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CHANGES IN SERUM TOTAL PROTEINS, PROTEIN FRACTIONS AND ALBUMIN-GLOBULIN RATIO DURING NEONATAL PERIOD IN GOAT KIDS AND THEIR MOTHERS AFTER PARTURITION

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Abstract

The aim of this study was to characterize the physiological changes in serum proteins of goat kids during the first month of life compared to changes in their mothers. The experimental subjects were five goats and their ten twin kids. Once a week, during the first month after parturition, serum total proteins and serum protein fractions were investigated. Two-way Repeated Measures ANOVA was used to evaluate the influence of sampling time and the differences between adults and kids. Kids and mothers showed a statistically significant effect of days after parturition ($P<0.01$) on albumin, alpha 1-, alpha 2- and gamma-globulins and total proteins. These showed a significant increase 21 days after parturition and a significant decrease to return to basal values on the last sampling day. A significant effect of age between mothers and kids ($P<0.001$) was found for all the parameters. The application of linear regression showed a significant correlation ($r = 0.58$) among postnatal age and gamma-globulins. It is concluded that although physiological protein concentrations are rarely influenced by age in adult animals, these are strictly dependent on days of life in the neonate. It is therefore essential that the normal electrophoretic pattern of each species be determined in neonates because this reflects the response to changes in homeostasis or disease.

Key words: serum proteins, electrophoresis, kids, neonatal period

In all animal species, the interval from birth to 28 days of age, known as the neonatal period, represents a delicate phase during which the metabolical profile, the serum and biochemical characteristics undergo a differentiation (Piccione et al.,

2006; Mohri et al., 2007; Piccione et al., 2008). The metabolic responses that occur during the transition from a fetal to neonatal life represent a transition phase from an unstable to a more stable status (Piccione et al., 2006). The newborn becomes engaged in a series of profound metabolic and morphological changes that are known as the adaptive period (Piccione et al., 2007). In fact, it is recognized as the most vulnerable period in the life of animals because of the high mortality and morbidity, which are more relevant during the first days of life (Piccione et al., 2008; Piccione et al., 2009; Piccione et al., 2010). Since diseases of newborns and neonatal mortality are a relevant cause of economic losses in livestock production many authors have investigated the causes of death, which can be broadly categorized as relating to the birth process and neonatal adaptation to postnatal life of lambs or kids (Mohri et al., 2007; Dwyer, 2008). Particularly, the determination of serum proteins has evolved into important diagnostic aids, showing that under either intensive or extensive conditions, kids with higher serum protein concentration than 800 mg/dl during the first 48 hours of life have lower morbidity and mortality rates than kids with lower serum protein levels (Kaneko, 1997; Mellado et al., 2008). Several studies indicated that an important proportion of kids does not reach this level of serum proteins shortly after birth (Mellado et al., 2008). Before birth, proteins represent nutrients and are an essential component for the dam and the growing fetus (Batavani et al., 2006; Mellado et al., 2008). In many newborn animals, colostrum is the main source of immunoglobulins and other proteins, necessary for the future life (Constant et al., 1994). Therefore, total proteins, both in colostrum and serum, also contribute profoundly to neonate immunity and growth, not only because of the immunoglobulin content, but possibly also due to other nutritional and physiological effects on the neonates (Chen et al., 1999; Piccione et al., 2007).

Because in ruminants the placenta interferes with the transfer of immunoglobulins from the dam to the fetus, the consumption of colostrum by the progeny of these species holds a fundamental role in the acquisition of immunity (Arguello et al., 2004).

Different studies showed that in kids, absorption of immunoglobulins from colostrum supplements has been reported to be poor (Constant et al., 1994; Zadoks et al., 2001). Some researchers demonstrated that parameters such as total serum proteins, globulins and other parameters are involved in the changes typical of the adaptive period, influenced also by the intake of the first colostrum (Gurgoze and Icen, 2010). Identifying and quantifying individual protein fractions and determining the normal serum protein pattern in a species enable the identification of individuals with altered patterns (Alberghina et al., 2011). For such considerations, the aim of this study was to investigate the physiological changes in serum protein patterns in the neonate kid to identify abnormalities in patient laboratory data.

Material and methods

The study was conducted in March on a farm in north-eastern Sicily (38˚2’N, 14˚40’E), Italy, at an altitude of 420 meters above the sea level. During the experi-

mental period, average ambient temperature and relative humidity were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK). The temperature-humidity index (THI) was then calculated with the following equation:

$$THI[°C] = t_{bs} - (0.55 - 0.55\ \varphi/100)\ (t_{bs} - 14.4)$$

where:
t_{bs} = dry-bulb temperature (°C),
φ = Relative Humidity (%).

The enviromental conditions are given in Figure 1.

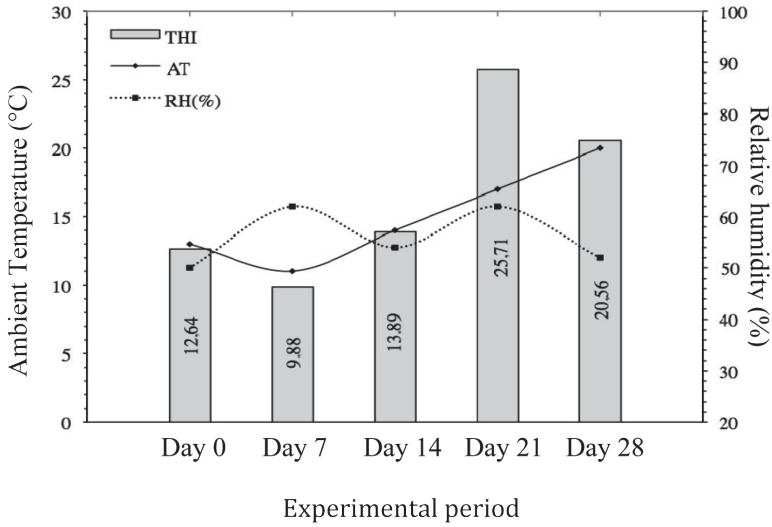


Figure 1. Graphical representation of the ambient temperature, temperature humidity index (THI) (expressed in °C), and relative humidity (expressed in %), recorded during the first month of life in twin kids and their five mothers after parturition

The experiment was carried out on five adult multiparous female goats (4 years old) of the Messinese breed, with an average body weight of 25.5±1.02 kg, and on their twin kids, born from each goat. All adult goats had free access to water and grazing, whereas the kids were fed only with colostrum and maternal milk and kept in a sheltered outdoor pen. Goats and kids were clinically healthy and the mothers were preventively treated for internal parasites at the start of the experiment. Their health status was evaluated before each sampling, based on behaviour, rectal temperature, heart rate, respiratory profile, cough, nasal discharge, ocular discharge, appetite, faecal consistency, navel examination and haematological profile. All kids were term born with an average birth weight of 3.07±0.30 kg and an average body weight of 7.17±0.40 kg at the end of the experimental period. Once a week at the

same hour in the morning (9:00 a.m.), during the first month after parturition, 5 ml of blood were collected from the external jugular vein of goats by vacutainer tubes (Terumo Corporation, Japan) with no additive. The first sampling in the kids was performed a few hours after the colostrum intake. From kids only 3 ml of blood were collected with the same modality. All the samples were allowed to clot at room temperature (20ºC) and centrifuged at 2081 g for 15 minutes to separate serum. The serum samples were not lipemic nor hemolyzed. They were dispensed into plastic tubes and stored at –20ºC pending analysis.

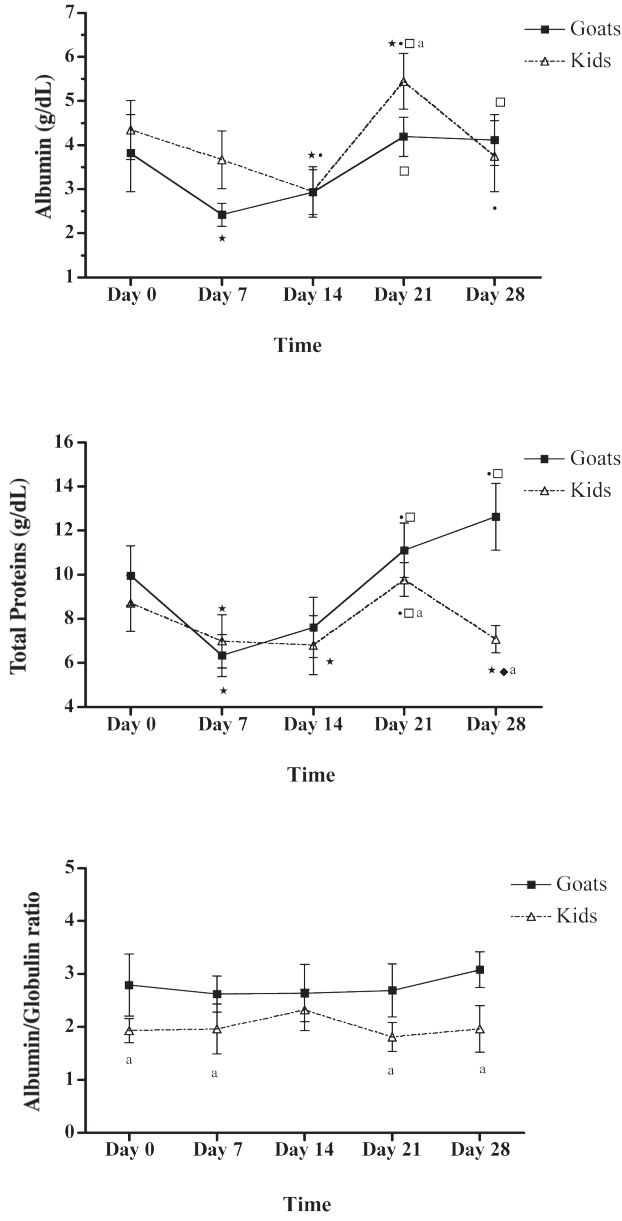
The total protein concentrations were determined in sera by the biuret method using a UV spectrophotometer (model Slim, SEAC, Florence, Italy). The protein standard was albumin (5.00 g/dL; Dasit, Milan, Italy). Electrophoresis was performed using a semiautomated AGE system (Selvet 24, Seleo Engineering, Naples, Italy) according to the procedures described by the manufacturer. For each sample, 25 μL of serum were applied to numbered sample wells. Each holder accommodated up to 24 samples. Films were electrophoresed for 28 min. at 450 V. After electrophoresis, films were simultaneously fixed using an automated system, stained in red stain acid solution for 10 minutes, and then dried at 37°C. After destaining in acetic acid and drying completely for 15 minutes films were scanned on a densitometer, and 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, alpha 1-, alpha 2-, beta- and gamma-globulins, respectively.

All results were expressed as mean ± standard deviation. On all data, normally distributed ($P<0.05$, Kolmogorov-Smirnov's Test), two-way Repeated Measure Analysis of Variance (ANOVA) was applied to evaluate the influence of the sampling time and the differences between goats and kids. If ANOVA showed an acceptable level of significance ($P<0.05$), Bonferroni's test was applied for post hoc comparison. Regression of the studied parameters on postnatal age (days of life), with 95% confidence intervals and the correlation coefficient (r), was determined. Data were analysed using Statistica 7 software package (Statsoft Inc., USA).

Results

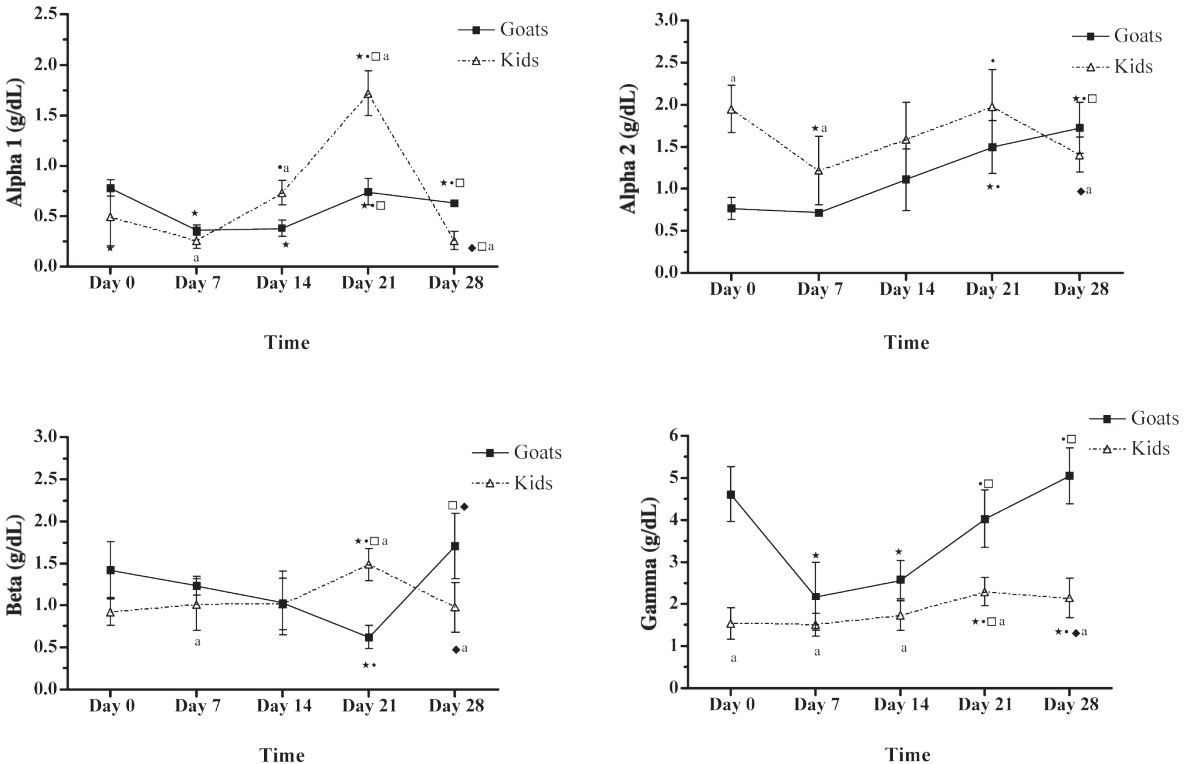
Figures 2 and 3 show the patterns of the mean values (±SD) of the studied parameters in kids and in their mothers, monitored during the experimental period with the statistical significances.

Both kids and goats showed a statistically significant effect of days after parturition on albumin ($P<0.0001$; $F_{(4;48)} = 21.71$); alpha 1-globulins ($P<0.0001$; $F_{(4;48)} = 125.20$); alpha 2-globulins ($P<0.0001$; $F_{(4;48)} = 10.06$); gamma-globulins ($P<0.0001$; $F_{(4;48)} = 26.34$) and total proteins ($P<0.0001$; $F_{(4;48)} = 9.03$). All the significant parameters showed a significant increase during the 21st day after parturition. The values decreased to return to the basal value on the last sampling day.



Significance of age: [a] vs goat (P<0.001).
Sampling time: *vs Day 0 (P<0.001); •vs Day 7 (P<0.001); □vs Day 14 (P<0.01); ◆vs Day 21 (P<0.001).

Figure 2. The pattern of mean values (±SD) of albumin, total proteins and albumin/globulin ratio, measured during the first month of life in ten twin kids, with the statistical significances



Significance of age: a vs goat (P<0.001).
Sampling time: *vs Day 0 (P<0.001); • vs Day 7 (P<0.001); ▫vs Day 14 (P<0.01); ◆vs Day 21 (P<0.001).

Figure 3. The pattern of mean values (±SD) of the serum protein fractions (alpha-1 globulins, alpha-2 globulins, beta-globulins and gamma-globulins) measured during the first month of life in ten twin kids, with the statistical significance

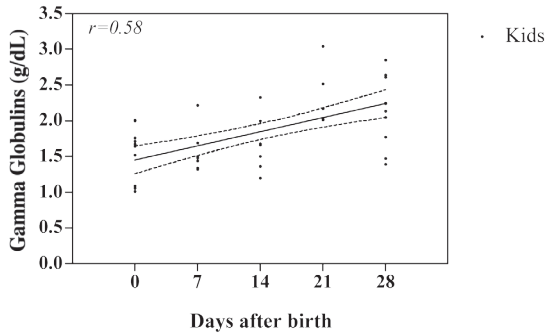


Figure 4. Regression line between days after birth and gamma-globulins (g/dL) of ten kids with 95% confidence interval and the correlation coefficient (r)

A significant effect of age between goats and kids was found on all the analysed parameters ($P<0.001$). The application of linear regression model ($y = a + bx$) showed a correlation only among postnatal age (days after birth) and gamma-globulins (Figure 4).

Discussion

Albumin showed a constant level from birth till the end of the experimental period. This is probably due to the metabolic pathways typical during the postnatal age. In fact, protein metabolism plays an important role in the regulation of physiological functions during pregnancy and lactation (Diogenes et al., 2010). The albumin is the most represented protein in the colostrum and in milk, as demonstrated by other authors (Rufibach et al., 2006; Mellado et al., 2008). The albumin levels showed a significant decrease in the first 14 days after birth, with a subsequent increase. This trend 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, 2004).

The alpha fractions showed substantial increases during the early days after birth and, if compared with serum levels found in mothers, were higher. The alpha fractions include two acute phase proteins (serum amyloid A and haptoglobin), which increase both with inflammatory status and stressors (Kaneko, 1997). In fact, as shown in other ruminant species, the environmental conditions represent an important trigger for the shift in acute phase proteins (Lomborg et al., 2008; Bagshani et al., 2008). During the experimental period, high THI (25.71ºC) was recorded at 21 days after birth. It seems that ambient temperatures and relative humidity have a considerable influence on farm animals, causing changes in feed intake, metabolism and heat balance (Bernabucci et al., 2009). Similarly the beta-globulin fractions showed an increase on day 21 after birth. This is because the most important proteins included

in beta fractions are the complement and the C-reactive protein, which like the acute phase proteins are involved in the environmental stress response (Kaneko, 1997; Bernabucci et al., 2009).

The gamma-globulin fraction is principally composed of immunoglobulins (Kaneko, 1997; Thrall, 2004; Alberghina et al., 2010). In the adult, the lymphoid tissues produce immunoglobulins as a response to antigenic stimulation, but in the neonatal period these are provided exclusively by colostrum so that the passive immunity can be acquired (Thrall, 2004). Our results showed a low, but constant, level of serum gamma-globulins, if compared with the mothers. Moreover, the gamma fraction is the only parameter that showed a positive correlation with the days of life of newborns. As shown in the calf, precolostral serum normally does not contain immunoglobulins, but these appear after a few hours from suckling colostrum and increase constantly during the postnatal period (Kaneko, 1997). This can be also related to the immunity of kids whose function during this period is not yet completely attributable to the lymphoid tissues, but it is principally due to colostrum-derived immunoglobulins. Instead, in the latter days of the experimental period a small increase is recorded and this can be related to the development of the lymphoid function. Total protein serum levels became higher at the beginning of the milking period and this variation is comparable to the goat's serum levels (Djuricic et al., 2010). Other authors related that a low serum level of total proteins corresponds with a low survival rate in newborns, and this is because the serum total proteins and immunoglobulin fractions are necessary for the neonate immunity and growth (Chen et al., 1999; Mellado et al., 2008).

In conclusion, we can affirm that although physiological protein concentrations are rarely influenced by age in the adult animals, in the neonate these are strictly dependent on days of life. Therefore, it is essential that the normal electrophoretic pattern of each species be determined in neonates because this reflects response to changes in homeostasis or disease.

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Zmiany poziomu białka całkowitego osocza krwi, frakcji białkowych i wskaźnika albuminowo-globulinowego w okresie neonatalnym u koźląt i ich matek po porodzie

STRESZCZENIE

Celem doświadczenia była charakterystyka fizjologicznych zmian w białkach osocza krwi koźląt w 1. miesiącu życia w porównaniu do zmian u ich matek. Materiał doświadczalny stanowiło 5 kóz i 10 ich koźląt z urodzeń bliźniaczych. Białka całkowite i frakcje białek osocza badano raz w tygodniu podczas pierwszego miesiąca po porodzie. Wpływ terminu pobierania próbek i różnice pomiędzy dorosłymi kozami a koźlętami oceniono przy pomocy dwuczynnikowej analizy wariancji z powtórzonymi pomiarami. U koźląt i ich matek stwierdzono statystycznie istotny wpływ dni po porodzie ($P<0,01$) na albuminy, alfa1-, alfa2- i gamma-globuliny oraz białka całkowite, których poziom wzrósł istotnie 21 dni po porodzie i zmniejszył się istotnie w ostatnim dniu pobierania próbek, powracając do wartości podstawowych. Istotny wpływ wieku matek i koźląt ($P<0,001$) stwierdzono dla wszystkich parametrów. Zastosowanie regresji liniowej wykazało istotną korelację ($r=0,58$) pomiędzy wiekiem po urodzeniu a poziomem gamma-globulin. W podsumowaniu stwierdza się, że choć wiek dorosłych zwierząt rzadko wpływa na fizjologiczne stężenia białka, u nowonarodzonych zwierząt stężenia te zależą ściśle od dnia życia. Należy zatem określić u noworodków zwierząt prawidłowy wzór elektroforetyczny dla każdego gatunku, ponieważ odzwierciedla on reakcję na zmiany homeostazy lub chorobę.