

Original study

Serum proteins profile in Comisana lambs during the first month of life

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Abstract

The aim of the present study was to measure the serum total proteins and the serum protein fractions (albumin, α 1-, α 2-, β - and γ -globulins) of ten newborn lambs (Comisana breed) during the first 30 days of life in order to obtain useful information for neonatal care. From each animal, blood samples were collected via jugular venepuncture at the same hour (9.00) every 3 days for 30 days starting from birth (day 0). The concentrations of serum total proteins and albumin, α 1-, α 2-, β -, γ - globulins and albumin/globulins ratio were determined using an automated electrophoresis system. One-way repeated measures analysis of variance (ANOVA) followed by Bonferroni test was used to determine significant differences between mean values of the studied parameters from the 1st to the 30th day of the experimental period. Data analysis of variance showed a statistical effect of days of life on total proteins, albumin, α 1-globulins, β -globulins, γ -globulins and albumin/globulins ratio during the first 30 days of life ($P < 0.0001$), while no statistical significant effect of days of life was observed on α 2-globulins during the experimental period ($P = 0.27$). The obtained results indicated that passive transfer status, determined from serum immunoglobulin concentration 24 h after birth, is a significant source of variation in pre-weaning growth performance in dairy lambs. These finding make a contribution to the knowledge of physiological adaptation in lambs during the first 30 days of life and give useful information for the diagnosis and treatment of neonatal diseases.

Keywords: neonatal period, lamb, total proteins, electrophoretic parameters

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Introduction

The neonatal period, known as »adaptive period«, is a transition phase during which all organ functions must adapt to the extra-uterine life. In fact, the birth and the subsequent 24 h are crucial for the newborn's survival because they represent a critical stage for the detection of health problems (Piccione *et al.* 2008, Piccione *et al.* 2010). In particular, lambs' survival is a complex trait influenced by many different factors that reflect the inherent variation in the ability of lambs to survive (Smith 1977, Christley *et al.* 2003, Everett-Hincks *et al.* 2005) as well as the variations in intra- and extra-uterine life and the postnatal maternal influences. Diseases of the newborn and neonatal mortality are major causes for economic loss in livestock production. In fact, although ovine neonates are relatively mature at birth with adequate thermoregulation and an active mechanism to compensate for physiological acid-base imbalances (Vannucchi *et al.* 2012), lambs' mortality rate is generally higher than in other farm species and reaches a peak during the perinatal period (Eales *et al.* 1983, Purvis *et al.* 1985, Nowak *et al.* 2000). Many studies have investigated the causes for lambs' death, which can be broadly categorized as relating to the birth process, neonatal adaptation to postnatal life, functional disorders or infectious disease (Dwyer 2008). Factors often associated with the risk of mortality in lambs are birth-weight, the serum immunoglobulin concentration (Christley *et al.* 2003) and the lambs' sex (males have a higher mortality than females; Gama *et al.* 1991). Newborn lambs are hypo-immune competent and they are characterized by a small store of energy for heat production and metabolism (O'Doherty & Crosby 1997). The most satisfactory way of providing the newborn with immunity against diseases is to ensure that it gets a large quantity of good quality ewe colostrum in early life because this transfers antibodies from the mother to the young (Napolitano *et al.* 2002, Nowak & Poindron 2006). Besser & Gay (1994) found that the mortality rate was lower in calves with a high serum immunoglobulin concentration than those with a low serum immunoglobulin concentration. So, the total serum globulin concentrations could provide an indication of an animal's humoral immune status or response (Chorfi *et al.* 2004). In particular, an abnormal serum protein profile could be identified with various disease processes and in this way provides the rationale for further definitive studies of the patient (Alberghina *et al.* 2010). Thus, the laboratory evaluation of serum protein concentration and protein fractions, typically parts of both basic haematology and biochemistry testing in animals, often provides important information that can be helpful in narrowing the list of diagnostic possibilities.

A better knowledge of ovine species' physiologic characteristics during the neonatal phase is economically important in the livestock production. Moreover, good management techniques and early intervention, diagnosis and treatment of situations that involve a high risk for the newborn could prevent a substantial proportion of neonatal diseases or death. Specific haematological and serum biochemical reference ranges could help to promote the ability of clinicians to interpret clinical pathology data more accurately and diagnose neonatal diseases (Piccione *et al.* 2008). On the basis of that, the aim of the present study was to measure the serum total proteins and the serum protein fractions (albumin, α 1-, α 2-, β -, and γ - globulins) of ten newborn lambs during the first 30 days of life in order to obtain useful information for neonatal care.

Material and methods

Ten clinically healthy female lambs (Comisana breed) were used in the present study carried out in Sicily (Italy) in spring. The animals with a mean body weight at birth of 5.6 ± 0.7 kg were fed only with colostrum and maternal milk and were kept in a sheltered pen. Their health status was evaluated daily based on behaviour, rectal temperature, heart rate, respiratory profile, cough, nasal discharge, ocular discharge, appetite, faecal consistency and navel examination. During the experimental period, no abnormalities such as fever, anorexia, depression, soft faeces or other conditions that can alter the studied parameters were observed.

For each lamb, blood samples were collected via jugular venepuncture using vacutainer tubes without anticoagulant (Terumo Corporation, Japan) at the same time (9.00) every 3 days for 30 days starting from birth. Blood samples were allowed to coat at room temperature (20°C) and were centrifuged at 3 000 rpm for 10 min. The serum samples were not lipaemic and haemolysed because of possible interference with the biuret method as well as the possibility that they would produce changes in the electrophoretogram. They were dispensed into plastic tubes and stored at -20°C until analysis. At the time of analysis, serum samples were thawed at 20°C for 30 min and the concentration of serum total proteins was determined by the biuret method using an automated analyser UV Spectrophotometer (SEAC, Slim, Florence, Italy).

The protein fractions were performed using an automated electrophoresis system (SelVet 24, SEAC, Italy) according to the procedures described by the manufacturer. For each sample, 25 µl of serum were applied to numbered sample wells. Films were electrophoresis for 30 min at 165 V. After electrophoresis, films were simultaneously fixed using an automated system, stained in red stain acid solution for 10 min and then dried at 37°C. After de-staining in acetic acid and drying completely for 15 min, films were scanned on a densitometer. Using the computer software SelVet-24, electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage and absolute concentrations (g/dl) were calculated using the total protein concentration. The major protein fractions were divided according to the recommendation by the manufacturer from cathode to anode as albumin α1-, α2-, β-, γ- globulins, respectively. All housing and care conformed to the standard recommended by the «Guide for the Care and Use of Laboratory Animals» and Directive 86/609 CEE.

Statistical analysis

All results were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) followed by Bonferroni test was applied to determine statistical differences between mean values of the studied parameters from the 1st to the 30th day of the experimental period. *P*-values <0.05 were considered statistically significant. Data were analysed using the software STATISTICA 7.5 (StatSoft, Inc. Tulsa, OK, USA).

Results

In Table 1 the mean values of serum total proteins and globulin fractions measured with the respective standard deviations are shown. Five fractions were clearly resolved (albumin, α1-, α2-, β-, γ- globulins) in ten dairy lambs (Figure 1).

Table 1

Mean values ± standard deviations of serum total proteins and their fractions (albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins and ratio albumin/globulins) obtained in ten newborn lambs during the first month of life (days 1, 4, 7, 10, 13, 16, 19, 22, 25 and 28)

Parameters g/dL	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22	Day 25	Day 28
Total proteins	6.21 ± 0.34	5.5 ± 0.45	6.66 ± 0.45	6.29 ± 0.32	6.39 ± 0.34	6.71 ± 0.47	6.36 ± 0.31	6.68 ± 0.48	3.35 ± 0.29	6.62 ± 0.39
Albumina	3.55 ± 0.33	2.84 ± 0.57	3.26 ± 0.24	2.99 ± 0.27	2.64 ± 0.30	2.79 ± 0.24	2.90 ± 0.23	3.08 ± 0.22	3.00 ± 0.25	3.14 ± 0.28
α1-globulins	0.33 ± 0.13	0.18 ± 0.06	0.26 ± 0.13	0.23 ± 0.09	0.29 ± 0.11	0.40 ± 0.15	0.35 ± 0.11	0.35 ± 0.14	0.27 ± 0.09	0.40 ± 0.13
α2-globulins	0.95 ± 0.12	0.87 ± 0.24	0.91 ± 0.16	0.89 ± 0.07	0.91 ± 0.12	0.86 ± 0.12	0.87 ± 0.13	0.93 ± 0.12	0.95 ± 0.09	0.86 ± 0.06
β-globulins	0.45 ± 0.10	0.56 ± 0.16	0.51 ± 0.10	0.54 ± 0.10	0.77 ± 0.17	0.57 ± 0.11	0.68 ± 0.14	0.52 ± 0.08	0.46 ± 0.07	0.52 ± 0.20
γ-globulins	0.92 ± 0.43	1.05 ± 0.18	1.71 ± 0.25	1.65 ± 0.31	1.77 ± 0.27	2.08 ± 0.24	1.56 ± 0.26	1.79 ± 0.27	1.68 ± 0.13	1.71 ± 0.32
Ratio albumin/ globulins	1.39 ± 0.44	1.13 ± 0.42	0.98 ± 0.17	0.93 ± 0.20	0.72 ± 0.15	0.72 ± 0.11	0.85 ± 0.13	0.87 ± 0.13	0.90 ± 0.12	0.91 ± 0.12

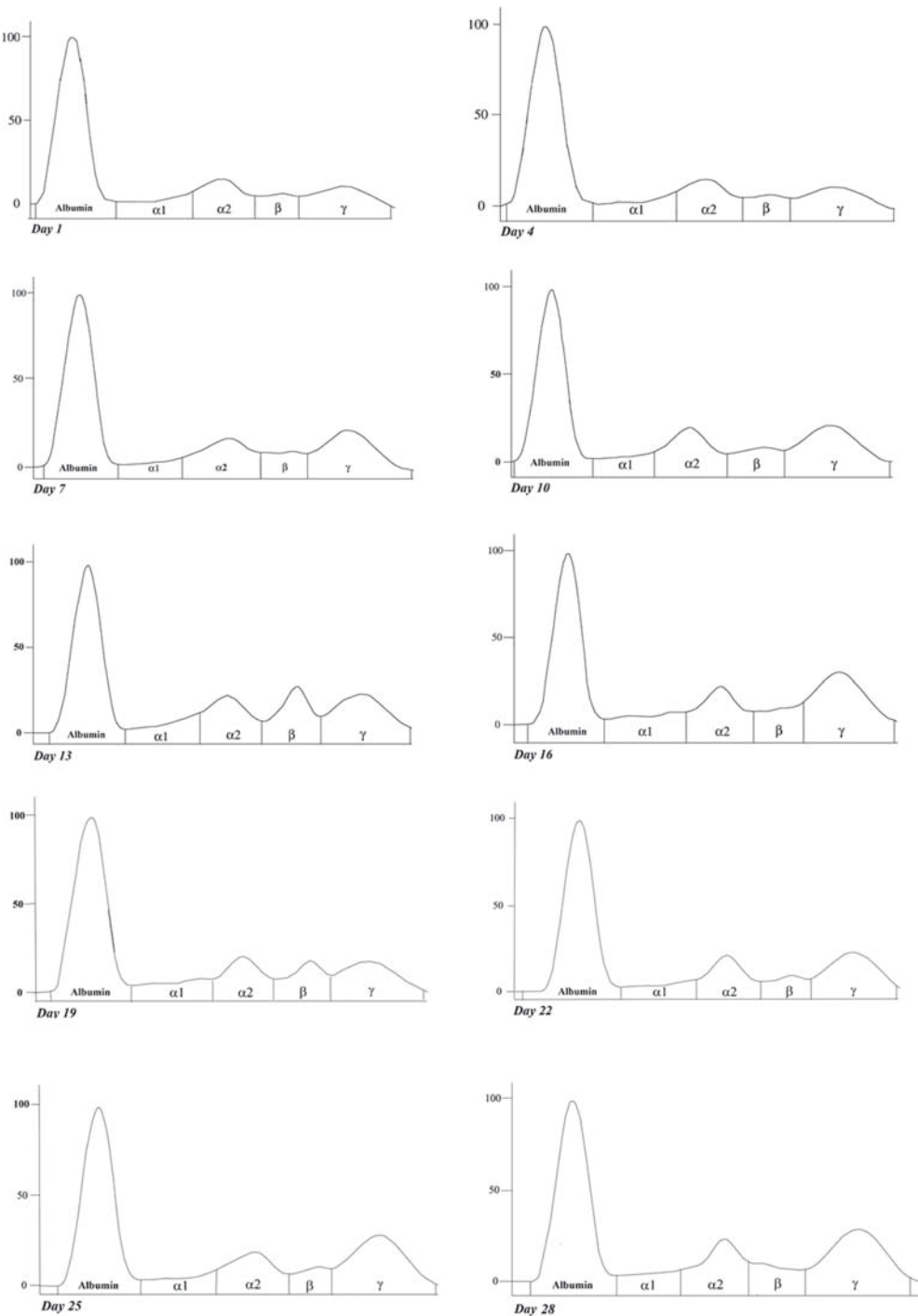


Figure 1
Serum protein electrophoretograms obtained in a lamb during the first month of life (days 1, 4, 7, 10, 13, 16, 19, 22, 25 and 28).

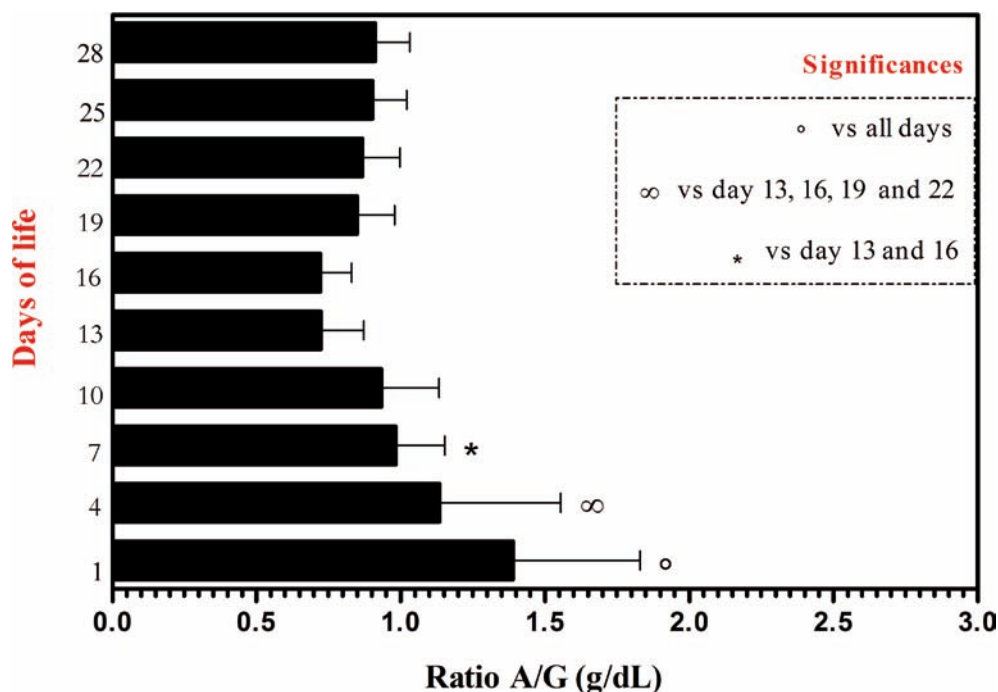


Figure 2

Mean values \pm standard deviation, together with statistical differences obtained in serum total proteins and their fractions (albumin, α 1-globulins, α 2-globulins, β -globulins, γ -globulins and ratio albumin/globulins) during the first month of life in ten newborn lambs.

As shown in Figure 2, ANOVA revealed a statistical effect of days of life on albumin, α 1-globulins, β -globulins, γ -globulins, albumin/globulins ratio and total proteins during the first 30 days of life ($P < 0.0001$), while no statistical significant effect of days of life on α 2-globulins appeared during the experimental period ($P = 0.27$).

Discussion

The neonatal period is very decisive in the rearing of lambs and during this period, mortality is a major factor limiting profitability in sheep farming. Immediately after birth, lambs are characterized by homeostatic physiological variability as shown in previous studies on different physiological parameters in these species during the first 4 weeks postpartum (Davey *et al.* 1998, Chen *et al.* 1999, Öztürk & Öxpınar 2006, Piccione *et al.* 2006, Piccione *et al.* 2007, Zumbo *et al.* 2011a, b). As previously demonstrated (Piccione *et al.*, Jezek *et al.* 2006, Mohri *et al.* 2007), our results confirm that blood parameters are in a developmental phase and a wide range of physiological values in different systems compensate for immaturity.

These results, obtained during the first period of life, showed a significant effect of days of life on total proteins, albumin, α 1-, β -, γ -globulins and albumin/globulins ratio during the first month of life while no difference was found for the α 2-globulins fraction. In particular, albumin, the main protein of mammal serum, essential for the regulation and keeping of oncotic pressure or osmotic pressure, necessary for the proper distribution of body fluids

in the vascular compartment and in tissues, showed a decrease during the first two weeks of life after the highest peak on the first day of life. Albumin started to increase constantly with the beginning of the 3rd week of life until the end of monitoring. This trend, according to a previous study on kids (Piccione *et al.* 2011), reflects the albumin's medium half-life that ranges from 14 to 16 days in ruminants, after which period the liver is responsible for albumin synthesis (Kaneko 1997, Thrall *et al.* 2004).

According to other authors that studied the course of these parameters in calf, foals (Bauer *et al.* 1985, Piccione *et al.* 2009) and lambs (Eales & Small 1981, Chen *et al.* 1999, Arguello *et al.* 2004, Nowak & Poindron 2006), total proteins and serum immunoglobulin levels showed the highest peak on the first day of life. In particular, our data showed that in the development from birth to the first months of life, total proteins exhibited a decrease on day 4 and then increase progressively until the end of the first month of life.

Alpha1-globulins, after a progressive decrease during the first two weeks of life, increased from the 3rd week of life to the end of monitoring. Beta- and γ -globulins increase progressively from 1st day to the end of the first month of life. These results could be explained by the first intake of colostrum during the first hours of life of lambs. In fact, it is well demonstrated, that newborns' physiological protein concentration and globulin fractions are variable during the first month of life as adaptive response to various environmental factors after birth including nutrition. In particular, the levels of these parameters are closely related to newborns' ingestion of colostrum within the first hours of life (Chen *et al.* 1999, Öztapak & Özpinar 2006, Massimini *et al.* 2006, Piccione *et al.* 2009). After the first colostrum intake, protein fractions are absorbed from the intestine, thus their concentration in the lamb's serum increase (Shubber *et al.* 1979a, Shubber *et al.* 1979b, Egli & Blum 1998, Piccione *et al.* 2009). This process of passive immunoglobulin absorption in the intestine ceases at about 24 h of age and is referred to as intestinal closure (Sawyer *et al.* 1977). Thus, the early mother-young interaction is crucial for the lamb's survival because it provides the lamb with colostrum to satisfy its metabolic needs and to protect it from aggression from the outside environment. Colostrum is produced just before parturition and contains dense nutrients as well as high levels of immunoglobulins, enzymes, hormones, growth factors and neuroendocrine peptides. It contains approximately 7 % fat, 4 % casein, 5 % lactose and 82 % water (Nowak & Poindron 2006) and because it is the unique source of food for the neonate, insufficient intake is the second major factor (after body reserve) that affects neonatal survival. Colostrum intake by newborn lambs immediately after birth and the following weeks significantly increases serum immunoglobulin concentration, which affects resistance to infectious diseases as well as survivability. Sufficient colostrum intake on the first days following birth plays an essential role in survival rate and resistance to infections of newborn lambs (Napolitano *et al.* 2002). In addition, as modifications of electrophoretic profile in the animals during the neonatal period can be used to aid the diagnosis and treatment of neonatal diseases, our study emphasizes the importance of establishing different reference intervals for these parameters in lambs based on their physiological status and diet.

In conclusion, the results obtained in this study, according to Massimini *et al.* 2006, indicated that passive transfer status, determined as serum immunoglobulin concentration 24 h after birth, is a significant source of variation in the pre-weaning growth performance of dairy lambs. The finding makes a contribution to the knowledge of physiological adaptation

in lambs during the first 30 days of life and gives useful information for the diagnosis and treatment of neonatal diseases.

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