

Research Article

Correlation among β Hydroxybutyric Acid (BHBA), Total Antioxidant Capacity (TAC) and Protein Profile Pre and Post-Partum in Shall Ewes

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Abstract

Nowadays, the role of pregnancy on antioxidant capacity of body in animals established in many studies. The aim of present study was to determine the correlation among β hydroxybutyric acid (BHBA), glucose, total antioxidant capacity (TAC) and protein profile pre and post-partum in shall sheep. In this study 112 heads sheep from shall breed in Qazvin city pre and postpartum to measurements amount of TAC, BHBA, glucose, protein profile and investigation of TAC modification were examined. The level of TAC prepartum significantly lower than postpartum ($P < 0.03$) and was found a negative correlation between BHBA and TAC pre ($r = -0.731$, $P < 0.000$) and post ($r = -0.390$, $P < 0.003$) partum. The concentration of TAC pre and post-partum with increase and decrease of BHBA changed, also were found a positive correlation between TAC and glucose pre ($r = 0.683$, $P < 0.000$) and post ($r = 0.393$, $P < 0.003$) partum. The value of total protein ($P < 0.05$), albumin ($P < 0.05$) and A/G ratio ($P < 0.05$) pre-partum were significantly higher than post-partum, whereas globulin levels ($P > 0.05$) have not significant changed.

ABBREVIATIONS

BHBA: β Hydroxybutyric Acid; TAC: Total Antioxidant Capacity; BCS: Body Condition Score; FRAP: Ferric Reducing Ability of Plasma.

INTRODUCTION

Measurement of biochemical markers in blood have an indispensable role for detection of metabolic diseases. One of the important characteristics of metabolic disorders is increased or decreased of blood biochemical factors. Ruminants for daily milk production and to maintain reproductive performance required high level of glucose or its precursors. The blood glucose concentration in ewes depending on various physiological conditions and the level of production, could be different [1], as well as in various stages of some metabolic diseases will be changed. In the absence of glucose, ketone bodies and urea

concentrations produced from fat and protein catabolism increase from normal condition up to different physiological status [2-6] and pathological condition [7,8]. Therefore, the appropriate information about the normal values of blood biochemical factors for prevention and control of many production diseases in farm animals is necessary and would be useful index for distinction of the physiological appearance in non-pregnant and pregnant ewes.

Protein profile of serum in ruminants with aging, some of diseases, hormonal and environmental changes associated with seasons can be different. Any alteration in normal range of serum protein reflects the pathological or physiological conditions of ruminants and can be a useful and inexpensive diagnostic methods for monitoring of livestock health [9-11].

Magnitude of negative energy balance during the last stage of pregnancy is considered as an important indicator to

the development of disease and hyperketonemia [12,13]. In hyperketonemic animals in which body fuel is mainly supplied from fat, the concentration of ketones begins to increase in the blood and other body fluids and may reach 10 mmol/L of blood, which is 10-fold higher than concentrations in healthy animals [14,15]. It was reported that ketone body, acetoacetate, can generate superoxide radicals that can then form hydroxyl radicals [16]. Markers of oxidative stress were increased in cows with subclinical ketosis [17] and in hyperketonemic compared with normoketonemic people with type 1 diabetes [16,18]. The amount of antioxidant capacity in the blood can be measured by ferric reducing ability of plasma (FRAP) method.

Pregnancy toxemia is a metabolic disorder with a high mortality rate and occurrence in end of gestation [19]. Characteristic symptom of the disease is maternal hypoglycemia and has been attributed to an increase in glucose uptake by the twin-bearing uterus [19]. Also some risk factors such as diseases, nutritional disorders or stress led to decrease energy intake and increase negative energy balance that seems to be critical for the progress of pregnancy ketosis [20]. Insulin exhibits selectivity among extra hepatic tissue and facilitates uptake of glucose by muscles [21,22] and adipose tissues [23,24].

Meat production is the first priority of sheep breeding in Iran and wool production is second or third purpose. The sheep population in Iran is about 53 million heads (14 breeds) [13] and more than 1.6 million people in Iran are directly involved in sheep and goats breeding which plays a significant economic role in rural and nomadic livelihood. Shall sheep with an approximate population is 1 million in Alborz and Qazvin province is one of the important meat breed in Iran, that more than 45% meat of Qazvin province comes from this breed [25]. Some characteristics of mentioned breed including: high percentage of twinning (approximately 30-35%), high conversion efficiency, high daily weight gain in the finishing period and about 1 kg wool production annually, led be superior to other meat breeds in Iran. The objective of current study was evaluation of possible correlation between BHBA, TAC and protein profile pre and post-parturition in Shall ewes.

MATERIALS AND METHODS

Animals

During this research in 2013 from January to March, 4 large flocks suburbs of Qazvin city, Iran with similar nutrition management program selected and 112 heads clinically healthy sheep from Shall breed (each flock 28 heads) studied. All flocks were in the winter pasture and the extra ration used consist of approximately 0.5 kg alfalfa hay per ewe, 1.5 kg wheat straw per ewe and 0.25 kg hand-made concentrate (containing 25% wheat bran, 20% corn, 20.5% wheat, 15% rice bran, 10% canola meal, 6% molasses, 1.5% calcium carbonate, 1% sodium bicarbonate, 1% enzymes and 0.5% sodium chloride per ewe) being offered daily. Water was given ad libitum. It should be mentioned before and after parturition extra ration have not changed.

Sample collection

Blood sample from jugular vein of sheeps, after overnight fasting in the morning by sterile venoject tubes (6 ml, MediPlus

Co., UK) collected. First blood sample 4-6 weeks before lambing and second blood sample 2 months after parturition were taken. Age and body condition score (BCS) (scale of 0 to 5 point) based on Russel, 1991 [17] of each sampled sheep recorded. Blood samples transferred to laboratory 30 min after collection beside ice pack, then serum isolated by centrifugation (1500× g for 15 min) and until 2 hours analyzed.

Analytical procedures

Biochemical analysis were carried out on the serum samples to measure of BHBA (Ranbut kit, Randox Laboratories Ltd., Ardmore, UK), glucose (Pars Azmoon Co, Iran), total protein (Pars Azmoon Co., Iran), albumin (Pars Azmoon Co., Iran) and TAC (FRAP) based on spectrophotometric methods. The automated method for measuring the FRAP or with other words the measurement of "antioxidant power" was modified by [26] to a manual assay. Our protocol is shortly as follows:

Making reagents: 1) Acetate buffer: 300 mmol/L pH 3.6 (3.1 g sodium acetate × 3 H₂O and 16 ml conc. acetic acid per 1 of buffer solution)

2) 40 mmol/L Dilute HCl: 1.46 ml conc. HCl (11M), distilled water (d.w.) to 1 liter store at room temperature

3) 10 mmol/L TPTZ (2,4,6-tri[2-pyridyl]-s-triazine): 0.031 g TPTZ in 10 ml of 40 mmol/LHCl, dissolved at 50°C in water bath, made fresh on day of assay in a new corning tube

4) 20 mmol/L Ferric chloride: 0.054 g FeCl₃ × 6 H₂O, dissolved in 10 ml distilled water, made fresh on day of assay in a new corning tube

Making standards: 1 mmol/L Ferrous sulphate solution: 0.278 g FeSO₄ × 7 H₂O in 1 liter distilled water dilute as follows to made a series of standards shown in Table 1, below:

Statistical evaluation

The results of biochemical examination of serum samples were analyzed with SPSS (SPSS, Ver. 16, Inc., Chicago, IL). Normal distribution of data with Shapiro-Wilk test checked, then descriptive statistics and paired sample T-test for compare means of biochemical factors before and after parturition done. Biochemical parameters changes between flocks pre and post-partum were compared by one way ANOVA. Pearson correlation tests were performed to evaluate the relationship among serum parameters in flocks. Value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Table 2 showed the range, min and max and mean±S.D serum value of BHBA, TAC, glucose, total protein, albumin, globulin and A/G ratio before and after parturition. Mean serum BHBA, TAC, glucose, Total protein, albumin, globulin and A/G ratio before parturition 1.11±0.30 mmol/L, 0.37±0.72 mmol/L, 51.78±3.84 mg/dl, 68.1±4.73, 39.7±6.51, 28.4±2.29 g/L, 1.39±0.78 and after parturition were 0.40±0.19 mmol/L, 0.42±0.99 mmol/L, 61.32±3.31 mg/dl, 65.3±3.28, 34.3±2.22, 31±4.16 g/L and 1.10±0.64 respectively. Statistic analysis shown that serum BHBA in pregnant sheeps was significantly higher than non-pregnant sheeps ($P < 0.01$), also the serum concentration of glucose in

Table 1: The composition of chemical substances to determine the standard concentrations in FRAP.

Standard Concentration (mmol/L)	FeSO ₄ × 7 H ₂ O solution (ml)	Distilled water (d.w.) (ml)
0.1	1	9
0.2	2	8
0.4	4	6
0.6	6	4
0.8	8	2
1.0	10	0

FRAP working solution: 200 ml acetate buffer [1], 20 ml TPTZ solution [3], 20 ml FeCl₃ × 6 H₂O solution [4] and 24 ml distilled water. Keep in a plastic bottle in water container at 37°C.

Table 2: Descriptive statistics of biochemical parameters before and after parturition.

Biochemical factors	Prepartum (No.:112)			Postpartum (No.:112)			p-value
	Range	Min - Max	Mean± S.D	Range	Min - Max	Mean± S.D	
BHBA (mmol/L)	1.15	0.75 - 1.90	1.11±0.3	0.81	0.11 - 0.92	0.4±0.194	< 0.01
TAC (mmol/L)	0.34	0.22 - 0.57	0.37±0.72	0.40	0.19 - 0.59	0.42±0.991	< 0.03
Glucose (mg/dl)	13.45	42.14-55.59	51.78±3.84	13.04	53.21 -66.25	61.324±3.31	< 0.01
Total Protein(g/L)	15.28	56.22-71.50	68.1±4.73	15.05	53.19- 68.15	65.31±3.28	< 0.05
Albumin (g/L)	11.73	32.87-43.34	39.7±6.51	14.10	26.11 - 40.21	34.3±2.22	< 0.05
Globulin (g/L)	7.05	29.10-36.15	28.4±2.290	9.04	26.16 - 35.20	31±4.160	ns
A/G ratio	0.069	1.129-1.198	1.397±0.788	0.144	0.998 - 1.142	1.10±0.645	< 0.05

No: number, ns: not significant at $p>0.05$.

non-pregnant sheeps was more than pregnant sheeps ($P< 0.01$) significantly. The value of TAC in pregnant sheeps was higher than non-pregnant sheeps significantly ($P< 0.03$). The level of total protein ($P< 0.05$), albumin ($P< 0.05$) and A/G ratio ($P< 0.05$) pre-partum were significantly higher than post-partum, while globulin values have not significant change pre and post-partum ($P> 0.05$). The serum concentration of biochemical parameters of 4 flocks studied shown with details in Table 3. There were a significant difference ($P< 0.05$) between concentrations of BHBA, TAC, glucose and albumin in all flocks pre and post-partum. The value of total protein was significantly lower ($P< 0.05$) post than pre-partum in flock C and globulin had significantly difference ($P< 0.05$) only in flock A pre-partum. A/G ratio in pre-partum was more than post-partum in flocks A and D ($P< 0.05$). Flock C showed significant changes than other flocks in BHBA and glucose concentrations pre and post-partum ($P< 0.05$) and TAC level Post-partum ($P< 0.01$). Significant difference ($P< 0.05$) observed in pre-partum levels of globulin and A/G ratio of flock C and in post-partum levels of globulin and A/G ratio in flock B than other flocks.

Correlation between biochemical parameters pre and post parturition shown in table 4. There are a strong negative correlation between BHBA and TAC in pregnant sheeps ($r=-0.731$, $P< 0.000$) and a weak negative correlation ($r=-0.390$, $P< 0.003$) in non-pregnant sheeps. Were found a very strong negative correlation between BHBA and glucose in pregnant sheeps ($r=-0.959$, $P< 0.000$) and in non-pregnant sheeps ($r=-$

-0.932 , $P< 0.000$) also, relative positive correlation between TAC and glucose ($r= 0.683$, $P< 0.000$) in pregnant sheeps and a weak positive correlation ($r= 0.393$, $P< 0.003$) in non-pregnant sheeps. There are relative positive correlation between albumin and globulin pre ($r= 0.403$, $P< 0.01$) and post ($r= 0.415$, $P< 0.01$) partum. A relative negative correlation between BHBA and albumin in pregnant sheep ($r=-0.387$, $P< 0.01$) and non-pregnant sheep ($r=-0.397$, $P< 0.01$) were found.

Several studies have shown that plasma concentrations of glucose in pregnancy and during early lactation is changed [27-29]. Serum glucose concentrations were significantly lower at pre-partum period than post-partum. The increase may reflect the recovery of feed intake and improving energy status of the ewe after lambing. Negative energy balance appears to be related to the glucose demands of the fetal-placental unit in pregnant ewes. The energy needs of the fetus and placenta are met almost entirely by glucose and lactate [30]. Serum glucose in current study had a significant negative correlation with BHBA pre and post-partum.

Measuring serum BHBA concentrations may serve as a useful method for monitoring the energy status in pregnant ewes [31]. Blood concentrations of BHBA between 0.8 to 1.6 mmol/L are indicative of a negative energy balance. In present study 51/120 heads sheep (42.5%) pre-partum and 15/120 heads sheep (12.5%) post-partum have a BHBA level more than 0.8 mmol/L that indicate of negative energy balance in studied sheep flocks

Table 3: Serum concentration of biochemical parameters before and after parturition in flocks (Mean±S.D) from each flock 28 sheep selected.

Flocks Factors	A	B	C	D
BHBA (mmol/L)				
Pre-partum	1.121±0.168	1.149±0.254	0.996±0.367*	1.159±0.414
Post-partum	0.384±0.169	0.397±0.115	0.375±0.228*	0.467±0.143
p- value	< 0.05	< 0.05	< 0.05	< 0.05
TAC (mmol/L)				
Pre-partum	0.374±0.653	0.367±0.605	0.380±0.141	0.365±0.293
Post-partum	0.428±0.123	0.419±0.260	0.461±0.578**	0.405±0.901
p- value	< 0.05	< 0.05	< 0.05	< 0.05
Glucose (mg/dl)				
Pre-partum	53.40±2.16	49.32±1.58	55.14±3.30*	49.26±2.91
Post-partum	62.21±3.35	61.34±4.31	65.29±2.68*	57.66±1.09
p- value	< 0.05	< 0.05	< 0.05	< 0.05
Total Protein (g/L)				
Pre-partum	67.31±1.24	65.93±2.61	70.66±0.94	68.52±2.01
Post-partum	65.62±1.39	63.53±0.11	66.71±0.69	65.40±2.87
p- value	> 0.05	> 0.05	< 0.05	> 0.05
Albumin (g/L)				
Pre-partum	39.73±1.25	39.62±1.16	38.54±1.39	40.91±0.89
Post-partum	33.21±0.64	36.52±0.76	34.39±1.18	33.11±1.23
p- value	< 0.05	< 0.05	< 0.05	< 0.05
Globulin (g/L)				
Pre-partum	27.58±0.46	26.31±1.03	32.12±0.87*	27.61±0.93
Post-partum	32.41±1.02	27.01±1.46*	32.32±0.51	32.29±1.74
p- value	< 0.05	> 0.05	> 0.05	> 0.05
A/G ratio				
Pre-partum	1.44±0.13	1.50±0.23	1.19±0.09*	1.48±0.18
Post-partum	1.02±0.17	1.35±0.62*	1.06±0.21	1.02±1.42
p- value	< 0.05	> 0.05	> 0.05	< 0.05

*P< 0.05 and ** P< 0.01 between flocks.

Table 4: Correlation between biochemical parameters pre and postparturition.

Biochemical parameters	BHBA	TAC	Glucose	TPP	Albumin	Globulin	A/G
BHBA							
prepartum		-0.731**	-0.959**	ns	-0.387*	ns	ns
postpartum		-0.390*	-0.932**	ns	-0.397*	ns	ns
TAC							
prepartum	-0.731**		0.683**	ns	ns	ns	ns
postpartum	-0.390*		0.393*	ns	ns	ns	ns
Glucose							
prepartum	-0.959**	0.683**		ns	ns	ns	ns
postpartum	-0.932**	0.393*		ns	ns	ns	ns
TPP							
prepartum	ns	ns	ns		ns	ns	ns
postpartum	ns	ns	ns		ns	ns	ns
Albumin							
prepartum	-0.387*	ns	ns	ns		0.403*	ns
postpartum	-0.397*	ns	ns	ns		0.415*	ns
Globulin							
prepartum	ns	ns	ns	ns	0.403*		ns
postpartum	ns	ns	ns	ns	0.415*		ns
A/G							
prepartum	ns	ns	ns	ns	ns	ns	
postpartum	ns	ns	ns	ns	ns	ns	

ns: not significant, p>0.05

significant correlations are indicated : * p<0.01, ** p<0.001

of Qazvin, which among them, 36/51 heads (70.6%) pre-partum and 12/15 heads sheep (80.0%) post-partum have a age more than 3 year, as a result a significant positive correlation ($r=0.60, P<0.01$) between age and negative energy balance were found. There are negative significant correlation between age with total protein pre ($r=-0.694, P<0.05$) and post ($r=-0.713, P<0.05$) partum. Our results about age-related alteration of total protein in shall ewes were similar with results of Alberghina et al. [32] in goats (*caprahircus*). In recent study of Qazvin, 23/120 heads sheep (20.5%) pre-partum and 7/120 heads sheep (5.8%) post-partum have a BCS 3 or greater than 3, but there was no significant correlation ($P>0.05$) between level of BHBA and BCS. Relationship between BCS and incidence of metabolic disease in cattle have been thoroughly reported with Ford et al. [33] and Schlumbohm and Harmeyer [34].

The metabolism of ewes pregnant with several fetuses is often severely stressed during the end of the pregnancy, which may cause pregnancy toxemia. The significant increase in the concentration of TAC and strong negative correlation with BHBA in ewes in present study are supported by results from a study of periparturient dairy cows [35].

Our results was consistent with the observations of Antunovic et al. and Deghnouche et al. [11,36], who shown high serum concentration of protein in the last trimester of pregnancy occur, but is in contradiction with results of Shetaewi and Daghash [27] who mentioned albumin value decrease in pregnancy compared with lactation period.

Several papers have been published comparing the different methods for total antioxidant activity measurement [37]. We used FRAP method which was elaborated by Benzie and Strain [38] for human cases originally. Our results shown that the rise and fall of BHBA level of serum leads to changes in the total antioxidant capacity of body before and after parturition. In recent study, we found that before parturition is characterized by depleted antioxidant status. This physiological phase can impose oxidative stress as indicated by a decrease of TAC. Bernabucci et al. [35] demonstrated that is the metabolic and endocrine adjustments, related to metabolism of fetus and mammary gland might be responsible for some variation of the oxidative status in transition cows. The results of biochemical analysis in late pregnancy appears to be a higher challenge to the energy metabolism in Shall ewes, and antioxidant capacity in late pregnancy dramatically decline, as a result, it is possible of lipolysis and release of free radicals was cause of this condition that maybe with balance of energy in end of pregnancy or increasing the amount of antioxidant in diet, this physiological situation be rather control.

CONCLUSION

This study approved that the physiological and hormonal changes in pregnancy have a remarkable effects on serum concentration of BHBA, TAC, glucose, total protein, albumin and A/G ratio in shall sheeps. Also, increasing BHBA value of serum has a significant influence on TAC concentration.

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