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RESEARCH ARTICLE

Investigation of Some Biochemical Parameters and Mineral Substance During Pregnancy and Postpartum Period in Awassi Ewes

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Summary

The aim of this study was to investigate the possible effects of the reproductive status on the serum chemistry and mineral substance in Awassi ewes at 21, 120 and 145 days of pregnancy and 7 and 14 days postpartum. All blood profiles were determined in 20 healthy pregnant Awassi ewes. Serum urea, total bilirubin, direct bilirubin, total protein, albumin, alanine transaminase (ALT), creatine kinase (CK) were higher in pregnancy while serum glucose, creatinine, alkaline phosphatase (ALP), P and Ca levels were higher in lactation. Neither the pregnancy nor the lactation effected AST, LDH, Fe and Mg levels. Lower (P<0.001) serum glucose levels were recorded on days 21, 120 and 145 of pregnancy, compared to days 7 and 14 postpartum. Blood total protein levels (P<0.01) decreased, while total bilirubin and direct bilirubin (P<0.01) increased, especially at 120 days of pregnancy. Serum urea (P<0.001) and ALT (P<0.05) levels increased on day 21 of pregnancy, compared with any other stages of gestation and days 7 and 14 postpartum. While serum CK (P<0.01) and albumin (P<0.001) levels were significantly higher on day 145 of gestation, serum ALP (P<0.05), and creatinine (P<0.001) levels were higher at 14 day postpartum. Although without statistical significance, the highest LDH concentration was found in the lactation periods. The mean level of serum Ca at 14 days postpartum was significantly (P<0.001) higher than prepartum period. The highest Mg levels were found at 14 days postpartum. Serum P levels were lower at 120 days of gestation and were higher 14 days postpartum. A gradual increase in serum Fe levels was recorded during pregnancy when compared to day 7 and 14 postpartum but it was not statistical significance. In conclusion, the present findings suggest that serum glucose, creatinine, urea, total bilirubin, direct bilirubin, total protein, albumin, ALT, CK, ALP, Ca and P concentrations of the ewes similarly fed, could alter depending on gestation period and parturition which have to be taken in to consideration for the correct interpretation of serum chemistry and elements status.

Keywords: *Blood metabolite, Pregnancy, Lactation, Ewes*

İvesi Koyunlarında Gebelik Sürecinde ve Postpartum Döneminde Bazı Biyokimyasal Parametreler ile Mineral Maddelerin Araştırılması

Özet

Bu çalışmada, İvesi koyunlarında gebeliğin 21, 120 ve 145. günleri ile doğum sonrası 7 ve 14. günlerde serum kimyası ve mineral madde düzeyleri üzerine reprodüktif durumun olası etkilerininin ortaya konulması amaçlandı. Tam kan profili 20 sağlıklı gebe İvesi koyununda tespit edildi. Serum üre, total bilirubin, direkt bilirubin, total protein, albumin, alanin transaminaz (ALT) ve kreatin kinaz (CK) düzeyleri gebelerde, serum glukoz, kreatinin, alkalin fosfataz (ALP), P ve Ca düzeyleri ise laktasyonda daha yüksekti. Ne gebelik ne de laktasyon AST, LDH, Fe ve Mg düzeylerini etkilemedi. En düşük (P<0.001) serum glukoz düzeyleri doğum sonrası 7 ve 14. günlerle karşılaştırıldığında gebeliğin 21,120 ve 145. günlerinde tespit edildi. Gebeliğin özellikle 120. gününde total bilirubin ve direkt bilirubin düzeyleri artarken (P<0.01) kan total protein düzeyleri azaldı (P<0.01). Serum üre (P<0.001) ve ALT (P<0.05) düzeyleri doğum sonrası 7 ve 14. günlerle ve gebeliğin diğer dönemleri ile karşılaştırıldığında gebeliğin 21. gününde arttı. İstatistiki açıdan en yüksek serum CK (P<0.01) ve albumin (P<0.001) düzeyleri gebeliğin 145. gününde, en yüksek serum ALP (P<0.05) ve kreatinin (P<0.001) düzeyleri ise doğum sonrası 14. günde saptandı. İstatistiksel öneme sahip olmamasına rağmen en yüksek LDH konsantrasyonu laktasyon peryodunda tespit edildi. Serum Ca düzeyleri doğum öncesine göre doğum sonrası 14. günde istatistiksel olarak daha yüksek (P< 0.001) bulundu. En yüksek Mg düzeyleri doğum sonrası 14. günde belirlendi. Serum P düzeyleri gebeliğin 120. gününde daha düşük, doğum sonrası 14. günde daha yüksekti. Serum Fe düzeylerinde gebelik esnasında postpartum 7 ve 14. günlere kıyasla istatistiksel olarak önemsiz ancak kademeli bir artış tespit edildi. Sonuç olarak, aynı şartlar altında beslenen koyunlarda serum glukoz, kreatinin, üre, total bilirubin, direk bilirubin, total protein, albumin, ALT, CK, ALP, Ca ve P düzeylerinin gebelik dönemi ve doğuma bağlı olarak değiştiği ve serum kimyası ile element durumunun doğru yorumlanabilmesi için bu durumun göz önünde bulundurulması gerektiği kanaatine varıldı.

Anahtar sözcükler: *Kan metabolitleri, Gebelik, Laktasyon, Koyun*

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INTRODUCTION

Major adaptations in maternal physiology and metabolism are required for successful pregnancy outcome. Hormonal changes initiated even before conception significantly alter maternal biochemistry early in pregnancy. As gestation progresses, reference ranges for the concentration of many biochemical parameters change significantly from those found in the nonpregnant state [1]. The identification of changes in the metabolism of sheep in various reproduction phases, the determination of abnormal metabolic states, and the prediction of some metabolic disorders, such as pregnancy toxaemia and fatty liver could provide some advantages to producers [2].

Metabolism of mineral substances plays a significant role in the regulation of physiological functions of the puerperal period. The ovarian activity of ruminants is influenced by mineral deficiency. Mineral deficiencies and imbalances are often cited as causes of poor reproduction. The nutritional requirements for these elements are small; however, these nutrients can greatly affect reproduction [3-6].

Blood glucose, blood urea nitrogen (BUN) and Ca concentrations were mainly recommended to diagnose pregnancy toxemia in ewes [7]. Blood glucose is the major metabolite used by the sheep fetus and the energy reguirements of the ewes increase during late pregnancy due to rapid growth of the fetus. As pregnancy progresses, there is a difference in serum glucose and total protein concentration as the need of the dam changes [2]. BUN concentration is protein metabolisms and indicates renal failure in the pregnancy toxemia [7].

Certain changes in values of liver function tests occur during normal pregnancy. Serum liver function tests are essential in the management of liver diseases during pregnancy. Routine liver function tests usually include total and conjugated bilirubin, amino-transferases, ALP.

The effects of reproductive status on the serum chemistry in Awassi ewes cannot be described adequately. This study was accomplished to compare the levels of metabolites in serum in the prepartum and postpartum periods. In this survey the levels of glucose, BUN, albumin, creatinin, total bilirubin, direc bilirubin, total protein, ALP, AST, ALT, CK, LDH, Ca, Fe, Mg and P in serum in the pregnant and lambed Awassi ewes comprised.

MATERIAL and METHODS

Experimental design

The study was carried out with Awassi ewes under natural conditions in research farm of Dicle University in the southeast of Turkey. This region is situated at 37°55′01″ N latitude and 40°16′46″ E longitude and at an altitude of 660m. The ewes grazed on natural pasture all day and water was offered ad libitum.

To understand the reproductive status on the serum chemistry and some macro element levels, ewes raised under the uniform pasture conditions. The study period started at May 2008 and ended at November 2008. A total 34 Awassi ewes clinically healthy, aged between 3-6 years, were used in the study. The treatment for estrous synchronization was performed using intravaginal progestagen sponges of 30 mg FGA for 12 days (Chronogest, Intervet International B.V., Boxmeer, Netherland). After wihtdrawall of sponges, 400 IU PMSG (Chorono-Gest/PMSG, Intervet International B.V., Boxmeer, Netherlands) was injected for enhancing pregnancy and rams were introduced. Pregnancies were diagnosed by transrectal ultrasonography (Pie medical LC 100) at 35 days after ram introduction. A total 20 ewes take into consideration because of pregnancies.

The blood samples were collected from jugular vein at days 21 before detection of pregnancies in all sponge treated ewes. Then, blood samples were collected from only pregnant ewes after detection of pregnancies by USG at days of 120 and 145 of gestation and, days of 7 and 14 of postpartum.

The serum was removed after centrifugation at 3000 rpm for 15 min at 4°C and aliquots were kept frozen at -20°C until the analysis.

Analytical procedures

The serum concentrations of glucose, BUN, albumin, creatinin, total bilirubin, direct bilirubin, total protein, ALP, AST, ALT, CK, LDH, Ca, Fe, Mg and P were detected using the commercial available kits (Biolabo SA, France). The analyses were carried out according to the manufacturer's instructions. Data were obtained using an autoanalyzer (AIRONE 200, Medisis Medical Systems Ltd.).

Statistical analysis

Data were analyzed at the different stages of

pregnancy, with the aid of a one-way analysis of variance (ANOVA), and the significance of differences with Duncan's test [8]. All values were presented as means ± S.E.

RESULTS

Serum urea, total bilirubin, direct bilirubin, total protein, albumin (P<0.001), ALT (P<0.05) and CK (P<0.01) concentrations increased significantly in pregnancy. Serum glucose, creatinine, Ca (P<0.001), ALP and P (P<0.05) concentrations were higher after parturation. AST and Mg remained unchanged in gestation and lactation. Serum Fe concentrations showed a tendency to increase during the gestation and LDH concentrations were somewhat increased in lactation, althougt the difference was not significant. The obtained results were summarized at *Table 1* and *2.*

DISCUSSION

There were significant differences in biochemical parameters parallel to changing physiology of the animals before and after parturition. The main objective of this study was to investigate possible effects of reproductive status on the serum chemistry and mineral concentrations in Awassi ewes.

The nutrient requirements of ewes increase during late pregnancy due to the rapid growth of the fetus. If ewes do not receive at least half of the required energy during this period, fat depots are mobilised in large quantities [11].

Some researchers [7,12-15] recorded plasma glucose concentrations to be higher during lactation than pregnancy in sheeps. In contrast, some authors [16,17] reported greater blood glucose levels in pregnant

Table 1. *Some biochemical parameters at 21, 120 and 145 days of pregnancy with 7 and 14 days postpartum in Awassi ewes (n:20)*
Tablo 1. *İvesi koyunlarında gebeliğin 21, 120 ve 145. günleri ile doğum sonrası 7 ve 14. günlerde bazı biyokimyasal parametreler*

Parameters	Days of Pregnancy			Days Postpartum		P	Physiological Levels (+)
	21	120	145	7	14		
ALT (IU/L)	25.00±4.14 ^{a}	17.06±1.30 ^{b}	18.35±1.35 ^{b}	16.00±1.26 ^{b}	15.94±1.55 ^{b}	*	14.8-43.8
AST (IU/L)	104.94±5.00	101.41±4.06	103.82±3.94	100.12±3.33	110.76±4.98	-	48.0-123.3
ALP (IU/L)	218.41±17.56 ^{ab}	148.88±16.08 ^{c}	165.47±17.92 ^{bc}	175.12±22.23 ^{bc}	224.06±22.83 ^{ab}	*	68-387
LDH (IU/L)	473.20±22.27	484.63±22.90	457.78±12.47	450.30±12.49	531.16±28.93	-	60-111
CK (IU/L)	11.11±1.55 ^{ab}	6.76±0.85 ^{c}	12.29±1.43 ^{a}	8.82±0.50 ^{bc}	9.41±0.70 ^{abc}	**	NA
BUN (mg/dl)	45.22±3.98 ^{a}	17.03±0.73 ^{c}	26.61±5.14 ^{b}	30.56±1.50 ^{b}	26.02±1.08 ^{b}	***	18-31
CRE (mg/dl)	0.50±0.03 ^{c}	0.64±0.01 ^{b}	0.65±0.03 ^{ab}	0.61±0.02 ^{b}	0.73±0.04 ^{a}	***	0.9-2.0
Glukoz(mg/dl)	33.82±1.55 ^{c}	32.18±1.60 ^{c}	39.41±2.29 ^{c}	49.47±2.85 ^{b}	56.65±3.68 ^{a}	***	44-81.2
Albumin(g/dl)	2.88±0.12 ^{c}	2.88±0.05 ^{c}	3.64±0.05 ^{a}	3.34±0.04 ^{b}	3.59±0.05 ^{a}	***	2.4-3.9
T. Protein(g/dl)	6.30±0.17 ^{cd}	5.96±0.08 ^{d}	7.01±0.12 ^{a}	6.10±0.09 ^{d}	6.58±0.08 ^{bc}	***	5.9-7.9
D. Bilirubin (mg/dl)	0.20±0.01 ^{b}	0.28±0.02 ^{a}	0.13±0.01 ^{c}	0.12±0.00 ^{c}	0.12±0.00 ^{c}	***	0-0.3
T. Bilirubin (mg/dl)	0.19±0.01 ^{d}	0.32±0.02 ^{a}	0.24±0.01 ^{bc}	0.19±0.01 ^{cd}	0.20±0.01 ^{cd}	***	0-0.5

The differences between the values marked with various letters in the same line are statistically significant (: P<0.05, **:P<0.01, ***: P<0.001)*
NA: not available
(+): Physiological levels were kindly obtained from some researchers [9,10]

Table 2. *Some mineral substance levels 21, 120 and 145 days of pregnancy with 7 and 14 days postpartum in Awassi ewes (n:20)*
Tablo 2. *İvesi koyunlarında gebeliğin 21, 120 ve 145. günleri ile doğum sonrası 7 ve 14. günlerde bazı mineral madde düzeyleri*

Parameters	Days of Pregnancy			Days Postpartum		P	Physiological Levels (+)
	21	120	145	7	14		
Ca (mg/dl)	8.57±0.36 ^{bc}	7.79±0.24 ^{c}	8.12±0.22 ^{c}	9.28±0.47 ^{b}	10.43±0.23 ^{a}	***	9.3-11.7
P (mg/dl)	4.32±0.17 ^{ab}	3.49±0.14 ^{c}	4.37±0.19 ^{ab}	4.05±0.29 ^{bc}	4.43±0.21 ^{ab}	*	4.0-7.3
Mg (mg/dl)	2.45±0.51	2.48±0.35	3.06±0.25	2.58±0.22	3.11±0.13	-	2.2-2.8
Fe (mcg/dl)	91.65±5.06	101.82±4.60	108.41±4.20	93.47±3.60	99.94±6.48	-	166-222

*The differences between the values marked with various letters in the same line are statistically significant (***:** P<0.05 *****:** P<0.001)*
(+): *Physiological levels were kindly obtained from some researchers [9,10]*

ewes. Balikci et al.[2] reported serum glucose levels to be lower on day 100 and 150 of pregnancy compared to 45 days postpartum. In our study, lower serum glucose levels were recorded on day 21, 120 and 145 of pregnancy compared to 7 and 14 days postpartum. Glucose concentrations were lower than reference value until parturation. The results of the present study were paralel to the literature [2,12-15]. Blood glucose is known as metabolic profile test, thus, it has distinguishable value in pregnancy toxemia, retarted growth, weight loss, production and reproduction defects [18,19]. The difference in glucose concentration between prepartum and postpartum periods reveals the consumption of glucose by fetus and milk yield, so glucose administration before and after parturition results reduction in hypoglycemia and pregnancy toxemia [20-24].

Plasma urea concentrations were determined as a significant indicator of dietary protein supply in both sheep and goats [25-27]. The mean level of BUN in prepartum period greater than postpartum period. Urea production raises to 67% during pregnancy and fall to 36% following parturition and lactation [24]. Also Brozostowski et al.[27] observed an increase in urea level during early pregnancy where there was a decrease below the reference levels in late pregnancy. El-Sherif and Assad [28] reported plasma urea to start increasing in pregnant ewes from 10th week of pregnancy, reaching a maximum level at parturition. Rodriguez et al.[29] stated the glomerular filtration and urea clearance in ewes to be reduced during late pregnancy and lactation and Shetaewi and Daghash [12] found the level of serum urea during pregnancy to slightly exceed that of early lactation but it was highest at 55 days of lactation. In this study, highest serum urea levels were recorded on day 21 of pregnancy compared to 120 and 145 days pregnancy and 7 and 14 days postpartum. Urea levels were higher than reference values on day 21 pregnancy and than returned to reference values on days 120 and 145 pregnancy and lactation periods. The reason for high urea concentration in pregnant ewes could be related to either high protein metabolism during pregnancy or nutritional management.

Doornenbal et al.[30] found the serum creatinine concentration decreased during lactation and increased during postweaning. Some researchers [31,32] said that the creatinin levels in cattle and sheep not varied with reproductive status. According to our results, it was significantly higher on day 14 postpartum than on days 21 and 120 pregnancy. Creatinine levels remained below reference ranges for sheep during pregnancy and lactation period.

Some autors [31,32] found the total protein concentration in cattle and sheep not to change with reproductive status while Balikci et al.[2] reported that it decreased on day 150 of gestation in ewes. Similarly, our results revealed that it was significantly lower on day 120 of gestation, compared to other stages of gestation and during the whole investigated period total protein concentration was in reference range for sheep. This decrease in serum total protein may be ascribed to the fact that the fetus synthesises all its proteins from the amino acids derived from the mother, and growth of the fetus increases exponentially reaching a maximum level, especially in muscles, during late pregnancy [33].

In the present study, serum total bilirubin and direct bilirubin levels were recorded to be significantly ($P<0.001$) higher at 120 days of pregnancy than at 21 and 145 days of gestation and they came in the reference range for sheep. Certain researchers also recorded higher plasma bilirubin levels in pregnant sheeps [2,14,34,35]. The higher plasma total and direct bilirubin levels in this study may be due to the fact that blood bilirubin levels during pregnancy increased as a consequence of additional bilirubin derived from degredation of fetal haemoglobin or due to inadequate glucuronic acid synthesis.

Some researchers [36-38] said that levels of albumin in cows were not change in late pregnancy and lactation period. In contrast, Sevinc et al.[39] reported the albumin levels 7 and 8 months of gestation in cows to be significantly high. Yildiz et al.[40] found the levels of albumin at prepartum period in cows were not significantly different compared to postpartum period despite tending to increased. Our results revealed that it was significantly ($P<0.001$) higher on day 145 of gestation, compared to other stages of gestation and was mainly in reference range for sheep.

Studies have observed an increase in CK and LDH levels at postpartum [32,40-43] while Sevinc et al.[39] found an decreased in that. In this study, serum CK levels were highest at 145 days of pregnancy but serum LDH levels were highest at 14 days postpartum. The activities of LDH in Awassi ewes during the whole investigated period were always above the reference range for sheep. Decreased protein uptake causes muscular destruction (MD) and then increases muscular enzyme activities [44]. In this respect, high level of serum CK activities is evidence supporting protein deficiency at late pregnancy. The other

evidence of the MD is the highest LDH activities that observed at lactation period.

The effects of pregnancy in serum AST and ALT activity levels are somewhat controversial. In a few studies, an increase in AST and ALT activities have been found at postpartum [45-47] while some researchers [37,39,48] determined a decreased in that. Jovanovic et al.[49] observed an increase in AST and ALT levels during pregnancy. However, in some published studies, serum AST activity levels do not change during pregnancy and the postpartum period [31,50-52]. The result of the present study were paralel to the literature [31,50-52]. The authors [31,32] suggested that levels of ALP in mid-pregnancy and late pregnancy were higher than lactation period. Our results showing that ALP activities were higher at 14 days postpartum. This increase during lactation is due to an increase in the production of the bone isoenzyme. In this study, in Awassi ewes the activities of AST, ALT and ALP were within reference range any gestation and lactation dependent changes.

Some researchers obtained different results about Ca levels during pregnancy and lactation. Kadzere et al.[53] reported that Ca concentrations in plasma increased as gestation progressed and decreased after kidding while Tanritanir et al.[54] said that no statistical differences between before and after parturation at Ca levels in goats. Yokus et al.[55] concluded that the levels of Ca decreased slightly than early pregnancy to late pregnancy and then increased at lactation period in sheep. In contrast some researchers said that [3,7,31,56] Ca levels were lower at postpartum than gestation period. The mean level of serum Ca at 14 days postpartum was significantly ($P<0.001$) higher than prepartum period. We did not have any explanation for increase in Ca levels at lactation periods. The levels of Ca were below reference range on days 21, 120 and 145 of pregnancy and within reference range at lactation periods. The reason for the conflicting result probably occurs because of the difference of race, regional feeding practice.

Mg is required for normal skeletal development and done of the most common enzyme activators. Yokus and Cakır [31] reported that Mg levels in cows decreased continually during the pregnancy period and the slightly increased at lactation period. In contrast, some studies have observed Mg levels reached peak 8 month of pregnancy in cows than it decreased in lactation periods [57,58]. The mean values of Mg during pregnancy and at 7 and 14 days post-partum ranged from 2.45 to 3.11 mg/dl. Although without statistical significance, the highest Mg levels were found at 14 days postpartum. The findings are in agreement with that reported by some autors [54,55]. Mg levels were above reference range on day 145 of pregnancy and 14 days postpartum.

Although, some researchers reported that no significant differences were observed at the P levels at the different stages of growth, reproduction, pregnancy and lactation [3,59], other researchers informed that P levels during late gestation and postpartum significantly increased in ewes and goats [54,59-62]. Yokus et al.[55] demonstrated in lactation to decrease the level of P when compared to pregnant ewes. But in another study [31] showed the P levels were unchanged in all gestation period and lactation in cattle. In this study serum P levels were lower at 120 days of gestation and were higher 14 days postpartum. The P levels were below reference range on day 120 gestation and within reference range at other periods.

Fe level in blood is a reliable diagnostic indicator of various disease cases and physiological stages. Gurdogan et al.[63] reported that in pregnant sheep, significant decreases in serum Fe levels, respectively at 60, 100 and 150 days of pregnancy and Fe concentrations increased steadily at 45 days of parturation compared to the pregnancy periods. A gradual increase in serum Fe levels was recorded during pregnancy when compared to day 7 and 14 postpartum. In our study, levels of Fe in the Awassi ewes were always below reference range at gestation and lactation periods and there was a low serum Fe concentration in early pregnancy, which might be caused by the high demand of Fe by fetus during early gestation. This result on Fe is in parallel to the findings of some studies [31,54,55,63].

In conclusion, serum urea, total bilirubin, direct bilirubin, total protein, albumin, ALT and CK levels were higher in pregnancy while serum glucose, creatinine, ALP, P and Ca levels were higher in lactation. Neither the pregnancy nor the lactation effected AST, LDH, Fe and Mg levels.

We suggest that gestation and lactation periods must be taken in to concideration for the correct interpretation of serum chemistry and elements status in Awassi ewes smilarly fed.

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