

Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period

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Assessed were changes in selected biochemical parameters taking place during different stages of pregnancy, *post-partum*, lactation and dry period in ewes. The observations were conducted on 10 clinically healthy Comisana ewes kept on one farm. Blood samples were withdrawn once before the morning feeding during *dioestrus* phase before pregnancy, and then on week 4, 11 and 18 of pregnancy, week 2 *post-partum*, week 5, 15 and 25 of lactation and finally once in a dry period (week 32). Blood serum total protein as well as serum albumin increased significantly during pregnancy compared to *dioestrus* phase. Compared to *dioestrus*, a significant increase during late gestation and a significant decrease during dry period was shown in blood urea. Total blood lipids showed a significant increase during pregnancy, *post-partum* and early lactation compared to *dioestrus*, while total cholesterol and triglycerides showed the opposite trend. The study showed marked changes in certain biochemical parameters of sheep blood serum during late pregnancy, *post-partum*, lactation and dry period.

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There are numerous studies on the effects of different phases of the reproduction cycle on biochemical parameters in domestic animal species. In sheep and goats they were carried out, among others, in relation to *oestrus* cycle, pregnancy and lactation [Krajničáková *et al.* 1993, Iriadam 2007].

Pregnancy and lactation are physiological statuses considered to modify metabolism in animals [Krajničáková *et al.* 2003, Iriadam 2007]. Blood biochemical parameters including total protein, triglycerides, free fatty acids and urea are important indicators of the metabolic activity in lactating animals [Karapehliyan *et al.* 2007]. During pregnancy, maternal tissues are involved in providing energy for reproduction processes, which may affect blood serum chemistry values, affected also by several other factors as breed, age, malnutrition, foetal growth, or season [Swanson *et al.* 2004, Yokus *et al.* 2006]. In sheep, during late pregnancy, blood serum lipids profile is characterized by increased concentration of total cholesterol, triglycerides and lipoproteins [Schlumbohm *et al.* 1997] due to the diminished responsiveness of target tissues towards insulin that, together with an increased mobilization of fatty acids from adipose tissue make available new sources for foetal growth. Variation in blood cholesterol content has been observed during *oestrus* and pregnancy, as precursor of the steroid hormones [Iriadam 2007]. Lipid profiles have been used to predict peripartum diseases; circulating blood triglycerides contribute significantly to milk fat synthesis [Nazifi *et al.* 2002]. Relative to protein metabolism, a decrease in blood protein concentration during the late stages of gestation was observed in sheep, witness the utilization of amino acids for protein synthesis in the foetal muscles [Antunovic *et al.* 2002]. It was also reported that plasma urea levels increased during week 10 of pregnancy, reaching a peak at parturition [El-Sherif and Assad 2001], which in domestic ruminants was ascribed to the cortisol-stimulated catabolism of proteins in the body [Silanikove 2000].

During lactation, the mammary gland secretory cells utilize 80% of the blood-circulating metabolites for milk synthesis, depending on the speed of infiltration of precursors of milk compounds (*i.e.* free amino acids, glucose and fatty acids). The strong reduction in lipogenesis and the increased fatty acid release, supported by norepinephrine and epinephrine stimulation, induce an increase in lipase activity of mammary gland, to provide the substrates for milk fat synthesis [Nazifi *et al.* 2002]. In lactating goats an increasing total protein level of serum was observed with the progress of lactation [Krajničáková *et al.* 2003] due to the catabolism of protein for milk synthesis.

This study attempted at providing a complete picture of dynamics of selected biochemical blood parameters in ewes from *dioestrus* to getting dry. This complete picture want to supply the normal range of the most commonly used serum biochemical parameters during the different reproductive stages, that should be considered as guidelines for the management strategies for ewes during farming condition. These guidelines should guarantee the metabolic needs of this animal and reduce the economic lost.

Materials and methods

Ten clinically healthy Comisana ewes were used (3 ± 0.6 years old, with a mean body weight of 52.1 ± 3.2 kg), free from internal and external parasites and kept on one farm. All ewes were housed in a barn and fed twice a day (08:00, 20:00) with straw and hay from first-crop polyphyte meadow and mix of cereals formulated according to their physiological and productive status: pregnancy (fresh matter: 17.90% crude protein, 2.80% crude fat, 8.60% crude fibre, 8.0% crude ash, 45,000 IU vit. A, 2,700 vit D, 68 mg Vit E, 150 mg vit PP, 5 mg copper, 188 mg zinc) and lactation (fresh matter: 14.30% crude protein, 3.90% crude fat, 7.90% crude fibre, 8.60% crude ash, 32,000 IU vit. A, 2,000 IU Vit D, 50 mg vit. E, 4 mg copper). During the trial, all animals were kept under natural photoperiod and ambient temperature, on a farm located in Sicily ($38^{\circ} 7' N$; $13^{\circ} 22' E$, 300 m above sea level).

The first blood samples were withdrawn once from all ewes, at the same hour (07:30), before pregnancy (during the *dioestrus* phase, 5 days after the last *oestrus*). Thereafter, blood was sampled on week 4, 11 and 18 of pregnancy, on week 2 *post-partum*, week 5, 15 and 25 of lactation and last during the dry period (week 32).

Blood samples, each of 10 ml, were withdrawn from jugular vein from each animal into vacuum glass tubes containing no anticoagulant. Following standing at room temperature for 20 min, the tubes were centrifuged at 3,000 rpm for 10 min and the serum samples stored at $-25^{\circ}C$ until analysed. Serum total protein, albumin, urea, creatinine, total lipids, triglycerides, total cholesterol and phospholipids contents were determined with the use of commercial kits (SEAC, Florence, Italy) and finally measured using the UV Spectrophotometer (SEAC, Slim, Florence, Italy).

All results were expressed as means and standard deviations (SD). The analysis of variance (ANOVA) was used to test the overall significance of differences among the means. Bonferroni's Multiple Comparison Test was applied for *post hoc* comparison. The software STATISTICA 5.5 (StatSoft Inc., Tulsa, OK, USA) was used for computations.

Results and discussion

Significant effect of time was identified on all serum parameters considered: **total protein** ($P<0.0001$, $F_{(8,72)}=9.52$), **albumin** ($P<0.0001$, $F_{(8,72)}=7.63$), **urea** ($P<0.0001$, $F_{(8,72)}=14.75$), **creatinine** ($P\leq 0.003$, $F_{(8,72)}=3.27$), **total lipids** ($P<0.0001$, $F_{(8,72)}=36.77$), **triglycerides** ($P<0.0001$, $F_{(8,72)}=7.06$), **total cholesterol** ($P<0.0001$, $F_{(8,72)}=7.51$) and **phospholipids** ($P\leq 0.004$, $F_{(8,72)}=3.07$).

Serum total protein showed a significant increase during all (early, mid and end) lactation and dry period compared to *dioestrus*, pregnancy and *post-partum* periods. Albumin increased significantly during the late gestation and end of lactation vs. *dioestrus* and dry period compared to *dioestrus*, pregnancy, *post-partum* and early lactation periods. Urea showed a significant decrease at the end of lactation and in dry

period compared to *dioestrus*, pregnancy, *post-partum*, early and mid lactation, and during mid lactation vs late gestation. Creatinine level significantly increased during dry period compared to the end of gestation (Tab. 1).

Table 1. Means and standard deviations (SD) for total protein, albumin, urea and creatinine contents of blood serum over 9 reproduction periods in Comisana ewes

Period		Total protein (g/l)	Albumin (g/l)	Urea (mmol/l)	Creatinine (μ mol/l)
<i>Dioestrus</i>	mean	69.60	24.05	10.55	133.92
	SD	7.60	3.13	2.69	16.18
Early gestation	mean	70.60	24.57	11.24	135.20
	SD	7.73	3.38	1.81	26.08
Mid gestation	mean	71.16	24.78	11.66	128.70
	SD	7.73	3.39	2.16	25.36
Late gestation	mean	72.10	25.06	12.47	119.40
	SD	7.20	3.07	2.00	25.44
<i>Post-partum</i>	mean	72.47	25.65	10.81	129.50
	SD	7.09	3.04	1.77	28.66
Early lactation	mean	73.07 ^a	26.20	10.19	138.90
	SD	6.83	3.11	1.60	27.67
Mid lactation	mean	73.95 ^{ab}	27.04	9.83	141.43
	SD	7.73	3.16	1.45	25.22
End of lactation	mean	74.98 ^{abc}	27.55 ^a	7.25 ^{abCdeFg}	142.00
	SD	7.56	3.25	1.50	26.87
Dry period	mean	76.40 ^{abcdE}	29.63 ^{abcdE}	6.74 ^{abCdeFg}	149.80 ^d
	SD	7.48	3.58	1.56	26.26

Significance of differences among means:

^avs. *dioestrus*, P<0.05; ^bvs. early gestation, P<0.05; ^cvs. mid gestation, P<0.01; ^dvs. late gestation, P<0.05; ^evs. *post-partum*, P<0.01; ^fvs. early lactation, P<0.05; ^gvs. mid lactation, P<0.05.

Total lipids level increased significantly from *dioestrus* to early lactation, and a significant decrease was found over the mid and the end of lactation and dry period vs. late gestation, post partum and early lactation. Triglyceride level was found to drop significantly during the late gestation, *post-partum*, and early and mid lactation compared to *dioestrus* and during the late gestation and *post-partum* vs. early gestation. In total cholesterol a significant decrease during the late gestation, *post-partum*, early and mid lactation compared to *dioestrus*, and during the *post-partum* and early lactation vs early gestation period, as well as significant increase during dry period compared to late gestation, *post-partum* and all lactation was found. In phospholipids, a significant increase during early lactation vs. early gestation, and a significant decrease during dry period vs. early lactation was identified (Tab. 2)

The results presented in Tables 1 and 2 show that the blood serum biochemical parameters considered in this report were affected by the reproduction stages of sheep. Mean values obtained under different conditions for the parameters studied occur similar to those established by Hindson and Winter [2002].

Table 2. Means and standard deviations (SD) for total lipids, triglycerides, total cholesterol and phospholipids contents of blood serum over 9 reproduction periods in Comisana ewes (n=10)

Period		Total lipids (g/l)	Triglycerides (mg/dl)	Total cholesterol (mmol/l)	Phospholipids (mg/dl)
<i>Dioestrus</i>	mean	1.73	87.30	1.77	90.71
	SD	0.17	7.10	0.36	6.27
Early gestation	mean	1.70	85.56	1.73	88.79
	SD	0.18	8.39	0.47	5.86
Mid gestation	mean	2.00 ^{ab}	83.40	1.60	93.47
	SD	0.22	8.78	0.36	8.56
Late gestation	mean	2.31 ^{abc}	77.08 ^{ab}	1.45 ^a	94.28
	SD	0.23	9.95	0.47	8.72
<i>Post-partum</i>	mean	2.41 ^{abc}	78.07 ^{ab}	1.41 ^{ab}	97.22
	SD	0.24	9.55	0.37	8.08
Early lactation	mean	2.43 ^{abc}	78.79 ^a	1.39 ^{ab}	99.70 ^b
	SD	0.16	9.43	0.32	8.50
Mid lactation	mean	1.83 ^{bDef}	79.40 ^d	1.46 ^a	94.16
	SD	0.22	9.45	0.35	7.45
End of lactation	mean	1.94 ^{DEf}	80.61	1.52	93.73
	SD	0.20	10.19	0.41	7.30
Dry period	mean	1.85 ^{DEf}	86.65 ^{DEfg}	1.85 ^{DEfgh}	90.00 ^f
	SD	0.15	8.55	0.41	9.19

Significance of differences among means:

^avs. *dioestrus*, P<0.05; ^bvs. early gestation, P<0.05; ^cvs. mid gestation, P<0.01; ^dvs. late gestation, P<0.05; ^evs. *post-partum*, P<0.01; ^fvs. early lactation, P<0.05; ^gvs. mid lactation, P<0.05; ^hvs. end of lactation, P<0.05.

In dry period an increase in **total protein** content was found, compared to *dioestrus*, gestation, *post-partum* and early lactation. Baumgartner and Pernthaler [1994] had not found a significant effect of the reproduction stage on the serum concentration of total protein in Karakul sheep. Maternal serum protein concentrations decrease due to an increased foetal growth, and especially the utilization of amino acids from the maternal circulation for protein synthesis in the foetal muscles [Antunovic *et al.* 2002]. The significant increase in early, mid and late lactation of serum total protein compared to *dioestrus* and early gestation could be due to a decrease in serum globulin [El-Sherif and Assad 2001]. The higher values of total protein in lactating ewes compared to *dioestrus* phase prove the high energy need due to milk synthesis which exists in animals, as confirmed by other authors, especially during the early lactation [Bremmer *et al.* 2000].

The significant increase in **albumin** level over dry period could be ascribed to the low protein intake during this period and dehydration [Yokus *et al.* 2006]. The increased total blood volume especially in late pregnancy induces an increase in glomerular filtration, which is also responsible for the increased values of albumin

and urea during late gestation [Yokus *et al.* 2006]. The significant increase of albumin in late gestation compared to *dioestrus* proves the higher energy requirement for the foetal growth [Durak and Altiner 2006].

A significant drop was found in **urea** content of serum at the end of lactation and over dry period, being in accordance with a study by Karaphelivan *et al.* [2007] on Tuj ewes and Yokus *et al.* [2006] on Sakiz-Awassi crossbreds. These recent reports support the hypothesis that changes in blood urea content during lactation could depend on milk synthesis [El-Sherif and Assad 2001]. It is probably associated with the use of urea for protein synthesis on the ruminohepatic pathway due to compensation of the low protein uptake during the dry period [Yokus *et al.* 2006]. The highest values of blood urea over the gestation period were reported by El-Sherif and Assad [2001] in Barki ewes in which plasma urea level started rising during week 10 of pregnancy and reached a peak at parturition, and by Durak and Altinek [2006] in Chios ewes. The reason for those highest values could be the increased cortisol level affecting the catabolism of protein in the body [Silanikove 2000].

The elevated values of urea during late gestation compared to *dioestrus* could be ascribed to the high thyroid activity in pregnant females which induces an increased protein catabolism. The high requirement for energy by pregnant sheep during their second half of pregnancy led to an increase in urea level which is evident during late pregnancy in this study. The highest values of blood urea in the last trimester of pregnancy were also observed by Antunovic *et al.* [2002].

Creatinine content of serum was statistically significant higher in dry period than in late gestation. The quantity of creatinine formed each day depends on the total body content of creatine, which in turn depends on dietary intake, rate of synthesis of creatine, and muscle mass.

Traditionally, the dry period has been thought to be necessary for replenishment of body reserves, regeneration of mammary tissue, and for maximal benefits from lactogenic endocrine events [Annen *et al.*, 2004]. Protein mobilisation is one of the events involved in this mechanism. A major source of mobilised amino acids is protein breakdown in skeletal muscles, although skin, uterine involution and myometrial protein degradation may have some contribution [Blum *et al.*, 1985; Bell *et al.* 2000]. In dairy cows, plasma creatinine and muscle diameter decreased through the period from week 1 before calving to week 4 after calving. Moorby *et al.* [2002] also reported that longissimus dorsi muscle diameter starts to decrease before calving and reaches a minimum between 4 to 7 week of lactation. Then, we can supposed that in ewes protein mobilisation start during gestation reaching its peak during late gestation, during which was observed the lower creatinine values. After calving protein mobilisation probably is reduced and creatinine reached its peak during dry period.

Significant increase in **total lipids** in the middle and at the end of pregnancy compared to *dioestrus* could be ascribed to the higher levels of free fatty acids (FFA) in pregnant than in non-pregnant ewes, caused by increased level of cortisol due to stress induced by pregnancy [Fleming 1997] as well as increased sensitivity of ewes

to epinephrine hormone, which leads to the increase in serum FFA concentrations in late gestation [Revell *et al.* 2000]. The elevated level of total lipids in late gestation compared to *dioestrus* is probably due to the reduced insulin-mediated inhibition of lipolysis observed in late pregnancy [Schlumbohm *et al.* 1997]. Lipogenesis stimulated by insulin is also responsible for the increased values of total lipids observed in ewes during early lactation. The significant *post-partum* increase in serum total lipids induces an increased FFA uptake by the liver from circulating plasma, with an increased triglyceride storage, as observed in cattle by Grummer [1993].

Significant decrease in serum **triglycerides** found in this study in late pregnancy is in accordance with increased concentration of these compounds in the ewes' liver as reported by Smith and Walsh [1975]. However, Kano *et al.* [1981] working on pregnant pony mares reported lower triglyceride values to occur compared to late pregnancy, probably due to species-specific factors. The significant decrease in serum triglycerides *post-partum* could be explained as the effect of increased lipolysis which is hormonally regulated, and not an expression of energy deficiency [Holtenius and Hjort 1990]. The adipose tissue metabolism is strictly related to insulin, which stimulate lipogenesis in pregnant ewes, while lactating individuals show a significant decrease in that compound's level. The significant decrease in triglyceride of serum during early and mid lactation of sheep has also been reported by Gradinski-Urbanac *et al.* [1986] while *post-partum* by Nazifi *et al.* [2002] who observed the lowest concentrations of the compound 2-3 weeks *post-partum*. This was in accordance with other authors working on goats who showed increased values of serum triacylglycerols to occur just before parturition [Hussein and Azab 1998]. During lactation the insulin stimulation of lipogenesis becomes inefficient what is confirmed by the significant decrease in serum triglycerides and total cholesterol *post-partum* compared to early pregnancy as reported by Watson *et al.* [1993] after foaling, because of an increased lipoprotein lipase activity consistent with the induction of the enzyme into mammary tissue to provide for milk fat synthesis. The decreasing pattern of serum triglycerides and total cholesterol in early lactation was also reported in dairy cows which showed the lowest values of these compounds at the onset of lactation for their growing requirement for energy [Marcos *et al.* 1990].

The significant decrease in **total cholesterol** in late pregnancy has also been reported in other species: by Tainturier *et al.* [1984] in Friesan cows at the end of pregnancy, Bekeová *et al.* [1987] before parturition in cows and Krajničáková *et al.* [2003] in goats. This is probably related to the role of the compound in ovary steroid genesis, so that the total cholesterol concentrations are under control of the complex of factors. The decrease observed during lactation compared to *dioestrus* could be ascribed to the increased cholesterol uptake by tissues involved in milk synthesis, because of the normal insulin responsiveness compared to late pregnancy [Nazifi *et al.* 2002].

The peak **phospholipids** content of serum occurred in early lactation, differing significantly from that found in early gestation and dry period. This can be interpreted

as a balance mechanism to compensate the decrease of triglycerides. Increase of phospholipids could also result from increases dietary ingestion, which is at its lowest at parturition time [Bennis et al. 1992]. Marked changes in the parameters described here were related to the physiological adaptations of ewes to subsequent reproductive stages. In fact, the gestation and lactation periods affect the protein and lipid metabolism of ewes, with requirement for energy varying in the different stages of reproduction. This complete picture want to supply the normal range of the most commonly used serum biochemical parameters during the different reproductive stages. and should be considered as guidelines for the management strategies for ewes during farming condition. However, further investigations are necessary for the correct interpretation of metabolic diseases in ewes, aimed at avoiding a decline of the productive performance and consequently economic lost.

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Wybrane wskaźniki biochemiczne surowicy krwi owiec podczas ciąży, po wykocie, w laktacji i w okresie zasuszenia

Streszczenie

Obserwacji dokonano na 10 klinicznie zdrowych maciorkach rasy Comisana na Sycylii, utrzymywanych na jednej farmie. Próbkę krwi pobrano jednorazowo od owiec jałowych w fazie *oestrus*, a dalej w 4, 11 i 18 tygodniu ciąży, w 2 tygodnie po wykocie, w 5, 15 i 25 tygodniu laktacji i jednorazowo po zasuszeniu. Zawartość białka całkowitego i albuminy w surowicy podczas ciąży okazała się istotnie wyższa niż w fazie *dioestrus*. W okresie późnej ciąży stwierdzono, w porównaniu z *dioestrus*, istotny wzrost zawartości mocznika w surowicy, a w okresie zasuszenia istotny jego spadek. Zawartość lipidów całkowitych podczas ciąży, po wykocie i we wczesnej laktacji była istotnie wyższa niż w *dioestrus*, podczas gdy w przypadku cholesterolu całkowitego i trójglicerydów stwierdzono zjawisko odwrotne.