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Evaluation the impact of the transition period on some hematobiochemical and hormonal parameters in Native sheep in Algab Alakhdar governorate in Libya

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ABSTRACT

This study aimed to provide a complete picture of selected hematological and serum biochemical parameters in Ewes from late pregnancy to early postpartum period. This study was carried out on 120 clinically healthy Native sheep in Elgab Al-Akhdar province in Libya. Animals were divided into three groups, group1 included 20 non-pregnant ewes, group2- included 50 pregnant ewes and group3 included 50 lactating ewes. Blood samples were collected three weeks before the expected parturition from group 2 and during the 1st month post-partum from group3. Whole blood samples were collected for hematological picture, while serum samples were collected for biochemical analysis which included, serum calcium, magnesium and phosphorus and serum glucose, AST, ALT, and cortisol. Significant changes between pre- and post-partum period (pregnant- lactating) were observed in values of hemogram and biochemical parameters including RBCs, MCV, WBCS count, and differential leucocytes show significant change in lymphocyte count, glucose, calcium, phosphorus, magnesium, AST, ALT, and cortisol. Non-significant changes were observed in Hb, HCT, Neutrophil, monocyte, eosinophil, platelet and basophil. These results concluded that transition period has an impact on some hematobiochemical parameters and ewes should be supplemented with minerals including calcium, phosphorus and magnesium. Investigations of major and minor elements may be considered as real indicators for health in transition period in ewes and helps to prevent many diseases at this stage.

1. INTRODUCTION

The periparturient period also known as the transitional period lasts from 3 weeks prepartum to 3 weeks postpartum (Roberts, 2012; Sordillo and Raphael, 2013; Drackley, 1999). The transition period is characterized by alterations in the concentrations of metabolites, minerals, enzymes, hormones and immune functions (Piccion et al 2012). Elevated energy demands during the periparturient period to sustain fetal development and milk production, as well as reduced feed intake around calving (Hayirli, 2002; Grummer et al. 1995). During the periparturient time, negative caloric intake or negative energy balance is characterized as an increase in energy output compared to energy input (Van Saun et al., 2014). The energy homeostatic processes that occur during the periparturient phase are regulated by hormonal changes around parturition.

The most prevalent periparturient diseases in sheep include metritis, pregnancy toxemia, mastitis, hypocalcaemia, retained placenta and lameness (Goff and Horst (1997), Van Saun and Sniffen (2014). To research ruminant metabolism disorders, metabolic blood profiles, including serum mineral and biochemical parameters, must be determined. Mineral quality and biochemical markers of sheep blood are commonly used as a result (Pastrana et al. 1991). During the transition period, hemato-biochemical

profiling of dairy cattle has been documented to provide new and valuable knowledge regarding animal health (Kevin and Ellen, 2012). The aim of this study was to evaluate the effect of the transition period on some hematobiochemical and hormonal parameters in sheep in Algab Alakhdar governorate in Libya.

2. MATERIAL AND METHODS

2.1 Animals:

This examination was done on 120 Native-sheep from different localities in Elgab al akhdar Libya. The animals aged from 2.5-4 years and weighed from 35-55 kg and body condition score were 3-4. Sheep were grazing on pasture in the morning and they were fed on barley overnight, each animal was supplied 1.5-2 kg in one shift, each group of animals was apparently clinically healthy after complete physical examination. Examination of the rectal temperature, pulse, respiration, conjunctival mucous membrane, superficial lymph nodes, lung and heart sounds were determined according to the method described by (Constable et al, 2017)

2.2 Sampling:

Two blood samples were taken from their jugular veins from all animals. The first sample included 3ml blood, on EDTA for hematological studies (complete CBC). The

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second sample included 5ml blood without anticoagulant for subsequent serum collection and analysis.

2.3 Hematological analysis:

Total erythrocyte count, mean corpuscular volume (MCV), Hemoglobin (Hb), Total leukocyte count (WBCs), Lymphocytes %, Monocytes %, neutrophils and eosinophils were measured by using Hematology Analyzer (Jain, 1993)

2.4 Biochemical analysis:

Glucose mg/dl was measured spectrophotometrically with special kits, following the method defined by (Trinder, 1969) The concentration of cortisol in (mmol/L) was calculated using spectrophotometric methods and special kits, as defined by (Wilson and Miles, 1977), GPT IU/L was determined spectrophotometrically by using of the special kits according to the method that described by (Thefeld, 1974), GOT IU/L was determined spectrophotometrically by using of the special kits according to the method that described by Reitman and Frankel (1957). Calcium mg/dl was determined spectrophotometrically by using of the special kits according to Tietz, (1986), Phosphorus mg/dl was measured spectrophotometrically using special kits according to the manufacturer's instructions (Young, 1975), Magnesium mg/dl was determined spectrophotometrically by using of the special kits according to Bohuon, (1962).

2.5 Statistical analysis:

The data were analyzed by using one-way analysis of variance (ANOVA). The means of groups were compared together and using one way ANOVA IBM (SPSS) software 20 values were represented by mean± standard error, all differences were considered statistically significant at $P < 0.05$ according to (Norman and Baily 1997).

3. RESULTS

3.1 Hematological parameters:

(WBC) count were significantly ($P \leq 0.05$) increased in pregnant ewes compared by that of the non-pregnant ones and lactating ones and did not show significant difference between lactating ewes and non-pregnant ewes Table(1), (RBC) count were significantly ($P \leq 0.05$) decreased in pregnant ewes compared by that of the non-pregnant ones and lactating ones and did not show significant difference between lactating ewes and non-pregnant ewes Table(1), (MCV) value were significantly ($P \leq 0.05$) lowered in lactating ewes compared by that of the non-pregnant ones and pregnant ones and did not show significant difference between pregnant ewes and non-pregnant ewes Table (1). Hb and HCT value results showed that did not significant different in between 3 group Table (2). Neutrophils were significantly ($P \leq 0.05$) decreased in non-pregnant ewes compared by that of the -pregnant ones and compared by lactating ones and did not show significant difference between lactating ewes and -pregnant ewes Table (1), Lymphocyte count were significantly ($P \leq 0.05$) increased in lactating ewes compared by that of the non-pregnant ones and showed significant ($P < 0.05$) increase in lactating ewes compared by that of the pregnant ones. And did not show significant difference between pregnant ewes and non-pregnant ewes Table (1).

result show no significant difference in Monocyte and Eosinophils and Platelet and Basophile between 3 period are presented in Table (1).

Table 1 Hematological parameters (Mean ± SEM) in non-pregnant, pregnant and lactating ewes (N= 120).

Parameters	Non-pregnant ewes (N=50)	pregnant ewes (N=50)	Lactating ewes (N= 20)
WBC ($10^6/\text{mm}^3$)	11.1± 0.56 ^a	12.3±0.24 ^b	11.48± 0.38 ^a
RBC ($10^3/\text{mm}^3$)	8.48± 0.15 ^a	7.81±0.17 ^b	8.19 ±0.18 ^a
Hb (g/dl)	10.7± 0.19 ^a	10.2±0.22 ^a	10.4± 0.21 ^a
HCT (%)	35.9± 0.73 ^a	34.9± 0.89 ^a	34.5± 1.01 ^a
Neutrophils (%)	30.4± 2.1 ^a	42.5 ± 0.96 ^b	41.5± 0.65 ^b
Lymphocytes (%)	51.3± 1.42 ^a	49.8±0.66 ^a	57.2± 1.44 ^b
Monocytes (%)	5.3±0.31 ^a	4.9± 0.2 ^a	4.4± 0.22 ^a
Eosinophils (%)	2.5±0.19 ^a	2.8± 0.14 ^a	2.7± 0.18 ^a
MCV (fL)	41.9± 0.37 ^a	41.4± 0.39 ^a	40.33± 0.15 ^b
Platelets ($10^3/\text{mm}^3$)	397± 19.2 ^a	427.8± 9.32 ^a	413± 5.1 ^a
Basophils (%)	0.40± 0.11 ^a	0.54± 0.07 ^a	0.44± 0.07 ^a

Means having the different letters are significantly different at ($P < 0.05$).

Table 2 Some biochemical parameters, cortisol and liver function tests (Mean ± SEM) in non-pregnant, pregnant and lactating ewes (N= 120).

Parameters	Non-pregnant ewes (N=50)	pregnant ewes (N=50)	Lactating ewes (N= 20)
Glucose (mg/dl)	57.9 ± 0.82 ^a	52.84±0.74 ^b	56.24±0.77 ^a
Magnesium (mg/dl)	2.22 ± 0.04 ^a	2.11±0.05 ^a	1.87±0.04 ^a
Calcium (mg/dl)	8.56±0.08 ^a	8.37±0.01 ^a	7.85± 0.08 ^b
phosphorus (mg/dl)	4.89±0.10 ^a	3.40±0.05 ^b	4.68±0.10 ^a
cortisol (mmol/l)	29.05 ± 0.84 ^a	40.69 ± 1.01 ^b	37.28± 0.86 ^c
AST (IU/L)	68.4±0.92 ^a	87.18±1.89 ^b	78.14±0.63 ^a
ALT (IU/L)	16.65 ± 0.44 ^a	23.28±0.73 ^b	19.86±0.37 ^a

Means having the different letters are significantly different at ($P < 0.05$).

3.2 Biochemical parameters:

Glucose concentrations were significantly decreased ($P < 0.05$) during pregnant period as compared by non-pregnant ewes and compared by lactating period are presented in Table (2), magnesium concentrations did not show any significant differences between pregnant period and non-pregnant period but showed significant decrease ($P < 0.05$) in lactating period compared by pregnant and non-pregnant period. Table (2), calcium concentrations in the lactating ewes were significantly decreased ($P < 0.05$) compared by pregnant period and compared by non-pregnant period, while there were no significant differences between pregnant ewes and non-pregnant ones Table (2). phosphorous concentrations in the pregnant ewes showed significant ($P < 0.05$) decrease in comparing with non-pregnant ewes and lactating ewes while there were no significant differences between lactating and non-pregnant ewes Table(2),cortisol in pregnant ewes was significantly ($P < 0.05$) increased in comparing with that of the non-pregnant ewes as well as there was significant ($P < 0.05$) increase in level of cortisol in lactating ewes than that of non-pregnant ones and there was significant ($P < 0.05$) increase in level of cortisol in pregnant ewes when it was compared by that of the lactating ewes Table (2), levels of AST showed significant ($P < 0.05$) increase during in pregnant ewes as compared by that of non-pregnant ones and showed significant increase ($P < 0.05$) in pregnant ewes than that of lactating ewes as well as lactating ewes showed significant increase ($P < 0.05$) in the serum level of AST than that of the non-pregnant ewes. Table (2), serum levels of ALT were significantly ($P < 0.05$) increased in pregnant ewes compared by that of the non-pregnant ones and compared by that of the lactating ones. While in the lactating ewes there was significant ($P < 0.05$) increase in the level of ALT compare by that of the non-pregnant ones Table(2).

4. DISCUSSION

The transition period, which lasts three weeks before and after parturition, is considered crucial since it is characterized by many metabolic changes and modifications to the animal's new physiological state. (Araújo et al., 2014). At this period, there is a higher risk of losses due to an imbalance in nutrient demand and availability, which is caused by the elevated food demand caused by the increased growth of the fetuses and mammary gland.

Hematologic examination is a valid method of determining an animal's health status (Cetin et al., 2009). PCV, RBC, and WBC levels measured during the late stages of pregnancy and early lactation may be indicators of anemia, parasites, digestive abnormalities, and reproductive and metabolic diseases in animals (Nozad et al., 2014). (RBC) was significantly decreased in the pregnant sheep compared by that of the non-pregnant and lactating ones. (MCV) were significantly decreased in lactating ewes compared by that of the non-pregnant ones and compare by that of the pregnant ones. Significant decrease in (RBC) count and the hemodilution effect can affect the (MCV) value., which is caused by an increase in plasma volume, has physiological meaning because it decreases blood viscosity, increasing blood supply in the narrow blood vessels of the uterus and udder, and is more noticeable in ewes with twins. (Iriadam, 2007). Significant reduction in the mean values of (MCV) was comparable to what was found by (Azab and Abdel-Maksoud, 1999) found that the red cell's osmotic tolerance is greater in pregnant ewes than in dry ewes.

(WBC) count was significantly increased in the pregnant sheep compared by that of the non-pregnant ones and compared by that of the lactating ones. The mean of Neutrophils was significantly decreased in non-pregnant ewes compared by that of the -pregnant ones and compare with lactating ones and did not show significant difference between lactating ewes and-pregnant ewes. Lymphocyte counts were significantly increased in lactating ewes compared by that of the non-pregnant ones and compared by that of the pregnant ones. This may be due to post parturient infection may be occurred.

These results are not similar to those observed by (Azab and Abdel-Maksoud, 1999; Mbassa and Poulsen, 1991). As well as (Leilson et al., 2017). Total leukocyte, neutrophil, and lymphocyte counts were shown to be affected by reproductive periods, with the total number of leukocytes during late pregnancy being slightly smaller than those seen in non-pregnant ewes, ewes at the beginning of pregnancy, and ewes in the middle of gestation. With advancing pregnancy, the breeds had similar In ewes, the neutrophil count reaches its peak in the postpartum period. While (Paape et al., 1992) During lactation, the amount of WBCs, basophils, and monocytes in ewes' blood decreased significantly, indicating that they migrated from blood to milk for more effective phagocytosis and mammary gland protection against pathogens. In lactating goat trials, a similar reduction in blood WBC, monocytes, and basophils was observed as lactation progressed. (Antunović et al., 2013).

The results of our study revealed that, serum glucose concentrations were significantly decreased during pregnant period and as compared by non-pregnant ewes and compared by lactating period during which glucose concentrations were no significantly difference compared by non-pregnant ones and lactating ones, as well as in the lactating period glucose concentrations show significant increase in comparing with that in pregnant period. The evolution of glucose levels during the late stage of

pregnancy and early lactation was similar observed by other studies in (Magistrelli and Rosi, 2014). The high concentration of glucocorticoid hormones including cortisol, which stimulates hepatic glycogenolysis and gluconeogenesis from glucose precursors, induces a rise in glucose concentration at parturition (Magistrelli and Rosi, 2014).

Furthermore, at the end of gestation, the decline in the response of peripheral tissues to insulin leads to a rise in blood glucose concentration when these tissues conserve their resources (Anwar et al., 2012). Serum magnesium and serum calcium concentrations showed significant decrease in lactating period compared by pregnant and non-pregnant period, While serum phosphorous concentrations in the pregnant ewes showed significant reduction in comparing with non-pregnant ewes as well as there was significant reduction in pregnant ewes than that of the lactating ones. These results may be attributed to negative Ca balance and this imbalance higher in twin pregnant sheep (Atilla and Fuat, 2004) Also, (Azab and Abdel-Maksoud, 1999) reported that the Increased demand for Ca due to mineralization of the fetal skeleton and milk synthesis may explain the decrease in Ca levels during late pregnancy and early lactation. Increased demand for phosphorus for mineralization of the fetal skeleton, which is more pronounced in ewes with twins, may be to blame for the decline in phosphorus levels. (Atilla and Fuat, 2004)

The decrease in serum phosphorous levels during late pregnancy is due to a similar rise in the rate of phosphorous migration out of the maternal bloodstream into the fetus, which is not offset by an increase in the rate of phosphorous absorption from the intestine or the bones of the dam. (Braithwaite, 1982). The mean values of serum magnesium level in pregnant ewes showed significant decrease than the non-pregnant. This result was agreed with (Atilla and Fuat, 2004; Azab and Abdel-Maksoud, 1999). The reduction in magnesium concentration may be linked to factors that affect magnesium absorption from the stomach. Which include dietary protein and ammonia contents as well as decrease in the gut motility which associated with late stage of pregnancy. Aside from that, the reduction in serum magnesium is possibly due to hemodilution, which happens often during late pregnancy. (Elnageeb and Adelatif, 2010).

Concentration of cortisol in pregnant sheep was significantly increased in comparing with that of the non-pregnant ewes and compared by lactating ones, as well as there was significant increase in level of cortisol in lactating ewes than that of non-pregnant ones. The mean serum cortisol levels obtained were comparable to those recorded by Tharwat et al., (2013) who discovered a peak in concentration during parturition in goats due to placental movement from the fetus to the dam, Cortisol levels rise in the mother's bloodstream, stimulating the development of prostaglandin F₂, which causes parturition. Suganya and Gomathy, (2009) and Magistrelli and Rosi, (2014) witnessed a parallel cortisol evolution during the transitional phase. Since this hormone serves as a signal of parturition and that there is a larger release of the glucocorticoid, cortisol levels in the blood rose during late pregnancy, more specifically at parturition (Araújo et al., 2014). The release of this hormone into the blood induces both hepatic glycogenolysis and gluconeogenesis from endogenous precursors, resulting in an increase in glycemia (Djoković et al., 2014).

In our study, significant increase (P <0.05) of AST and ALT in pregnant ewes as compared by that of non-pregnant ones and compared by lactating ones. Significant increase

in AST and ALT activities during lactation in relation to pregnant was additionally detailed by (Sadjadian et al., 2013). Increased gluconeogenesis during periods of high energy demand, such as lactation, can be responsible for an increase in serum activity of this enzyme as a result of the increased hepatic metabolism (Iriadam, 2007). In periparturient animals, AST activity is an indication of liver function; however, despite the rise, AST serum activity remained below the normal values for the species referred to (Sadjadian et al., 2013b), and in accordance with the result obtained by Elzein et al., (2016). However, El-Sherif and Assad, (2001) found that this enzyme's activity increased during pregnancy and that high concentrations were maintained during lactation in sheep.

5. CONCLUSION

Ewes showed hematobiochemical adaptation in late pregnancy and early lactation (transition period) characterized by some biochemical and hormonal changes including changes in calcium, phosphorous, magnesium, glucose, AST, ALT and cortisol as well as hematological changes including RBCs, WBCs, neutrophil, lymphocytes, PCV and Hb. These changes can be used as indicators of the healthy status of ewes during the transition period (late pregnancy and early lactation).

Late pregnancy and early lactations (transition period) has an impact on the relationships between some hematobiochemical parameters and ewes should be supplemented with minerals including calcium, phosphorous and magnesium. Investigation of major and minor elements may be considered as real indicators for healthy in transitional period in ewes and helps prevent of many diseases at this stage.

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