.00^{ns}	-0.08^{ns}	0.04^{ns}	-0.08^{ns}	-0.02^{ns}	-0.12^{ns}	-0.04^{ns}	0.05^{ns}	0.05^{ns}
BW9	0.05^{ns}	0.02^{ns}	0.00^{ns}	0.09^{ns}	0.01^{ns}	-0.21^{ns}	-0.15^{ns}	0.02^{ns}	0.19^{ns}
BW12	0.04^{ns}	0.04^{ns}	-0.04^{ns}	0.14^{ns}	0.07^{ns}	-0.13^{ns}	-0.10^{ns}	-0.05^{ns}	0.17^{ns}

TP: total protein (g/dL); A: albumin (g/dL); G: globulins (g/dL); A/G: albumin/Globulins ratio; α1, α2, β and γ: α1, α2, β and γ Globulins (g/dL) respectively; IgG: immunoglobulin G (g/dL) and BW0, BW3, BW6, BW9 and BW12: birth, 3, 6, 9 and 12-month body weights, respectively.
* ($P<0.05$) and ** ($P<0.01$).
NS: non significant.

However, depending on the species, each fraction may be more divided as α1, α2, β1, β2, γ1 and γ2 fractions (Eckersall, 2008). In the present study, the α globulins migrated into two zones (α1 and α2). Generally acute inflammations, resulted by acute-phase reactants, can increase the α1 protein fraction, which is composed of α1-antitrypsin, thyroid-binding globulin and transcortin. On the other hand, ceruloplasmin, α2-macroglobulin and haptoglobin contribute to the α2-protein fraction which is increased as an acute-phase reactant (O'Connell *et al*. 2005). Therefore, the most likely reason for elevation of α-globulin fraction is release of acute phase reactants, such as α1 antitrypsin and α1acid glycoprotein in response to inflammation process (Esmaeilnejad *et al*. 2014). In the present study, average concentration of α1 globulin was lower than the α2 globulin, which agrees with previous observations in sheep, goat and cattle (Nagy *et al*. 2015).

The β fraction is mainly composed by transferrin, β-lipoprotein, IgA, IgM and sometimes IgG, along with complement proteins (O'Connell *et al*. 2005). Similar to the α globulins, average level of the β globulins in sick animals may be higher than the healthy animals (Esmaeilnejad *et al*. 2014). The γ globulin fraction is mainly composed by various immunoglobulins, including IgG, IgA, IgM, IgD and IgE, as a part of the immune system (Tothova *et al*. 2016a). Thus, γ globulin is most attended fraction in interpretation of serum protein electrophoresis (O'Connell *et al*. 2005). Average γ globulin level in this study (2.82 g/dL) was lower than the reported values in Santa Inês lambs (Alves *et al*. 2015) and higher than other values reported in Iranian West Azerbaijan sheep (Esmaeilnejad *et al*. 2014), Sarda and Lacaune lactating ewes (Miglio *et al*. 2015), Merino adult female sheep (Nagy *et al*. 2015) and Comisana ewes (Nagyová *et al*. 2017). Average of IgG level in the present study (1.56 g/dL) was similar to the reported value in non-pregnant Santa Inês ewes (Sabes *et al*. 2017) and lower than the values reported in pregnant Lori ewes (Koushki *et al*. 2019) and young colostrum fed Santa Inês lambs (Alves *et al*. 2015). The observed variations of serum proteins in this study and other reports in literature (Table 4) could be attributed to breed properties, health and physiological conditions, such as pregnancy and lactation, environmental factors, age, and different methods used to measure the protein fractions.

Based on the observed correlation coefficients between different serum protein fractions (Table 2), albumin and IgG levels were almost independent from other protein fractions. The IgG is the most common antibody in blood and its level depends on health and disease status, thus its independence from other protein fractions which are mostly related to metabolic status, is not unexpected. Moreover, the IgG was measured directly, using an ELISA reader equipment, while other serum protein fractions were estimated by electrophoresis method. Different methods used for determination of serum protein fractions may influence the correlation coefficients between different fractions. In a study on Santa Inês lambs a low positive correlation was found between serum IgG and total protein measured by the biuret method (0.44), but there was not any significant correlation between IgG and total protein determined by refractometry (Alves *et al*. 2015). On the other hand, independence of albumin from most of other protein fractions in the present study contradicts a study on Iranian Ghezel and Mehraban sheep, which serum level of albumin was significantly correlated with α2 and γ globulins (Nazifi and Mostaghni, 1995). However, many correlation coefficients between different serum protein fractions in the present study, including high correlation of total protein and γ globulin, significant correlations between total protein and albumin, albumin/globulin ratio, α2, β and γ globulins, not significant correlations of albumin with α1 and α2 globulins, significant correlations of α2 globulin with albumin/globulin ratio and α1, β and γ globulins are confirmed by Nazifi and Mostaghni (1995).

As it was mentioned in the results section, total globulins, albumin/globulins ratio, α2 globulin and γ globulin were significantly associated with age. Generally, age is an important factor which may affect concentrations and electrophoretic patterns of different serum protein fractions (Tothova *et al*. 2016a). Significant effects of age on different serum protein fractions have been shown in several studies. However, in many cases, serum protein levels were studied in early ages (Piccione *et al*. 2013; Carlos *et al*. 2015; Tothova *et al*. 2016b; Da Cruz *et al*. 2017; Rahman *et al*. 2018) or compared between young and adult animals (Tothova *et al*. 2013).

In the first stages of life, most of acute phase proteins increase from birth to the subsequent days. Because liver, as the main site of acute phase proteins synthesis, is less mature in newborn animals (Tothova *et al*. 2016a). However, all serum protein fractions do not have a same trajectory. In a study on lambs, total proteins and γ globulin concentrations increased one day after colostrum intake and then decreased gradually till the next month, but albumin concentration showed an opposite trend. Whereby albumin concentration decrease done day after colostrum intake and then progressively increased till the end of the studied period. In that study, relative concentration of α1-globulin decreased while, relative values of α2 and β globulins increased during the first month of life (Nagy *et al*. 2014). Significant changes of total proteins, albumin, α1-globulins, β-globulins, γ-globulins and A/G ratio in the first 30 days of life were also reported in Comisana lambs (Piccione *et al*. 2013).

Table 3 Results of the GLM analysis and least square means comparisons for the studied serum protein fractions

Variable	TP	A	G	A / G	α1	α2	β	γ	IgG
Age (year)									
2-3	5.521	2.042	3.470^{ab}	0.618^{c}	0.030	0.570^{a}	0.106	2.778^{ab}	1.526
4	5.996	2.121	3.877^{a}	0.561^{c}	0.067	0.501^{ab}	0.137	3.181^{a}	1.549
5	5.624	2.301	3.291^{ab}	0.711^{bc}	0.039	0.376^{b}	0.115	2.754^{ab}	1.507
6	5.303	2.452	2.833^{b}	0.815^{ab}	0.060	0.389^{b}	0.104	2.219^{b}	1.799
7+	5.387	2.468	2.904^{b}	0.897^{a}	0.073	0.394^{b}	0.111	2.334^{b}	1.519
SEM	0.280	0.145	0.218	0.053	0.015	0.046	0.040	0.206	0.118
Birth season									
Spring	5.293	2.233	3.031	0.755	0.044	0.482	0.095	2.405^{b}	1.601
Winter	5.839	2.331	3.519	0.686	0.064	0.410	0.134	2.901^{a}	1.558
SEM	0.235	0.121	0.183	0.045	0.013	0.039	0.034	0.173	0.099
Birth type									
Singleton	5.626	2.313	3.285	0.723	0.054	0.453	0.150	2.611	1.577
Twin	5.506	2.240	3.265	0.718	0.053	0.439	0.079	2.695	1.582
SEM	0.230	0.118	0.179	0.044	0.012	0.038	0.033	0.169	0.097
P-values									
Age	0.2642	0.0744	0.0007	< 0.0001	0.0886	0.0046	0.9610	0.0013	0.1529
Birth season	0.0960	0.5220	0.0571	0.2647	0.2556	0.1830	0.4133	0.0409	0.7484
Birth type	0.6858	0.6315	0.9323	0.9202	0.9470	0.7716	0.1019	0.7016	0.9623
BV_{BW}	0.5043	0.7015	0.2665	0.0710	0.6617	0.4921	0.2464	0.4372	0.5275

TP: total protein (g/dL); A: albumin (g/dL); G: globulins (g/dL); A / G: albumin / globulins ratio; α1, α2, β and γ: α1, α2, β and γ globulins (g/dL), respectively; IgG: immunoglobulin G (g/dL) and BV_{BW}: breeding value for body weight.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 Comparison of the studied serum protein variables between Lori-Bakhtiari sheep with the reported values in literature

Source	Serum protein variables								
	TP	A	G	A/G	α1	α2	β	γ	IgG
Current study	5.82	2.35	3.47	0.69	0.06	0.42	0.15	2.82	1.56
Al-Hadithy and Badawi (2015)	7.08	3.37	3.70	-	-	-	-	-	-
Alves *et al*. (2015)[1]	5.46	2.00	3.46	0.58	-	-	-	3.46	1.83
Alves *et al*. (2015)[2]	5.80	2.13	3.67	0.58	-	-	-	3.66	2.96
Borjesson *et al*. (2000)[3]	7.40	3.30	4.00	0.90	-	-	-	-	-
Carlos *et al*. (2015)	5.61	2.95	2.66	1.62	-	-	-	-	-
Durak *et al*. (2015)	7.60	3.10	4.10	-	-	-	-	-	-
Esmaeilnejad *et al*. (2014)	7.10	3.20	-	-	0.27	0.80	0.36	1.69	-
Jin *et al*. (2019)	7.15	2.49	4.86	-	-	-	-	-	-
Koushki *et al*. (2019)	3.23	-	-	-	-	-	-	-	2.25
Lepherd *et al*. (2009)	5.80	3.40	2.40	1.40	-	-	-	-	-
Miglio *et al*. (2015)[4]	7.29	3.86	3.43	1.10	0.33	0.76	0.54	1.79	-
Miglio *et al*. (2015)[5]	7.89	3.60	4.29	0.90	0.35	0.82	0.87	2.17	-
Murariu *et al*. (2014)[6]	4.73	3.33	-	-	-	-	-	-	-
Murariu *et al*. (2014)[7]	4.56	2.22	-	-	-	-	-	-	-
Nagy *et al*. (2015)	6.98	3.90	3.08	1.28	0.41	0.78	0.33	1.57	-
Nagyová *et al*. (2017)	6.89	2.72	4.17	0.66	-	-	1.54	2.06	-
Njidda *et al*. (2014)[8]	8.60	2.90	5.70	-	-	-	-	-	-
Njidda *et al*. (2014)[9]	8.30	2.30	5.60	-	-	-	-	-	-
Njidda *et al*. (2014)[10]	5.70	2.70	3.00	-	-	-	-	-	-
Rahman *et al*. (2018)	6.30	3.20	-	-	-	-	-	-	-
Sabes *et al*. (2017)	7.06	4.41	-	-	-	-	-	-	1.56
Zamani *et al*. (2016)	7.07	3.38	3.54	0.99	0.22	0.84	0.30	2.22	-

TP: total protein (g/dL); A: albumin (g/dL); G: globulins (g/dL); A / G: albumin / globulins ratio; α1, α2, β and γ: α1, α2, β and γ globulins (g/dL), respectively and IgG: immunoglobulin G (g/dL).
1 and 2: male and female Santa Inês young lambs, respectively; 3: reported as median values; 4 and 5: Lacaune and Sarda ewes, respectively; 6 and 7: Karakul and Tzurcanaewes, respectively and 8, 9 and 10: Yankasa, Ouda and Balami ewes, respectively.

No-significant effects of age on total protein and albumin in the present study is in agreement with a previous observation made by Durak *et al*. (2015). Significant effects of age on γ globulin level and albumin/globulin ratioare also supported by a study on Iranian Ghezel and Mehraban breeds of sheep (Nazifi and Mostaghni, 1995). In both studies, minimal albumin/globulin ratio was observed in 4 years of age and γ globulin level increased till 4 years of age and then decreased afterward. Nazifi and Mostaghni (1995) reported an increasing trend of albumin level in higher ages, which agrees with the result of the present study. However, in the present study, increase of albumin levels in higher ages was near to significant level ($P<0.10$; Table 3).

A general concept is that increase of age should elevate total protein level, as result of a small decrease in albumin and a progressive increase in globulin levels (Eckersall, 2008). But these trajectories apparently contradict the results of the present study. The main reason of this inconsistency is that this trajectory is expected for the first few months after birth, while in the present study only adult animals were evaluated. Serum protein profile in newborn and adult animals are completely different. For example, in a study on three Iranian native breeds of sheep, serum levels of total protein, albumin, total globulins, α, β and γ globulins in adult animals were significantly higher than young lambs (Khazraei Nia *et al*. 1998). No significant effect of age on serum IgG level in the present study is in agreement with previous reports on primiparous and multiparous Santa Inês ewes (Alves *et al*. 2015) and different age classes in Ethiopian local sheep (Aynalem Gemechu and Kibeb, 2017).

As it was mentioned previously, birth season had a significant effect on blood serum level of γ globulins, which could be attributed to different environmental conditions of winter and spring born lambs, such as different health conditions of barn and pasture, and different feeding regimes in winter and spring. This observation indicates higher immune activity of the winter-born animals, which is probably due to unsuitable environmental conditions of winter-born lambs in closed barns with high-density flocks, insufficient ventilation and humid litter in winter. No report about effect of birth season on serum protein profile was found in literature and this observation is probably the first evidence. However, it must be noticed that the data should be accurate and relevant to show physiological status of the animal and help clinicians to differentiate between sickness and health conditions (Nagyová *et al*. 2016). Moreover, the samples should be handled and analyzed according to the specified procedures and every step of the analytical process needs careful attention (Tothova *et al*. 2010). Changes of the serum protein pattern in the stored samples should be also considered before interpretation of the results, because some serum protein electrophoretic fractions may be influenced by storage time (Tothova *et al*. 2010; Piccione *et al*. 2014; Nagyová *et al*. 2016).

CONCLUSION

This study revealed significant effects of age and birth season on serum protein profile. Where by, winter-born ewes had higher levels of γ globulins and probably higher immune system activity. Older ewes had lower levels of α2, γ and total globulins and higher albumin/globulin ratios. Body weight and birth type did not have any significant association with the studied serum protein fractions. The results of the present study suggest that age and birth season should be considered for interpretation of the serum protein profiles.

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REFERENCES

Alberghina D., Casella S., Vazzana I., Ferrantelli V., Giannetto C. and Piccione G. (2010). Analysis of serum proteins in clinically healthy goats (*Capra hircus*) using agarose gel electrophoresis. *Vet. Clin. Pathol*. **39,** 317-321.

Al-Hadithy H.A. and Badawi N.M. (2015). Determination of serum proteins and glucose concentrations in clinically normal and anemic Awassi sheep. *World's Vet. J*. **5,** 1-6.

Almasi M., Zamani P., Mirhoseini S.Z. and Moradi M.H. (2020). Genome-wide association study for weaning traits in a meat type sheep. *Ann. Anim. Sci*. **20,** 811-824.

Alves A.C., Alves N.G., Ascari I.J., Junqueira F.B., Coutinho A.S., Lima R.R., Pérez J.R., De Paula S.O., Furusho-Garcia I.F. and Abreu L.R. (2015). Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. *J. Dairy Sci*. **98,** 3706-3716.

Aynalem Gemechu G. and Kibeb L. (2017). Effect of age, sex and altitude on the normal physiological and biochemical parameters in apparently healthy local breed sheep in Shebedinodistrict in Sidam zone, Ethiopia. *BioChem. Indian J*. **11,** 122-128.

Borjesson D.L., Christopher M.M. and Boyce W.M. (2000). Biochemical and hematologic reference intervals for free-ranging desert bighorn sheep. *J. Wildlife Diseas*. **36,** 294-300.

Burtis C.A. and Bruns D.E. (2014). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. Elsevier, Amsterdam, The Netherlands.

Carlos M.M.L., Leite J.H.G.M., Chaves D.F., Vale A.M., Façanha D.A.E., Melo M.M. and Soto-Blanco B. (2015). Blood parameters in the Morada Nova sheep: Influence of age, sex and body condition score. *J. Anim. Plant Sci*. **25,** 950-955.

Da Cruz R.E.S., Rocha F.M., Sena C.V.B., Noleto P.G., Guimarães E.C., Galo J.A. and Mundim A.V. (2017). Effects of age and sex on blood biochemistry of Dorper lambs, Semin. Cie. Agr. **38,** 3085-3093.

Durak M.H., Erkan R.E.C., Çelik R., Yokuş B., Kurt D. and Gürgöze S. (2015). The effects of age and gender on some biochemical serum parameters in Zom sheep raised in the vicinity of Karacadağ. *Israel J. Vet. Med*. **70(2),** 33-39.

Eckersall P.D. (2008). Proteins, proteomics, and the dysproteinemias. Pp. 117-155 in Clinical Biochemistry of Domestic Animals. J.J. Kaneko, J.W. Harvey and M.L. Bruss, Eds. Academic Press, San Diego, USA.

Esmaeilnejad B., Tavassoli M., Asri-Rezaei S., Dalir-Naghadeh B. and Pourseyed S.H. (2014). Evaluation of serum total protein concentration and protein fractions in sheep naturally infected with *Babesia ovis*. *Comp. Clin. Pathol*. **23,** 151-155.

Geffré A., Friedrichs K., Harr K., Concordet D., Trumel C. and Braun J.P. (2009). Reference values: A review. *Vet. Clin. Pathol*. **38,** 288-298.

Ghasemi M., Zamani P., Vatankhah M. and Abdoli R. (2019). Genome-wide association study of birth weight in sheep. *Animal*. **13,** 1797-1803.

Ignjatovic V., Lai C., Summerhayes R., Mathesius U., Tawfilis S., Perugini M.A. and Monagle P. (2011). Age-related differences in plasma proteins: How plasma proteins change from neonates to adults. *PLoS One*. **6(2),** e17213.

Jin Y.M., Zhang X.Q., Badgery W.B., Li P. and Wu J.X. (2019). Effects of winter and spring housing on growth performance and blood metabolites of Pengbo semi-wool sheep in Tibet, *Asian-Australasian J. Anim. Sci*. **32,** 1630-1639.

Khazraei Nia P., Mojabi A., Abasalipour Kabireh M. and Nowrouzian I. (1998). Determination of serum total protein and different blood serum protein fractionslevels using cellulose acetate electrophoresis method in Iranian sheep. *Anim. Sci. J. (Pajouhesh and Sazandegi)*. **38,** 118-121.

Koushki R., Mansoori Yarahmadi A., Khaldari M., Fakhraei L. and Karkoodi K. (2019). Milk yield and blood metabolite profile in late pregnancy in Lori ewes receiving diets containing undegradable protein sources. *Iranian J. Appl. Anim. Sci*. **9,** 643-650.

Lepherd M.L., Canfield P.J., Hunt G.B. and Bosward K.L. (2009). Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. *Australian Vet. J*. **87,** 5-11.

Merlot A.M., Kalinowski D.S. and Richardson D.R. (2014). Unraveling the mysteries of serum albumin - more than just a serum protein. *Front. Physiol*. **5,** 299-305.

Meyer K. (2007). WOMBAT: A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). *J. Zhejiang Univ. Sci. B*. **8,** 815-821.

Miglio A., Antognoni M.T., Maresca C., Moncada C., Riondato F., Scoccia E. and Mangili V. (2015). Serum protein concentration and protein fractions in clinically healthy Lacaune and Sarda sheep using agarose gel electrophoresis. Vet. Clin. Pathol. **44,** 564-569.

Murariu O.C., Lazăr R., Murariu F., Paul C. and Boişteanu P.C. (2014). Variation of biochemical parameters of ovine blood according to genotype and sex after the stalling period, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. *Anim. Sci. Biotechnol*. **71,** 73-75.

Nagy O., Tóthová C., Nagyová V. and Kováč G. (2015). Comparison of serum protein electrophoretic pattern in cows and small ruminants. *Acta Vet. Brno*. **84,** 187-195.

Nagy O., Tothova C., Nagyova V., Kovac G. and Posivak J. (2014). Changes in the serum protein electrophoretic pattern in lambs during the first month of life. *Acta Vet. Brno*. **83,** 187-193.

Nagyová V., Arfuso F., Rizzo M., Nagy O. and Piccione G. (2017). Stability of ovine serum protein fractions under different storage conditions. *J. Vet. Diagn. Invest*. **29,** 312-315.

Nagyová V., Arfuso F., Rizzo M., Zanghì E., Nagy O. and Piccione G. (2016). Stability of total proteins and their electrophoretic fractions in goat serum (*Capra hircus*), maintained under different condition. *Small Rumin. Res*. **144,** 145-148.

Nazifi S. and Mostaghni K. (1995). Electrophoresis of serum proteins in Ghezel and Mehraban sheep. *(Pajouhesh and Sazandegi)*. **29,** 118-125.

Njidda A.A., Shuai'bu A.A. and Isidahomen C.E. (2014). Haematological and serum biochemical indices of sheep in semi-arid environment of northern Nigeria. *Global J. Sci. Front. Res. D Agric. Vet*. **14(2),** 49-56.

NRC. (2007). Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, D.C., USA.

O'Connell T.X., Horita T.J. and Kasravi B. (2005). Understanding and interpreting serum protein electrophoresis. *American Family Phys*. **71,** 105-112.

Piccione G., Arfuso F., Faggio C., Casella S., Zumbo A. and Panzera M. (2013). Serum proteins profile in Comisana lambs during the first month of life. *Arch. Anim. Breed*. **56,** 742-750.

Piccione G., Casella S., Giannetto C., Panzera M., Pennisi P. and Alberghina D. (2014). Influence of short-term storage on electrophoretic profile of bovine serum proteins. *J. Appl. Anim. Res*. **42,** 123-125.

Rahman M.K., Islam S., Ferdous J., Uddin M.H., Hossain M.B., Hassan M.M. and Islam A. (2018). Determination of hematological and serum biochemical reference values for indigenous sheep (*Ovies aries*) in Dhaka and Chittagong districts of Bangladesh. *Vet. World*. **11,** 1089-1093.

Sabes A.F., Girardi A.M., Fagliari J.J., de Oliveira J.A. and Marques L.C. (2017). Serum proteinogram in sheep with acute ruminal lactic acidosis. *Int. J. Vet. Sci. Med*. **5,** 35-40.

SAS Institute (2013). Users Guide, Version 9.4: Statistics. SAS Institute, Cary, NC, USA.

Tothova C., Mihajlovičová X. and Nagy O. (2017). The electrophoretic pattern of serum proteins in dairy cows with inflammatory diseases. *Acta Vet*. **67,** 178-190.

Tothova C., Nagy O. and Kovac G. (2016a). Serum proteins and their diagnostic utility in veterinary medicine: A review. *Vet. Med*. **61,** 475-496.

Tothova C., Nagy O., Nagyova V. and Kovac G. (2016b). The concentrations of selected blood serum proteins in calves during the first three months of life. *Acta Vet. Brno*. **85,** 33-40.

Tothova C., Nagy O., Seidel H. and Kovac G. (2010). The effect of storage on the protein electrophoretic pattern in bovine serum. *Iranian J. Vet. Sci. Technol*. **2,** 77-84.

Tothova C., Nagy O., Seidel H. and Kovac G. (2013). Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. *Comp. Clin. Pathol*. **22,** 15-20.

Tschuor A.C., Riond B., Braun U. and Lutz H. (2008). Hematological and clinical biochemical reference values for adult goats and sheep. *Schweiz. Archiv fur Tierheilkd*. **150,** 287-295.

Zamani P., Salehian Dehkordi H., Doosti A. and Abdolmohammadi A. (2016). Ovine DRB1 polymorphism and its associations with body weight, milk contents and immunological parameters. *Ann. Anim. Sci*. **16,** 425-438.

Zeder M.A. (1999). Animal domestication in the Zagros: A review of past and current research. *Paléorient*. **25(2),** 11-26.