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The comparison of some biochemical parameters in hyperketonemic and normal ewes

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ABSTRACT

The objective of this study is to assess and compare the alterations in some biochemical parameters in normal and ewes affected by subclinical pregnancy toxemia. Pregnancy toxemia in sheep and goats is a multifactor disorder of metabolism usually occurs in the last month of pregnancy. Hypoglycemia and hyperketonemia are the primary metabolic disturbances in pregnancy toxemia. Pregnant ewes in their last month of gestation were classified based on their BHBA levels in two groups of normoketonemic and hyperketonemic ewes. Serum concentrations of NEFA, BUN, ALT, AST, Cortisol, Calcium, Glucose, total Protein and Ceruloplasmin were determined and compared between these two groups. Serum levels of NEFA, Cortisol and Ceruloplasmin were significantly ($p<0.05$) higher in hyperketonemic ewes than normal ewes. Glucose and Calcium concentrations were significantly lower than normal ewes. Our results indicated that the hyperketonemic ewes are more likely to have hypoglycemia, hypocalcemia and hypercortisolemia compared to the normal ewes during the last month of pregnancy. Furthermore, it can be concluded that hyperketonemic ewes might have higher levels of serum Ceruloplasmin concentrations implicating the role and importance of stress in the pathophysiology of the condition.

Key words: Biochemical parameters, Hyperketonemia, Subclinical Pregnancy Toxemia

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INTRODUCTION

Pregnancy toxemia (ketosis, hepatic lipidosis) typically develops during the final trimester of pregnancy in ewes and does. The condition usually is seen in females carrying multiple fetuses and may result from their inability to consume adequate energy to match metabolic demands. Conditions that increase energy demands or decrease energy intake also can predispose affected animals to this disease. A negative energy balance in late gestation results in changes in the insulin-glucagon ratio and activates lipases that mobilize fatty acids and glycerol from body energy reserves. The liver uses these fatty acids and glycerol as energy for fetal growth. If the energy demands are greater than the supply, the liver cannot produce enough glucose and may become overwhelmed with free fatty acids, resulting in the production of ketones [1]. The developing fetuses depend upon glucose (maternal hepatic gluconeogenesis) for their energy needs. Ketone bodies and free fatty acids do not cross the placenta in any substantial quantities [2]. Since, meeting the metabolic needs of the fetuses even at the expense (if necessary) of the dam is crucial; Thus, it is expected the late pregnant dam to often be subclinically ketotic [3].

Biochemical parameters for assessing energy status of pregnant ewes are mainly serum concentrations of glucose, BHBA and NEFA. Generally, serum concentrations of BHBA have been used to determine hyperketonemic state and subclinical ketosis, but there are different ideas about cut-off point of BHBA. This rise in serum BHBA is a compensatory mechanism and a reflectionary response to carbohydrate deficiency and inhibition of Kreb's cycle [4]. So far, the effects of hyperketonemic state in pregnancy toxemia on various biochemical parameters have been

studied in several researches. Considering the different cutoff points and controversial results obtained by these studies, it is still worthwhile to investigate the influences of hyperketonemia on different biochemical parameters and the results could contribute in clarifying the pathophysiology of the disease. Therefore, the objective of this study is to assess and compare the alterations in some biochemical parameters in normal and subclinically ketotic ewes.

MATERIALS AND METHODS

The study was performed on the total number of 20 pregnant Afshari breed ewes. Based on the serum BHBA concentrations and the cutoff point of 0.8 mmol/L, they were categorized in 2 groups of ten ewes: A) hyperketonemic ewes, affected by subclinical pregnancy toxemia and B) healthy normoketonemic ewes. Animals showing abnormal clinical signs were excluded from the study. Subsequently, serum concentrations of NEFA, BUN, ALT, AST, Cortisol, Calcium, Glucose, Total Protein and Ceruloplasmin were determined and compared between these two groups.

The ewes were selected from 4 flocks located near Tehran, the capital of Iran. They were multiparous, aged 3 – 5 years, with body condition score of 3.5 – 4 (on scale of 1 -5). By ultrasonography, their pregnancy status and gestational age were determined. All of them were in the last month of their gestational period and bearing just one embryo. The flocks were being hand fed at the time of sampling, although they were being grazed most of the year, and had regular vaccination and deworming programs.

Blood samples were taken from jugular vein and submitted to the laboratory. Serum concentrations of mentioned biochemical parameters were determined using available commercial kits.

Statistical analysis was performed using SPSS 20.0. The Shapiro-Wilk test was used to evaluate the distribution normality of data. Means of biochemical parameters were compared by independent samples t student test between the groups. Pearson's correlation coefficient was performed to assess the presence of linear correlation between the parameters. $p<0.05$ was regarded as significant.

RESULTS

The obtained results were summarized in tables3-1 and 3-2.

Descriptive Statistics						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
BHBA (mmol/L)	10	.36	.63	.4590	.02618	.08279
NEFA (mmol/L)	10	.06	.61	.2150	.05222	.16514
Glucose (mg/dL)	10	43.00	60.00	49.9000	1.81016	5.72422
Cortisol (µg/dL)	10	.50	2.02	1.1010	.16806	.53146
Total Protein (g/dL)	10	6.30	8.50	7.3700	.23620	.74692
BUN (mg/dL)	10	15.26	26.07	20.9510	1.14796	3.63017
Calcium (mg/dL)	10	9.21	11.60	10.1000	.25883	.81848
AST (U/L)	10	70.00	134.00	99.8000	6.73597	21.30102
ALT (U/L)	10	18.00	33.00	24.1000	1.52352	4.81779
Ceruloplasmin (mg/dL)	10	10.00	14.00	11.6500	.41533	1.31339

Table 3-1. Biochemical parameters in normoketonemic ewes

Descriptive Statistics						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
BHBA (mmol/L)	10	.88	1.70	1.2210	.10016	.31674
NEFA (mmol/L)	10	.38	1.30	.9030	.10177	.32184
Glucose (mg/dL)	10	26.00	62.00	39.4000	3.46795	10.96662
Cortisol (µg/dL)	10	.92	8.50	4.0000	.78250	2.47447
Total Protein (g/dL)	10	6.20	8.30	7.3400	.19276	.60955
BUN (mg/dL)	10	17.02	40.66	23.5180	2.31906	7.33352
Calcium (mg/dL)	10	8.40	10.30	9.3110	.21764	.68823
AST (U/L)	10	89.00	220.00	135.4000	15.63202	49.43278
ALT (U/L)	10	20.00	37.00	27.0000	1.58465	5.01110
Ceruloplasmin (mg/dL)	10	11.00	17.00	13.5400	.58256	1.84222

Table 3-2. Biochemical parameters in hyperketonemic ewes

Serum levels of NEFA, cortisol and ceruloplasmin were significantly ($p<0.05$) higher in hyperketonemic ewes than normal ewes. Glucose and calcium concentrations were significantly lower in hyperketonemic ewes than normal ewes.

Table3-3 summarizes the presence of significant ($p<0.05$) correlations between parameters.

Biochemical Parameters		Correlation coefficient (r)
AST	Ceruloplasmin	0.503
ALT	NEFA	0.558
BHBA	ALT	0.504
BHBA	Calcium	-0.467
BHBA	Ceruloplasmin	0.481
BHBA	Glucose	-0.549
BHBA	NEFA	0.789
BHBA	Cortisol	0.831
BUN	Ceruloplasmin	0.703
BUN	AST	0.633
Glucose	NEFA	-0.600
Glucose	Cortisol	-0.583
NEFA	Cortisol	0.647

Table 3-3. Significant correlations between biochemical parameters and their correlation coefficient (r)

DISCUSSION

As mentioned earlier, there are different parameters and cut-off points to define hyperketonemia in sheep and goats. In addition the results obtained by various studies are controversial and the pathophysiology of pregnancy toxemia still need to be clarified. Therefore, this study designed in such a way to assess and compare changes in mentioned biochemical parameters in normal and subclinically ketotic ewes.

Biochemical parameters for assessing energy status of pregnant ewes are mainly serum concentrations of glucose, BHBA and NEFA. Ruminants appear to be well adapted to a carbohydrate economy based on their ability of gluconeogenesis. But while, fetal glucose demands increase with increasing body size, it can increase susceptibility to pregnancy toxemia. A fetus of sheep in late pregnancy utilizes about one-third to one-half of the daily glucose turnover of 100 g [5].

Generally, researchers believe that BHBA concentration is a golden marker for diagnosis of pregnancy toxemia and/or ketosis in ewes and cattle [5]. It is the most stable ketone and accounts for approximately 85% of the total ketones in sheep with pregnancy toxemia [6] but there are different ideas about cut-off point of BHBA. Smith (1996) introduce levels higher than 1 mmol/l as a cut-off point [7]. However in this study, the cut-off point of 0.8 mmol/L was chosen to differentiate between hyperketonemic and normal ewes.

In animals affected by clinical pregnancy toxemia, blood glucose levels are variable and the animals may or may not be hypoglycemic [1,3]. Based on the results obtained by this study, glucose levels were significantly ($p<0.05$) lower in hyperketonemic than normal ewes. These results were consistent with the findings of studies by Bani Ismail et al (2008), Lacetera et al (2001) and Schlumbohm and Harmeyer (2004) [8-10]. Schlumbohm and Harmeyer believe that due to a metabolic adaptation, efficiency of hepatic gluconeogenesis from glucose precursors increases during pregnancy but negative feedback of hyperketonemia on glucose production renders the pregnant or lactating ruminant into a vicious circle. It is also proposed that the reduced ability of the late gestating ewes to utilize BHBA promote hyperketonemia [11]. However, results of present study were different from the results obtained by Rezapour and Taghinejad (2011). They showed that food restriction were tolerated by animals and could not decrease serum concentration of glucose significantly in contrast to the control group [12].

The findings of this study as we expected showed that there is a significant ($p<0.05$) negative correlation between glucose and BHBA ($r = -0.549$, $p<0.05$), as well as glucose and NEFA ($r = -0.600$, $p<0.01$).In the study conducted by Roofi et al (2013),blood glucose levels was negatively correlated with serum NEFA. In addition, a positive correlation was shown between NEFA and BHBA, both confirming the results of this study [13].On the other hand, in the present study, serum levels of NEFA were significantly ($p<0.05$) higher in hyperketonemic ewes than normal ewes. The increase in serum NEFA may be ascribed to the fact that the deposit fat is used to generate energy for the energy restricted sheep and fetus and growth of the fetus which increases exponentially during late pregnancy. In addition, there was a strong positive correlation between NEFA and BHBA ($r = 0.789$, $p<0.01$) in this study. As there is strong positive correlation between BHBA and NEFA, where it is possible, assessment of NEFA concentration can be useful.

Plasma calcium and inorganic phosphate concentrations are influenced by food supply. A significant decrease in plasma calcium concentration was observed in hyperketonemic ewes in the present study and this agreed with the results of other studies [10 ,14-15]. In addition, the results of this study showed that calcium was negatively correlated ($r=-0.467, p<0.05$) with BHBA. During the last trimester of pregnancy, the growing fetus also retains an increasing amount of calcium for the circulation, which is required for skeletal development and ewes that carry twins are in even greater need of calcium and are at the same time at a higher risk of developing pregnancy toxemia than ewes with only one offspring [14].

The bioactivity of liver enzymes increases in response to damage of liver parenchyma and release of these enzymes. In the study carried out by Hefnawy et al (2011), there was a marked drop in serum total protein, globulin, albumin, cholesterol and total lipids with significant increase in AST and ALT in goats affected by clinical pregnancy toxemia which could throw some light on the hepatic origin of pregnancy toxemia that may be attributed to fat mobilization associated with inadequate dietary intake or due to hepatic damage or hepatic lipidosis. Reference range of serum AST and ALT activities in sheep is reported as 60 to 280 U/L and 22 to 38 U/L, respectively [16]. In this study, the activities of liver enzymes, AST and ALT, did not exceed the reference range and did not differ significantly between the two groups. Therefore, the results were similar to the study by Rezapour and Taghinejad-roudbaneh (2011). Based on the results of this study, a significant positive correlation was observed between AST and Ceruloplasmin($r = 0.503, p<0.05$). Also there was a significant positive correlation between ALT and NEFA($r = 0.558, p<0.05$).

Azotemia, both from dehydration and secondary to renal disease, is a common finding in pregnancy toxemia, and even a fatal uremia may occur [1]. Urea and creatinine concentrations were significantly higher in clinically affected animals in various studies [14, 17-18]. The findings of this study showed that BUN concentration between normal and hyperketonemic subclinically affected ewes did not differ significantly and this agreed with the findings of Rezapour and Taghinejad-roudbaneh (2011). Therefore, on the basis of absence of azotemia and normal serum total protein concentration, it can be concluded that the subclinically affected animals are not as dehydrated as clinically affected animals. BUN concentration was positively correlated with ceruloplasmin ($r = 0.703, p<0.01$) and AST ($r = 0.633, p<0.01$). In contrast to the findings of Bani Ismail et al, 2008, there was no significant correlation between BUN concentration, blood glucose and BHBA concentrations in this study.

Cortisol was significantly ($p<0.05$) higher in hyperketotic ewes than normal ewes. In addition the activity of cortisol has strong positive correlation with BHBA ($r = 0.831, p<0.01$) and NEFA ($r = 0.647, p<0.01$) and also was negatively correlated with glucose concentration ($r = -0.563, p<0.01$). In agreement with our study, Hefnawy et al (2010) and Bani Ismail et al (2008) reported the presence of significant positive correlations between BHBA and Cortisol concentrations in experimentally pregnant toxemic goats and subclinical pregnancy toxemic goat does, respectively [8,19]. In cases of clinical pregnancy toxemia, cortisol-induced changes in the hemogram (neutrophilia, lymphopenia, eosinopenia) can be expected [3]. In addition, cortisol stimulates the gluconeogenesis in the liver by stimulating the breakdown of substrate, for example promoting lipolysis. The insulin-mediated glucose uptake by muscle, adipose tissue and other tissue that uses glucose is inhibited, which reduces the use of glucose in the body.Cortisol is necessary for epinephrine, growth hormone and other lipolytic substances stimulate the hydrolysis of stored TAG at maximal rates [20-21].

Ceruloplasmin is a major protein carrying various functions which its main function is to transport 90 – 95% of copper in serum. In addition, ceruloplasmin works as a moderate acute phase protein and is synthesized by liver in response to the tissue damage and inflammation. It is an important intravascular antioxidant and inhibits the lipid peroxidation [22]. Serum ceruloplasmin increases during stress conditions and it is suggested that adrenal steroids are involved in stress-induced increase in ceruloplasmin activity. On the last week before parturition, stress level increases in the pregnant animal. In addition to the feed deficiency as a separate stress factor, normal pregnancy is also associated with the increase in oxidative stress and lipid peroxidation. Gursel et al (2010) suggested that serum ceruloplasmin level can be used as a sensitive indicator of feed deficiency in the last days of pregnancy [23]. In this study serum levels of ceruloplasmin was significantly higher in hyperketonemic ewes than normoketonemic ewes. In addition, there was a significant positive correlation between the activity of ceruloplasmin, BHBA ($r = 0.481, P<0.05$), BUN ($r = 0.703, p<0.01$)and AST ($r = 0.503, p<0.05$) concentrations. In the study carried out by Sakha et al (2013), it was shown that serum ceruloplasmin concentrations of ewes after the induction of subclinical pregnancy toxemia were significantly higher than before the induction. They concluded that circumstances such as feed restriction and hyperketonemia could increase ceruloplasmin levels [24].

CONCLUSION

In summation, our results indicated that the hyperketonemic ewes are more likely to have hypoglycemia, hypocalcemia and hypercortisolemia compared to the normal ewes during the last month of pregnancy. Furthermore, it can be concluded that hyperketonemic ewes might have higher levels of serum ceruloplasmin concentration simplicating the role and importance of stress in the pathophysiology of the condition. The findings of this study might be useful for veterinary researchers and practitioners in their understanding of pathophysiological changes that occur in pregnant ewes.

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