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INFLUENCE OF AGE ON PROFILE OF SERUM PROTEINS IN THE CALF

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The authors studied the course of serum proteins during the first week and the first month of life in the calf in order to obtain useful information for neonatal care. Eight Limousine calves, four males and four females, clinically healthy, were used. From all animals blood samples were collected from the external jugular vein from the day after the birth every day for one week and every five days for thirty days. Blood samples were taken at the same hour (09:00), and the serum concentrations of albumin, globulins (α1-globulins, α2-globulins, β-globulins, γ-globulins) and total proteins were determined by means of automated multiparametric agarose gel electrophoresis system. One-way repeated measures analysis of variance (ANOVA), followed by the Bonferroni's test, was used to determine significant differences. Data analysis of variance showed a significant effect of days of life, with $p <$ 0.05, only on albumin, α1-globulins and α2-globulins during the first month of life. These results confirm that despite the fact that newborns must adapt to various environmental factors after birth, including nutrition that can influence the serum protein profile during neonatal period, the evaluation of electrophoretic's parameters during the first months of life results useful for the diagnosis and treatment of neonatal diseases.

Key words: electrophoretic parameters, calf, neonatal period, perinatal period, total proteins

INTRODUCTION

The most vulnerable period in the life of any animal is the period after birth. In all species, the neonatal period represents a critical time during which all organ functions must adapt to the extra-uterine life; it is a transition phase from the sheltered intra-uterine to the exposed extra-uterine environment (Piccione *et al*., 2008). Nearly half of all pre-weaning lamb deaths occur on the day of birth, whilst the rate of lamb losses is greatly reduced beyond the first week of life (Nowak *et* *al*., 2000; Sawalha *et al*., 2007). Many studies have investigated the causes of lamb deaths, which can broadly be categorised as relating to the birth process, neonatal adaptation to postnatal life, functional disorders or infectious disease (Dwyer, 2008). In particular the determination of serum proteins has evolved into important diagnostic aids in clinical biochemistry (Kaneko *et al*.,1997). This has occurred even though a specific diagnosis can seldom be made with serum proteins. Abnormal serum profiles can be identified with general types of disease processes and in this way provide the rationale for further definitive studies of the patient. Normal physiological variations within an individual are relatively constant over a considerable period of time, even minor changes in the serum proteins profile can be of significance and warrant close scrutiny (Kaneko *et al.*,1997).

Laboratory evaluation of serum protein concentrations typically is a part of both basic haematology and biochemistry testing in animals. Measurement of serum protein concentrations often yields important informations that can be helpful in narrowing the list of diseases to be considered and, in some cases, in revealing the presence of a specific disease.

Only few studies have been carried out with the purpose of underling the influence of age and development on the concentration of total proteins and on protein fractions (Bauer *et al*., 1985; Carcangiu *et al*., 2002; Tumbleson and Kalish, 1972), in order to be useful for precise interpretation of laboratory results. Instead, the influence of age on biochemical, haematological and physiological profile were demonstrated (Knowles *et al*., 2000; Mohri *et al*., 2007; Piccione *et al.*, 2007) and it was determined that the physiological status of calves at birth and the perinatal factors might predispose newborn calves to debility and death (Diesch, 2004).

On the basis of such considerations, the serum protein profile (albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and total proteins) was studied during the first week and the first month of life in Limousine calves, in order to obtain useful information for neonatal care.

MATERIAL AND METHODS

Eight clinically healthy and full-term-born Limousine calves (4 males: mean body weight 43.75 $\pm$ 6.29 Kg ; 4 females: mean body weight 40.00 $\pm$ 4.08 Kg) were used for our study, which was carried out in spring in Sicily, Italy. Calves were fed only with maternal milk and were kept in a sheltered outdoor pen. Their health status was evaluated daily based on behaviour, rectal temperature, heart rate, respiratory profile, cough, nasal discharge, appetite, fecal consistency, navel examination and haematological profile. Blood samples were taken from the external jugular vein by vacutainer tubes (Terumo Corporation, Japan) at the same hour (09:00). Blood samples were centrifuged at 3000 r.p.m. for 10 minutes and the obtained sera were separated and stored until analyses at -20^{o}C. Serum concentrations of albumin, globulins (α1-globulins, α2-globulins, β-globulins, γ-globulins) and total proteins were assessed by means of an automated multiparametric agarose gel electrophoresis system (Hydrasys, Sebia, France). The parameters studied were assessed for each subject in the following experimental conditions: the day after birth every day for one week and every five days for thirty days on all animals. Statistical analysis of the data for each parameter was based on the average values obtained. All results were expressed as mean ± standard error of the means (SEM). One-way repeated measures analysis of variance (ANOVA) was used to determine significant effects of days of life during the first week and the first month of life. P values <0.05 were considered statistically significant. The Bonferroni's test was applied for post hoc comparison. Data were analyzed using the software STATISTICA 5.5 (Statsoft Inc., USA).

RESULTS

Tables 1 and 2 show the mean values of parameters considered, together with their standard errors of the means (SEM), and with the statistical significances obtained during the first week and the first month of life in eight Limousine calves.

In figures 1 and 2, the pattern of mean values (±SEM) of albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and total proteins, expressed in g/dL, observed in eight Limousine calves during the first week and the first month of life is presented.

In figures 3 and 4 are given serum protein electrophoretograms observed in a calf during the first week and the first month of life.

Table 1. Average values (± standard errors) of electrophoretic parameters studied, expressed in their conventional units of measurement, in eight Limousine calves during the first week of life

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Albumin (g/dL)	1.69±0.02	1.73±0.04	1.79±0.05	1.87±0.08	1.83±0.12	1.86±0.15
α1-globulins (g/dL)	0.98±0.09	1.07±0.11	1.06±0.11	0.99±0.11	0.98±0.10	0.98±0.12
α2-globulins (g/dL)	0.82±0.05	0.76 ± 0.04	0.70 ± 0.04	0.82 ± 0.06	0.83 ± 0.06	0.82 ± 0.10
β–globulins (g/dL)	0.84±0.14	1.21±0.25	1.24±0.24	1.23±0.21	1.05±0.21	1.07±0.20
γ–globulins (g/dL)	1.15±0.19	1.32±0.29	1.36±0.24	1.33±0.20	1.09±0.24	1.21±0.20
Total Proteins (g/dL)	5.34±0.34	6.10±0.66	6.16±0.62	6.25±0.59	5.85±0.69	5.80±0.70

Table 2. Average values (± SE) of protein fractions (g/dl), and statistical significances observed in eight Limousine calves during the first month of life

Parameters	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Albumin (g/dL)	1.69 ± 0.02	1.83 ± 0.12	2.05 ± 0.11	2.30 ± 0.15$^{*\bullet}$	2.29 ± 0.14$^{*\bullet}$	2.81 ± 0.15$^{*\bullet\#\lozenge}$	2.41 ± 0.20$^{*\bullet}$
α1-globulins (g/dL)	0.98 ± 0.09	0.98 ± 0.10	0.65 ± 0.08$^{*\bullet}$	0.63 ± 0.07$^{*\bullet}$	0.52 ± 0.07$^{*\bullet}$	0.51 ± 0.03$^{*\bullet}$	0.44 ± 0.04$^{*\bullet}$
α2-globulins (g/dL)	0.82 ± 0.05	0.83 ± 0.06	0.87 ± 0.12	0.76 ± 0.11	0.58 ± 0.08	0.66 ± 0.09	0.54 ± 0.04$^{\#}$
β-globulins (g/dL)	0.84 ± 0.14	1.05 ± 0.21	0.97 ± 0.16	0.87 ± 0.11	0.84 ± 0.08	0.82 ± 0.04	0.74 ± 0.04
γ-globulins (g/dL)	1.15 ± 0.19	1.09 ± 0.24	1.01 ± 0.20	0.86 ± 0.17	0.95 ± 0.19	1.31 ± 0.19	0.88 ± 0.20
Total Proteins (g/dL)	5.34 ± 0.34	5.85 ± 0.69	5.46 ± 0.53	5.55 ± 0.51	5.20± 0.37	6.13 ± 0.22	5.03 ± 0.43

Significances: *vs day 1 $p<0.05$; $^{\bullet}$vs day 5 $p<0.05$; $^{\#}$vs day 10 $p<0.05$; $^{\lozenge}$vs day 15 $p<0.05$; vs day 20 $p<0.05$

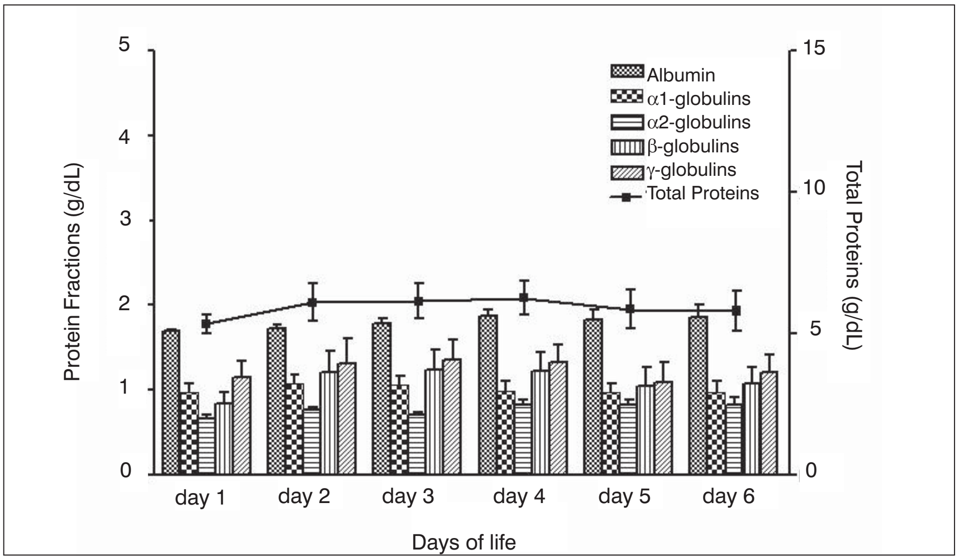


Figure 1. The pattern of mean values (±SEM) of albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and total proteins, expressed in g/dL, observed during the first week of life in eight Limousine calves

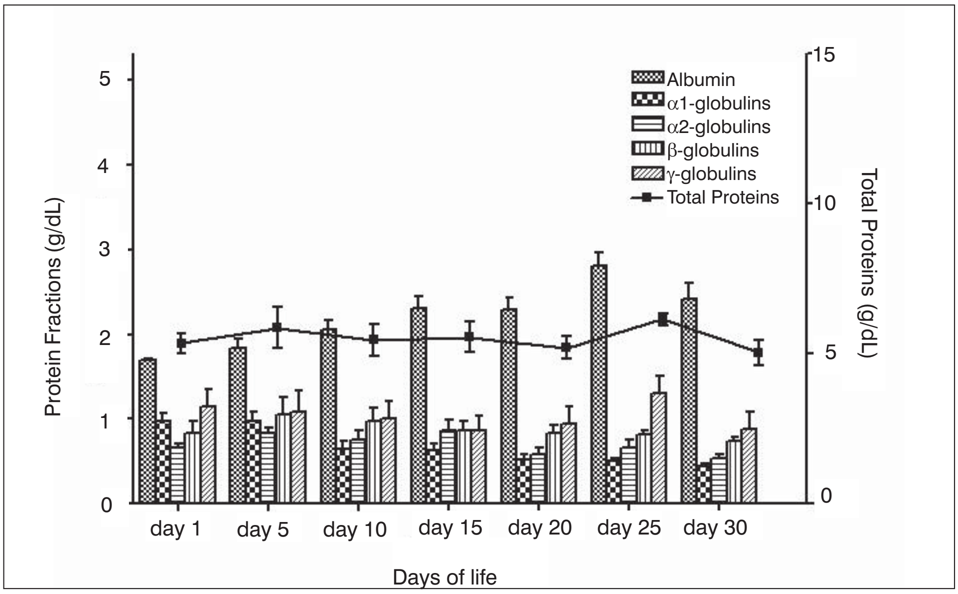


Figure 2. The pattern of mean values (±SEM) of albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and total proteins, expressed in g/dL, observed during the first month of life in eight Limousine calves

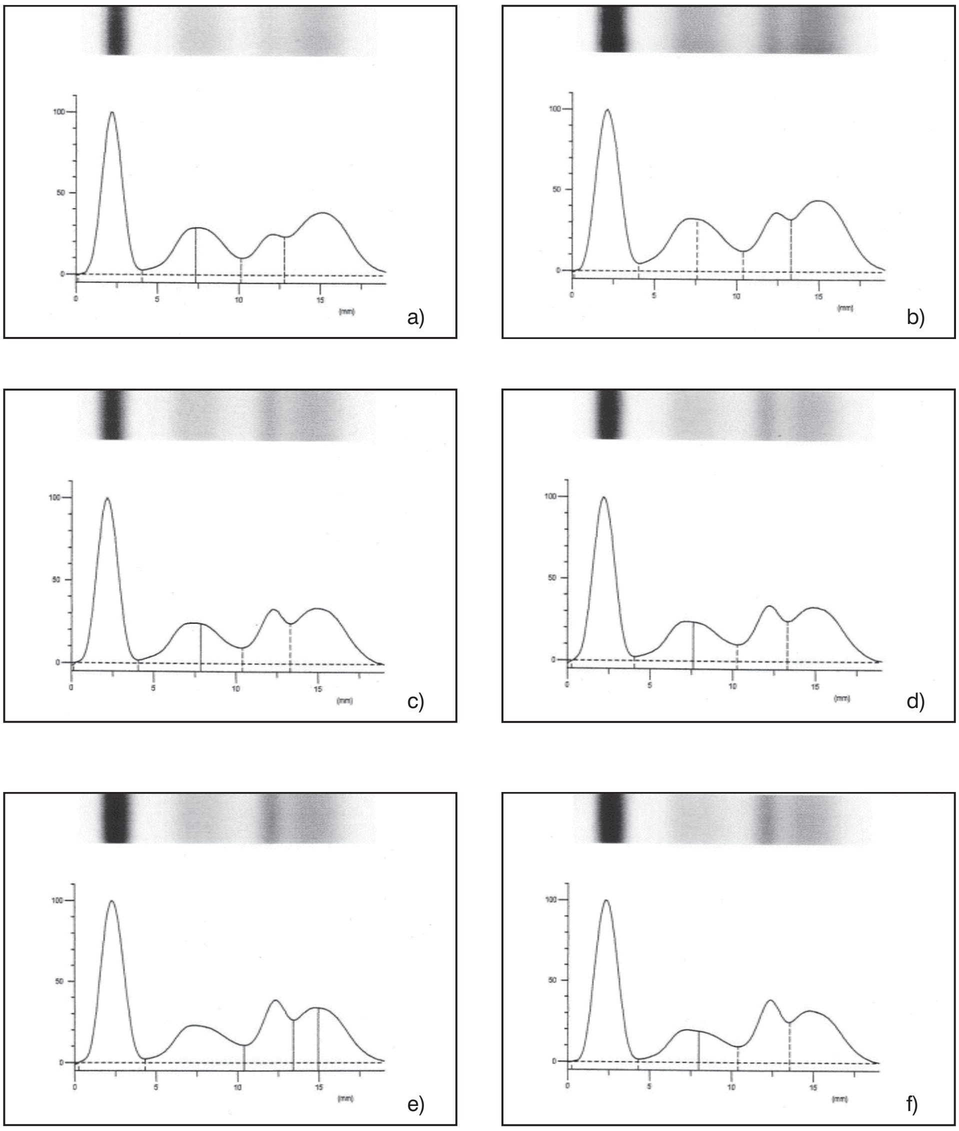
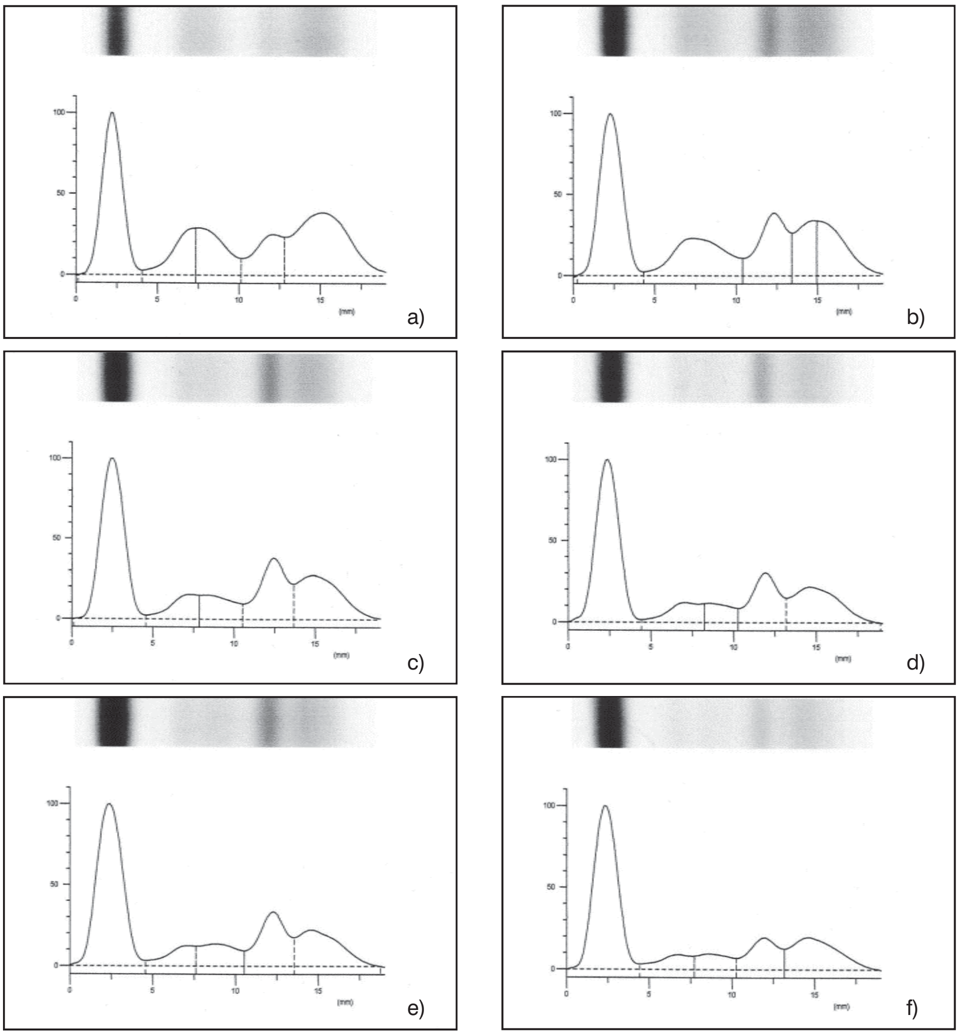


Figure 3.Serum protein electrophoretograms observed in a calf during the first week of life in days 1 (a), 2 (b), 3 (c), 4 (d), 5 (e) and 6 (f)



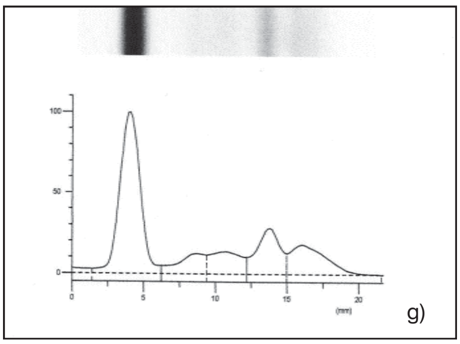


Figure 4. Serum protein electrophoretograms observed in a calf during the first month of life in days 1 (a), 5 (b), 10 (c), 15 (d), 20 (e), 25 (f) and 30 (g)

One-way repeated measures analysis of variance (ANOVA) showed a statistical effect of days of life on albumin ($F_{(6,42)}$=16.17, $p<0.0001$), α1-globulins ($F_{(6,42)}$=11.89, $p<0.0001$) and α2-globulins ($F_{(6,42)}$=3.55, $p<0.0001$) during the first month of life. ANOVA showed no statistical significant effect of days of life on all parameters studied during the first week of life and showed no statistical significant effect of days of life on β-globulins, γ-globulins and total proteins during the first month of life.

DISCUSSION

In animals, there is a general modification in serum proteins with advancing age and in the very old; thus, age is an important consideration in the interpretation of the serum proteins (Kaneko *et al.*, 1997).

Our results did not show a significant effect of days of life on serum protein profile (albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and total proteins) during the first week of life; however, a significant effect of days of life on some of these parameters was found in the first month of life.

At birth, serum protein levels of most animals are quite low due to the minimal quantities of immunoglobulins and low albumin. Modifications of total protein concentrations can result from variations of concentrations of albumin, globulins, or both. An increased albumin or globulin concentration, however, does not always produce detectable increases in total protein concentrations (Thrall *et al*., 2004). Our results show no variations of total proteins during the neonatal period, but only of some protein fractions. The concentration of albumin progressively increases with a decrease of α-globulins and an absence of variations in β-globulins and γ-globulins during the first month of life. According to other authors, that studied the course of these parameters in foals, in the development from birth to first months of age a progressive increase in albumin concentration is seen (Bauer *et al*., 1985). After the first colostrum intake, protein fractions are absorbed from the intestine thus their concentration in the calf serum increase (Boediker, 1991; Egli and Blum, 1998). Later on, the concentrations of albumin are influenced not only by age but also by nutrition (Kaneko *et al*., 1997; Knowles *et al*., 2000; Birgele and Ilgaža, 2003). The majority of biochemical parameters in young animals differ from normal values for adults because are changing with the age and are influenced by colostrum intake (Ježek *et al.*, 2006). In particular, the mean level of albumin increased slowly from birth trough the whole investigated period in accordance to some authors (Knowles *et al*., 2000) that found lowest concentration of albumin to be after birth; then it increased and reached the level of cows by the age of 10 days, then it increased till the end of the investigation.

Bovine colostrum contains various nutrients (proteins, essential and nonessential amino acids and fatty acids, lipids, lactose, vitamins, minerals, oligoelements), as well as non-nutrient substances such as immunoglobulins, enzymes, nucleotides, peptides, polyamines, growth factors, hormones and cytokines, which are important for defence, growth and development, i.e. for overall adaptation of neonatal calves to the new environmental factors after birth related to the drastic change from primarily parenteral nutrition during the fetal period to exlusively enteral provision of nutrients at birth (Blum and Hammon, 2000; Blum and Baumrucker, 2002; Blum et al., 2002; Levieux, 1999). However, in neonatal calves "gut closure" with respect to protein macromolecules such as globulins occurs in neonatal period (Blum *et al*., 2002; Georgiev, 2005), this could explain the decrease of á-globulins that we observed during the first month of life.

Furthermore, since loss of water from the blood causes an increased concentration of albumin molecules, an increase in albumin could occur in dehydration. Instead a decreased α-globulins concentration alone does not result in a decreased globulin concentration and further studies are needed for a diagnosis on these changes.

In conclusion we can affirm that newborn calves must adapt to various environmental factors after birth, including nutrition (Hamman and Blum, 2008), for this reasons it is possible to find changes in the parameters studied during the neonatal period. However, as sometimes these parameters indicate diseases, modifications of electrophoretic profile in the calf during the neonatal period can be used to aid the diagnosis and treatment of neonatal diseases.

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UTICAJ STAROSNE DOBI NA PROTEINSKI PROFIL SERUMA TELADI

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SADRŽAJ

U ovom radu su prikazani rezultati proučavanja proteinskog profila seruma teladi tokom prve nedelje i prvog meseca života. Za ispitivanja je korišćeno osam klinički zdrave teladi rase limuzin (četiri muška i četiri ženska). Uzorci krvi su prikupljani svakog dana u isto vreme (9h) tokom prve nedelje života a zatim svakih pet dana do isteka perioda od mesec dana. U svakom uzorku su određivane koncentracije ukupnih protetina, albumina i globulina (α1, α2, β i γ) automatizovanom metodom elektroforeze u gelu agaroze. Tokom prvog meseca života teladi, metodom analize varijanse su dokazane statistički značajne razlike samo u koncentraciji albumina, α1 i α2 globulina.