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Variations of Energy Related Biochemical Metabolites During Periparturition Period in Fat-Tailed Baloochi Breed Sheep

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Abstract

Negative energy balance in pregnant ewes during last two month of gestation could result in fatal pregnancy toxemia and affects ewe and lamb health. The understanding of variations of energy related metabolites during this critical period may cast light upon to a better management of the situation. This study describes the variations of serum energy related biochemical metabolites at a period of 45 days before to 21 days after parturition in Baloochi breed ewes. Blood from the jugular vein was collected from each sheep at days 45, 30, 20, 10, 1 prior to parturition, and days 4, 7, 13, 19 and 21 post-partum to determine total protein, albumin, urea , cholesterol, glucose, triglyceride, aspartate aminotransferase (AST), β-Hydroxybutyrate (BHBA) and non-esterified fatty acid (NEFA). NEFA and BHBA concentrations reached peak levels at parturition. However, AST activity had highest levels at the first and second week after lambing. AST activity significantly correlated to energy metabolism indicators. This enzyme had positive significant correlations with NEFA and BHBA and negative correlations with cholesterol and triglyceride. NEFA concentrations had a positive correlation with BHBA and negative correlation with glucose and cholesterol. At pre-partum period NEFA correlation with BHBA and AST were stronger than post-partum. This finding indicates that NEFA testing at pre-parturition days is a reliable predictor of fat mobilization and energy status of pre-parturition period in sheep. Urea and total protein concentrations reached lowest levels at parturition and then gradually increased afterwards.

Keywords: Fat tailed sheep, pregnancy, negative energy balance, energy metabolites

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Introduction

Iranian fat-tailed sheep are reared under the different climatic conditions of Iran. The physiological importance of the fat tail is to provide energy during drought seasons and conditions of feed deprivation which are not uncommon under the climatic conditions of Iran (Nazifi *et al*., 2002). The Baloochi breed is a breed of sheep that originated in the eastern and south eastern area (Sistan and Baloochestan) and kavir markazi of Iran. This breed is one of the most prevalent sheep breeds in the region. They are fat-tailed sheep using for dairy, meat and wool (with good quality carpet wool). Milk yields per lactation about 50 kg and average of yearly fleece weight 1.55 kg each sheep (Sattari, 1988).

Pregnancy and parturition are accompanied by endocrine changes that exceed those occurring in time. Concurrent with shifts in endocrine profile are metabolic changes that facilitate diversion of nutrients away from maternal stores toward fetus during pregnancy and milk synthesis and nurturing of the newborn after parturition.

The identification of changes in the metabolism of such sheep in various reproduction phases, the determination of abnormal metabolic states, and the prediction of some metabolic disorders, such as pregnancy toxemia and fatty liver could provide some advantages to producers (Balikci *et al*., 2007). To the knowledge of authors, there is no paper published on the variations of energy related metabolites around parturition in fat-tailed ewes.

The present study is derived retrospectively from samples collected during a previously reported clinical trial to determine the effect of monensin, administered 40 days before parturition, on energy metabolism, blood electrolytes, reproductive performance, and milk production (Taghipour *et al*., 2010). Samples and data collected during the trial provided an opportunity to study the variations of energy related biochemical metabolites during periparturition period in fat-tailed Baloochi breed sheep.

Materials and Methods

Details of the experimental study, data collection, laboratory and statistical analysis were reported in Taghipour *et al*. (2010). Briefly, Eighteen Baloochi breed pregnant ewes with average age of 4-6 years, average body weight of 50 ± 6.25 Kg were enrolled in an experimental trial. Baloochi sheep is the dominant fat-tailed breed in north-east of Iran. Nine ewes were received monensin as its 10% sodium salt in the amount of 23mg/sheep/daily which was solved in distilled water and fed by drencher individually. The sheep were received monensin for the average of 40 days (median, 42 days) before parturition. Nine ewes were received only distilled water as placebo. The diet was formulated to meet all nutrient requirements for pregnant ewe with an average body weight of 50 Kg. Nine ewes were fed restricted diet for last 30 days of pregnancy and 9 sheep were continuing feeding ordinary diet. The restricted diet was formulated to reach 30% less dry matter (0.945 Kg) than the ordinary diet. The ingredients and chemical composition of ordinary and restricted diets are shown in Table 1.

The present outcomes were not affected by the administration of monensin and feeding restricted diet in the underlying clinical trial, except BHBA. BHBA concentrations were significantly affected by monensin administration. For the present study BHBA amounts in monensin supplemented group were omitted.

Blood sampling and biochemical analysis

Blood from the jugular vein was collected from each sheep at days 45, 30, 20, 10, 1 prior to parturition, and days 4, 7, 13, 19 and 21 post partum. The blood sample was collected into 10-ml vacuum tube and was chilled immediately after sampling and transported to the laboratory within 1 hour after collection. Serum was harvested after centrifugation, frozen and stored at -20° C until analysis.

The concentrations of cholesterol, triglyceride, blood urea nitrogen (BUN), total protein, albumin, glucose, and aspartate aminotransferase (AST) were determined by an automated biochemical analyzer (Biotecnica, Targa 3000, Rome, Italy) using commercial kits (Parsazmoon, Tehran, Iran). β-Hydroxybutyrate (BHBA) and non-esterified fatty acid (NEFA) were determined by a D-3-hydroxybutyrate kit and a NEFA Kit (Randox Laboratories Ltd, Ardmore, UK). Blood chemistry was analyzed according to the following colorimetric methodologies, total protein: Biuret reaction (Tomas, 1998), albumin: Bromcresol green (Johnson et al., 1999), BUN: urease UV (Tomas, 1998), cholesterol: CHOD-PAP (Deeg and Ziegenhorn, 1983), glucose: glucose oxidase (Barham and Trinder, 1972), triglyceride: GOD-PAP (Cole *et al*., 1997), aspartate aminotransferase (AST): IFCC (Bergmeyer *et al*., 1986). β-Hydroxybutyrate (BHBA) (McMurray *et al*., 1984) and non-esterified fatty acid (NEFA) (DeVries *et al*., 1976) were determined with commercial colorimetric Kits (Randox Laboratories Ltd, Ardmore, UK). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy. All parameters showed a within-run impression of less than 10% (total protein: 0.95%, albumin: 0.91%, BUN: 5.41%, cholesterol: <3%, glucose: <3%, triglyceride: <3%, AST: 2.5%, BHBA: <5% and NEFA: <5%).

Data management and statistical analysis

The data were checked for errors and compared with written reports; outliers were rechecked to ensure that values were accurate. Because serum metabolites were measured over time, a repeated measures approach using ANOVA with mixed linear models used in SAS (The SAS system for Windows, Version 8.2, Cary, NC). All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The distributions of glucose, cholesterol, BUN, were normal. Serum concentrations of BHBA, NEFA, triglyceride, albumin, total protein, and AST were skewed to the right. Therefore, a logarithmic transformation was performed on each of these dependent variables, which resulted in a normal distribution. The following model was fitted to all metabolites that had repeated measurements over time:

$$Y_{ijk} = \mu + M_i + R_j + T_k + (M \times R)_{ij} + (M \times T)_{ik} + (M \times R \times T)_{ijk} + e_{ijk}$$

Where Y_{ijk} = the dependent variable, μ = overall mean, M_i = effect of monensin (I = 1, 2), R_j = effect of ration restriction (j = 1, 2), T_k = effect of time of sampling (k = 1 to 17), $(M \times R)_{ij}$ = effect of monensin × restriction interaction, $(M \times T)_{ik}$ = effect of monensin × time, $(M \times R \times T)_{ijk}$ = effect of monensin × restriction × time interaction, and e_{ijk} = random residual error.

The compound symmetry covariance structure was used for each model. The interaction term was removed from the model when $P > 0.25$. All reported values are least squares means.

Data was analyzed for statistical correlation of energy related metabolites using Pearson coefficient. Significance was declared $P \leq 0.05$.

Results and Discussion

NEFA

NEFA concentrations increased before lambing reached peak levels on parturition and started to decrease thereafter (Table 2). NEFA reflects the magnitude of fat mobilization from fat stores in response to negative energy balance (LeBlanc, 2006). The gradual increase of plasma NEFA during the final days pre-partum may be explained by the gradual depression of DMI observed during this time (Bertics *et al*., 1992). NEFA levels had significant positive correlations with BHBA (r = 0.37) and AST (r = 0.2) and a significant negative correlation (r = -0.31) with glucose (Table 3). The correlation with glucose was higher at post-parturition period (Table 4).

Table 1: Raw material and chemical composition of the experimental diets.

	Pre-parturition		Post-parturition
	Ordinary Diet	Restricted Diet	All Animals
Dry Matter Intake (Kg/day)	1.35	0.945	1.8
Ingredient (%DM)			
Alfalfa hay	45	45	45
Barely grain	20	20	25
Soya bean meal	4.5	4.5	4.5
Wheat bran	9.5	9.5	4.5
Wheat straw	20	20	20
Vitamin and minerals	1	1	1
Chemical Composition			
Energy Intake NE (Mcal/day)	3.45	2.4	4.5
NE Mcal/kg	2.4	2.4	2.5
CP (% DM)	13.1	13.1	13
ADF (% DM)	28.3	28.3	27.9
NDF (% DM)	43.1	43.1	42
EE (% DM)	2.4	2.4	2.3
Ca (% DM)	0.8	0.8	0.9
P (% DM)	0.5	0.5	0.5

Table 2: Least square of means (LSM) and standard error (SE) of biochemical metabolites during periparturition period.

Blood Metabolite		Days relating to lambing							*P* value
		-45	**-30**	**-15**	**-1**	**+7**	**+13**	**+21**	
NEFA mmol/l	LSM	0.23	0.34	0.18	0.48	0.29	0.443	0.21	
	SE	0.08	0.08	0.08	0.11	0.08	0.08	0.08	0.0078
BHBA mmol/l	LSM	0.59	0.61	0.64	0.81	0.80	0.84	0.71	
	SE	0.09	0.09	0.09	0.11	0.10	0.09	0.09	0.0419
Glucose mmol/l	LSM	2.45	2.14	2.89	3.07	3.35	3.29	3.56	
	SE	0.16	0.16	0.15	0.20	0.16	0.15	0.15	<0.0001
Cholestrol mmol/l	LSM	1.77	1.64	1.56	1.47	1.33	1.38	1.43	
	SE	0.08	0.08	0.08	0.09	0.08	0.08	0.08	<0.0001
Triglyceride mmol/l	LSM	0.20	0.18	0.21	0.20	0.06	0.06	0.07	
	SE	0.02	0.02	0.02	0.03	0.02	0.02	0.02	<0.0001
AST iu/l	LSM	73.54	81.54	71.87	67.89	94.41	101.68	93.85	
	SE	4.91	5.00	4.82	6.14	4.91	4.82	4.82	<0.0001
Urea mmol/l	LSM	4.84	5.18	4.80	4.61	5.04	5.24	5.30	
	SE	0.25	0.25	0.24	0.31	0.25	0.24	0.24	<0.0001
Albumin g/l	LSM	32.64	33.18	32.56	32.38	32.00	31.69	31.42	
	SE	0.91	0.92	0.90	1.11	0.91	0.90	0.90	0.4806
T.Protein g/l	LSM	71.16	69.07	65.61	58.93	66.95	68.50	69.41	
	SE	2.04	2.00	2.00	2.31	2.02	2.00	2.00	<0.0001

Table 3: Correlation matrix of energy related metabolites for ewes at pre and post-parturition periods.

	Peri-parturition Period				
	Glucose	NEFA	BHBA	Triglyceride	Cholesterol
AST	-0.01	0.2**	0.25**	-0.29**	
Cholesterol	-0.10	-0.12	-0.11	0.27**	
Triglyceride	-0.2**	-0.09	-0.02		
BHBA	-0.01	0.37**			
NEFA	-0.31**				

Table 4: Correlation matrix of energy related metabolites for ewes at pre and post-parturition periods.

	Glucose	NEFA	BHBA	Triglyceride	Cholesterol
Pre-parturition Period					
AST	-0.29**	0.28**	0.11	-0.017	0.05
Cholesterol	-0.08	-0.14	-0.30**	-0.06	
Triglyceride	0.05	-0.07	0.06		
BHBA	0.005	0.4**			
NEFA	-0.30**				
Post-parturition Period					
	Glucose	NEFA	BHBA	Triglyceride	Cholesterol
AST	0.05	0.12	0.34**	-0.00	-0.20*
Cholesterol	0.17	-0.03	0.11	0.09	
Triglyceride	0.15	0.08	0.02		
BHBA	-0.08	0.34**			
NEFA	-0.46**				

* $P<0.05$
** $P<0.01$

BHBA

BHBA concentrations were low before parturition and reached peak level on lambing; then, gradually decreased (Table 2). However, these variations were not statistically significant. BHBA had significant correlations with NEFA and AST at peri-parturition (Table 3). Measuring serum BHBA concentrations may serve as a useful method for monitoring the energy status in pregnant ewes (Edmondson and Pugh, 2009). Blood concentrations of BHBA of 0.8 to 1.6 mmol/ L are indicative of a negative energy balance (Navarre and Pugh, 2002).

AST

AST activity was the lowest at d 1 before lambing and highest at d 13 postpartum (Table 2). In addition, AST significantly correlated to triglyceride (r = - 0.29), BHBA (r = 0.25), NEFA (r = 0.20), and cholesterol (r = - 0.27). The level of this enzyme was determined to provide an estimate of liver function. This was the enzyme that correlated best with reduced hepatic function in fatty liver disease in other investigations (Herdt *et al*. 1988) and has been selected in herd monitoring programs for the occurrence of fatty liver (Reid *et al*. 1983, Sommer, 1995). In cattle, the sensitivity of AST is reported to be 94% for hepatic lipidosis (Kaneko *et al*., 2008).

In a previously reported study, AST showed no significant differences at pre- and post-lambing stage, but were slightly higher at pre-lambing period (Khan *et al*., 2002). They discussed that low enzyme levels during the post-partum period could be attributed to the corticosteroid levels.

Glucose

Several authors recorded plasma glucose concentrations to be higher during lactation than pregnancy in sheep (Henze *et al*., 1994; Shetaewi and Daghash, 1994; Takarkhede *et al*., 1999; Balikchi *et al*., 2007). In a study, peak plasma glucose levels were observed on the last day of pregnancy (Charismiadou *et al*., 2000). Serum glucose concentrations were significantly lower at pre-partum period than post-partum and reached the highest level at 21 days post-partum in the present study. 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 (Bauman and Currie, 1980). Serum glucose had a significant negative correlation with NEFA pre- and post-partum.

Cholesterol and Triglyceride

Serum cholesterol and triglyceride

concentrations gradually decreased during pregnancy and reached low levels after lambing. Some researchers reported that serum cholesterol and triglyceride concentrations to be higher in pregnant compared to non-pregnant sheep (Hamadeh *et al*., 1996; Al-Dewachi, 1999). Nazifi *et al*. (2002) stated that pregnancy had a significant effect on the serum lipids and cholesterol concentrations in lipoproteins of Iranian fat-tailed sheep, as with progression in the pregnancy period there was an increase in the cholesterol and triglyceride concentrations. These variations appear to reflect increased hepatic triglyceride synthesis and VLDL secretion, because of the activities of lipoprotein lipase and hepatic lipase, the enzymes responsible for the catabolism of VLDL and their remnants (Watson *et al*., 1993). In contrast to sheep, cholesterol concentrations are higher in lactating cows than pregnant non-lactating cows (Seifi *et al*., 2007). Negative energy balance is prevalent in dairy cows during early lactation because of rapid increase in energy demands for milk production (Herdt and Gerloff, 2009). However, Negative energy balance appears to be related to the energy demands of the fetal-placental unit in pregnant ewes.

BUN, Albumin and Total Protein

Blood urea concentrations reached to lowest level at parturition. The decrease of serum BUN around parturition may be associated with the decline of feed intake due to stress and hormonal changes during lambing.

Serum total protein concentrations gradually decreased during pre-partum period and reached the lowest level at parturition and then slowly increased afterward. This decrease in serum total protein may be ascribed to the fact that the fetus synthesizes all its proteins from the amino acids derived from the dam, and growth of the fetus increases exponentially reaching a maximum level, especially in muscles, during late pregnancy (Jainudee and Hafez, 1994). However, serum albumin concentrations did not significantly change in the present study. It could be concluded that the globulins were responsible for significant variations of total proteins levels. Drainage of globulins to mammary glands for colostrums synthesis may be considered as a main factor for this difference. Shetaewi and Daghash (1994) demonstrated that lactation could increase globulin when compared to pregnant ewes.

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تغییرات متابولیت‌های بیوشیمیایی انرژی در حول و حوش زایش در گوسفند نژاد بلوچی

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چکیده

موازنه منفی انرژی در میش‌های آبستن طی دو ماه آخر آبستنی می‌تواند منجر بـه مسـمومیت آبسـتنی مهلـک شـده و بـر سلامتی میش و بره آن اثر گذارد. شناخت تغییرات متابولیت‌های مربوط به انرژی در طی این دوره بحرانی ممکـن اسـت سـبب مدیریت بهتر آن گردد. در مطالعه حاضر متابولیت‌های بیوشیمیایی وابسته به انـرژی ۴۵ روز قبـل تـا ۲۱ روز پـس از زایـش در میش‌های آبستن نژاد بلوچی بررسی گردید. خون ورید وداج از هر گوسفند در روزهـای ۴۵، ۳۰، ۲۰، ۱۰ و ۱ قبـل از زایـش و روزهای ۴، ۷، ۱۳، ۱۹ و ۲۱ پس از زایش اخذ گردید. متابولیت‌هائی که در سرم اندازه گیری شد عبارت بودند از پـروتئین تـام، آلبومین، اوره، کلسترول، گلوکز، تری گلیسیرید، آنزیم آسپارتات آمینوترانس فـراز، بتاهیدروکسـی بـوتیرات و اسـیدهای چـرب غیر استریفیه. بتاهیدروکسی بوتیرات و اسید های چـرب غیـر اسـتریفیه در هنگـام زایمـان بـه بـالاترین مقـدار خـود در دوره حول و حوش زایش رسیدند. فعالیت آنزیم آسپارتات آمینوترانس فراز در نخستین و دومین هفتـه پـس از زایـش بـه بیشـترین مقدار خود رسید. فعالیت این آنزیم به طور معنی‌داری با شاخص‌هـای متابولیسـم انـرژی همبسـتگی داشـت. آنـزیم آسـپارتات آمینوترانس فراز دارای همبستگی مثبت با بتاهیدروکسی بوتیرات و اسـید هـای چـرب غیـر اسـتریفیه و همبسـتگی منفـی بـا کلسترول و تری‌گلیسیرید نشان داد. مقادیر اسید های چرب غیر استریفیه با مقادیر بتاهیدروکسـی بـوتیرات دارای همبسـتگی مثبت و با مقادیر گلوکز و کلسترول همبستگی منفی داشت. در دوره قبل از زایش همبستگی اسیدهای چرب غیر اسـتریفیه بـا مقادیر بتاهیدروکسی بوتیرات و فعالیت آنزیم آسپارتات آمینوترانس فراز قوی تر از دوره پـس از زایـش بـود. ایـن یافتـه نشـان می‌دهد که اندازه گیری اسیدهای چرب غیر استریفیه در روزهای قبل از زایش شاخص معتبری برای تحرک چربـی و وضـعیت انرژی در دوره قبل از زایش در گوسفند محسوب می‌گردد. مقادیر اوره و پروتئین تام در هنگام زایش کمترین مقدار داشته کـه به تدریج پس از آن افزایش می‌یابد.

واژگان کلیدی : گوسفند دمبه‌دار، آبستنی، موازنه منفی انرژی، متابولیت‌های انرژی