

Comparison of energy related metabolites during peri-parturition period in single and twin-bearing Lori-Bakhtiari ewes

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Abstract:

BACKGROUND: The Lori-Bakhtiari sheep breed is one of the most common native breed in the south-western parts of Iran. The peri-parturition period (2 weeks before to, 2 weeks after parturition), is generally of critical importance regarding the health, production and profitability of the ewes. **OBJECTIVES:** This study was carried out to compare the serum concentrations of glucose, non-esterified fatty acid (NEFA), β -hydroxybutyrate (BHBA), cholesterol, blood urea nitrogen (BUN) and calcium (Ca) during peri-parturition period in 30 single and 30 twin-bearing Lori-Bakhtiari ewes. **METHODS:** All blood profiles were determined in healthy ewes on days 14, 7 prepartum, and days 7 and 14 postpartum. **RESULTS:** Serum glucose levels were lower in twin-bearing ewes, compared to single-bearing ewes on prepartum and day 7 after lambing. Serum NEFA, BHBA and cholesterol levels were higher in twin-bearing ewes compared to single-bearing ewes during the peri-parturition period. Lower serum Ca levels were recorded 7 days before and after parturition in twin-bearing ewes, compared to single-bearing ewes. **CONCLUSIONS:** The results showed that NEFA and BHBA recorded significant ($p < 0.05$) changes during the peri-parturition period in twin-bearing ewes. These significant differences could be related to increased metabolic demands of the fetuses in twin-bearing ewes.

Introduction

The Lori-Bakhtiari sheep breed is one of the most common native breeds in the south-western parts of Iran. Majority of the sheep population is managed under a nomadic system, utilizing natural pastures as the major source of nutrition. This breed also has the largest fat-tail among all breeds in Iran (Shadnough et al., 2004; Vatankhah and Zamani, 2007). The physical characteristics and productive perfor-

mances of the Lori-Bakhtiari sheep include an adult live weight of 68-77 kg; height of 70-75 cm; fat-tail weight of 8-10 kg; a milk yield of 85-100 L per 6 months; 1.17 ± 0.38 number of lambings per year and a fleece weight of 2.5-3 kg per year.

The peri-parturition period (2 weeks before to, 2 weeks after parturition) is generally of critical importance regarding the health, production and profitability of the ewes. Pregnancy toxemia is the most common metabolic

disorder encountered in late-term gestation of ewes carrying multiple fetuses, exacerbated by an inability to consume adequate energy to match their metabolic demands. In late gestation, ewes carrying twins require 180% more energy than those carrying singletons. In addition, 80% of fetal growth occurs during the last 6 weeks of gestation (Edmondson and Pugh, 2009). The metabolic capacity of ewes is generally under severe strain during late pregnancy, with the increased size of the fetus(es) increasing the tension on the maternal carbohydrate metabolism (Sigurdsson, 1988). Environmental stresses, chronic illnesses, depressed appetite, and a negative energy balance lead to alterations in insulin-glucagon ratio. Fatty acids and glycerol are mobilized from body fat to be used in energy production. A large portion of the fatty acids are extracted by the liver and used in ketone body synthesis (Edmondson and Pugh, 2009).

The determination of serum glucose, non-esterified fatty acid (NEFA), β -hydroxybutyrate (BHBA), cholesterol, blood urea nitrogen (BUN) and calcium (Ca) concentrations during peri-parturition period, particularly in the case of twin-pregnancy, can provide important information for estimating the animal's state of metabolism in late pregnancy. Many health disorders can occur during the peri-parturition period, an increased understanding of the biology at this time, can help decrease health problems and increase the profitability of the ewes. The aim of this study was to compare the energy-related metabolites during this peri-parturition period in single and twin-bearing Lori-Bakhtiari ewes.

Materials and Methods

The study was conducted between October 2010 and March 2011 at Sholi Station of the Lori-Bakhtiari Sheep Breeding Research Center located in Charmahal and Bakhtiari province of Iran. From the flock at the station,

60 healthy pregnant Lori-Bakhtiari ewes (30 bearing singles and 30 bearing twin lambs) weighing 71.5 ± 11.2 kg, aged between 2 and 6 years, were used in this experiment. Ewes were classified as either single or twin-bearing ewes following ultrasonography 35 days after synchronized mating. The diet used was administered daily and it contains 1.2 kg alfalfa hay, 0.8 kg wheat straw and 0.7 kg concentrate containing 26.5% wheat bran, 21% corn, 21% wheat, 12% rice bran, 9% canola meal, 6% molasses, 1.5% calcium carbonate, 1% sodium bicarbonate, 1% enzymes, 0.5% vitamins and 0.5% sodium chloride per ewe. Blood samples were collected from the jugular vein at 5-6 a.m. (before feeding) on days 14 and 7 before the expected date of delivery, and days 7 and 14 postpartum. Blood sample was taken into a plain glass tube where it was allowed to coagulate. Serum was collected after centrifugation; it was frozen and stored at -70°C until analyzed.

Blood chemistry was analyzed according to the following colorimetric methodologies, glucose: glucose oxidase, cholesterol: CHOD-PAP, BUN: urease UV and calcium: arsenazo. To determine these parameters, commercial kits (Parsazmoon, Tehran, Iran) were used by an automated biochemical analyzer (Biotechnica, Targa 3000, Rome, Italy). β -hydroxybutyrate and NEFA were determined by a D-3-hydroxybutyrate kit and a NEFA Kit (Randox Laboratories Ltd, Ardmore, UK).

The data were checked for errors and were compared with written reports; outliers were rechecked to ensure that the values were accurate. Because serum metabolites were measured over time, repeated measures approach using ANOVA with GLM models and logistic regression were used in SAS (Version 8.2). All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The distributions of all variables were normal. Data were analyzed using One Way ANOVA at dif-

ferent stages of peri-parturition period. The T-test was used to compare serum metabolite levels between single and twin-bearing ewes. Values of $p < 0.05$ were considered significant.

Results

The serum metabolite concentrations during peri-parturition period are presented in Table 1. Serum glucose levels were lower in twin-bearing ewes, compared to single-bearing ewes on prepartum and day 7 after lambing. Serum NEFA, BHBA and cholesterol levels were higher in twins compared to singletons during peri-parturition period. Lower serum Ca levels were recorded 7 days before and after parturition in twin-bearing ewes, compared to single-bearing ewes. The results showed that NEFA and BHBA had significant effects ($p < 0.05$) on twin-bearing ewes during the peri-parturition period.

Discussion

In the present study no significant changes in blood glucose concentrations was noticed during the peri-parturition period in both groups of ewes with single and twin lambs. However, the glucose concentrations were significantly lower in twin-bearing ewes in comparison to single-bearing ewes on prepartum and day 7 postpartum, without diagnosing pregnancy toxemia in the ewes. This indicates that the feeding regime had been sufficient to meet the maintenance, pregnancy and lactation requirements of the ewes, with no real negative energy balance. Vernon et al. (1981) and Firat and Özpınar (2002) also reported that changes in the serum glucose levels during pregnancy and lactation were not significant. Several researchers have reported that serum glucose levels are higher during lactation than for pregnancy in ewes (Henze et al., 1994; Takarkhede et al., 1999; Balıkcı et al., 2007; Moghaddam and Hassanpour, 2008). Lower serum glucose

levels were recorded in twin-bearing ewes than in single-bearing ewes on day 150 of gestation and this may be ascribed to the larger metabolic demand in the twin-bearing ewes (Balıkcı et al., 2007). The blood glucose concentration is always significantly related to the rate of glucose production and the greatest stress-induced reductions in glucose concentration and glucose production rate are seen during late gestation in twin-bearing ewes (Schlumbohm and Harmeyer, 2008). The glucose homeostatic system of ewes bearing twin is significantly more susceptible to hypoglycemic stress than that of ewes bearing single lambs, the primary cause of hypoglycemia in twin-pregnant ewes at late gestation is an increased susceptibility to stress related reduction in glucose production rate (Schlumbohm and Harmeyer, 2008).

In the present study, significant changes in blood NEFA and BHBA concentrations were recorded during the peri-parturition period only in the group of ewes with twin lambs. Blood NEFA and BHBA levels were significantly higher in twin-bearing ewes, compared to single-bearing ewes. Moallem et al. (2012) reported that blood NEFA concentrations in pregnant ewes were greater as litter size increased and the highest concentrations of NEFA were seen during the last week of pregnancy, but no significant differences in NEFA concentrations were observed between twins and singletons in late gestation. High values of NEFA indicate a negative energy balance that occurs in animals which are not prone to any disease (Radostits et al., 2007).

In normal fed, ruminants' ketone bodies are released at about similar rates by the rumen epithelium (from acetate and butyrate) during the absorption process and by the liver (from NEFA and acetoacetate). The rate of hepatic ketone body production usually increases 4-5 times in ewes during late gestation and lactation. Under conditions of insufficient energy supply, alimentary ketogenesis decreases and the rate of hepatic ketogenesis from NEFA

Table 1. Mean (\pm SE) serum metabolite concentrations during peri-parturition period in single (n=30) and twin-bearing (n=30) ewes. Means in the same row and within the same variable with different superscripts differ significantly ($p < 0.05$). (*) represent $p < 0.05$, (**) represent $p < 0.01$ and (***) represent $p < 0.001$ in columns.

Parameters		Days relating to lambing			
		-14	-7	+7	+14
Glucose (mg/dl)	Single	67.51 \pm 1.8	69.16 \pm 1.8	66.16 \pm 1.8	63.65 \pm 1.8
	Twin	58.36 \pm 1.8	57.14 \pm 1.8	59.33 \pm 1.8	61.85 \pm 1.8
	Single vs. Twin	**	***	*	
NEFA (mmol/l)	Single	0.39 \pm 0.05	0.42 \pm 0.05	0.35 \pm 0.05	0.31 \pm 0.05
	Twin	0.70 \pm 0.05 ^{ab}	0.78 \pm 0.05 ^a	0.62 \pm 0.05 ^b	0.49 \pm 0.05 ^c
	Single vs. Twin	***	***	***	*
BHBA (mmol/l)	Single	0.51 \pm 0.04	0.53 \pm 0.04	0.51 \pm 0.04	0.48 \pm 0.04
	Twin	0.96 \pm 0.04 ^{ab}	1.0 \pm 0.04 ^a	0.88 \pm 0.04 ^b	0.72 \pm 0.04 ^c
	Single vs. Twin	***	***	***	***
Cholestrol (mg/dl)	Single	62.83 \pm 2.0	62.83 \pm 2.0	65.07 \pm 2.0	64.03 \pm 2.0
	Twin	73.64 \pm 2.0	73.90 \pm 2.0	73.34 \pm 2.0	72.38 \pm 2.0
	Single vs. Twin	**	**	*	*
BUN (mg/dl)	Single	16.69 \pm 0.7	17.55 \pm 0.7	17.31 \pm 0.7	17.10 \pm 0.7
	Twin	20.18 \pm 0.7	20.77 \pm 0.7	20.88 \pm 0.7	20.77 \pm 0.7
	Single vs. Twin				
Ca (mg/dl)	Single	9.25 \pm 0.18	9.52 \pm 0.18	9.36 \pm 0.18	9.24 \pm 0.18
	Twin	8.77 \pm 0.18	8.77 \pm 0.18	8.84 \pm 0.18	9.04 \pm 0.18
	Single vs. Twin		*	*	

disproportionately increases. Such metabolic changes are associated by marked increases in the rate of mobilization of long chain fatty acids from adipose tissues and marked rises of circulating concentrations of NEFA and ketone bodies (Harmeyer and Schlumbohm, 2006). Measuring serum BHBA concentration may thus serve as a useful method in monitoring the energy status of pregnant ewes. Values of 0.8 to 1.6 mmol/L are indicative of a negative energy balance (Edmondson and Pugh, 2009). Significant higher blood levels of BHBA in late pregnant ewes have been reported by Hamadeh et al. (1996). Ewes with two lambs, had significantly higher BHBA concentrations in late gestation and during lactation than in the dry period, this was not the case for ewes bearing only one lamb (Harmeyer and Schlumbohm, 2006). Moallem et al. (2012) demonstrated that the concentrations of BHBA in blood increases as the number of fetuses increased, but no significant differences in BHBA concentrations were observed between twins and singletons in late gestation. High BHBA concentrations significantly sup-

press the endogenous glucose production, but have no effect on glucose utilization. The suppressive effect of hyperketonemia on hepatic glucose production has resulted in a significant reduction of serum glucose concentration and was qualitatively similar in late pregnancy and lactation (Schlumbohm and Harmeyer, 2004). The reduced ability of the late gestating ewe to utilize BHBA promotes hyperketonemia and this reduction is greater in twin-bearing ewes than in single-bearing ewes (Harmeyer and Schlumbohm, 2006).

In the present study significant lower serum Ca levels were recorded 7 days before and after parturition in twin-bearing ewes, compared to single-bearing ewes. Moallem et al. (2012) showed that there were no significant differences in the serum concentrations of Ca between twin and single-bearing ewes in late gestation. For ewes, especially those carrying twins, meeting the sudden increase in fetal skeletal demand for Ca often presents a greater challenge to Ca homeostasis than lactation. Estrogen, which dramatically increases during late gestation, decreases osteoclast

activity. This reduces the ability to use bone Ca reabsorption to meet fetal skeletal Ca demands (Goff, 2009). As a result, most primary hypocalcemic disorders of the ewe occur in late gestation. During the last three months of gestation, hyperketonemia in ewes is often associated with hypocalcemia. Hypocalcemia lowers the endogenous production of glucose and induces a decline in serum glucose concentration during prepartum and postpartum period in normoketonemia and hyperketonemia. Pregnant normoketonemic ewes are able to compensate for the hypoglycemic effect of hypocalcemia and keep the endogenous production at the normocalcemic level. Hypocalcemia does not promote the onset of pregnancy toxemia per se, but in combination with hyperketonemia it facilitates the development of the disease when it is present (Schlumbohm and Harmeyer, 2003).

In the present study, serum cholesterol levels were significantly higher in twin-bearing ewes compared to single-bearing ewes during peri-parturition period. Nazifi et al. (2002) reported that pregnancy have a significant effect on the serum lipids and cholesterol concentrations of lipoproteins in Iranian fat-tailed sheep. Serum cholesterol levels were recorded to be higher in twin-bearing ewes than in single-bearing ewes on day 150 of pregnancy (Balıkcı et al., 2007). In contrast, there were significant lower blood levels of cholesterol in late pregnant ewes carrying two fetuses than in ewes carrying one fetus as reported by Moallem et al. (2012). Several researchers recorded higher blood cholesterol concentrations during late pregnancy (Hamadeh et al., 1996; Nazifi et al., 2002, Balıkcı et al., 2007). The increase in cholesterol concentration may be due to insulin, which plays a direct role in the metabolism of adipose tissue during pregnancy. Its responsiveness is significantly reduced in ewes during late pregnancy. The diminished responsiveness of the target tissue to insulin during late pregnancy predisposes the ewes to

an increase in blood cholesterol and lipoprotein concentrations (Schlumbohm et al., 1997).

In the present study, insignificant differences were seen for serum BUN concentrations between twin and single pregnancies. This is in agreement with the studies of Fırat and Özpınar (2002) and Balıkcı et al. (2007). In Corriedale ewes, the glomerular filtration and urea clearance were significantly reduced during late pregnancy and lactation (Rodriguez et al., 1996). El-Sherif and Assad (2001) recorded a maximum urea concentration at parturition in Barki ewes and they concluded that protein catabolism and high energy need by pregnant ewes during the second half of pregnancy led to an increase in urea concentrations to an extent above the ability of kidneys to eliminate excess amounts from plasma.

The results of this study showed significant differences in energy-related blood metabolite concentrations around parturition between single and twin-bearing Lori-Bakhtiari ewes. These significant differences could be associated with increased metabolic demands of the fetuses in twin-bearing ewes.

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مقایسه متابولیت‌های وابسته به انرژی در دوره حوالی زایش در میش‌های آبستن تک قلو و دوقلو از نژاد لری-بختیاری

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چکیده

زمینه مطالعه: نژاد لری-بختیاری یکی از متداولترین نژادهای بومی گوسفند در بخش‌های جنوب غربی ایران است. به طور کلی دوره حوالی زایش (از ۲ هفته قبل تا ۲ هفته بعد از زایمان) از نظر سلامتی، تولید و سودآوری میش‌ها اهمیتی بحرانی دارد. هدف: این تحقیق با هدف مقایسه غلظت سرمی گلوکز، اسیدهای چرب غیراستریفیه (NEFA)، بتا هیدروکسی بوتیرات (BHBA)، کلسترول، نیتروژن اوره خون (BUN) و کلسیم در دوره حوالی زایش در ۳۰ رأس میش آبستن تک قلو و ۳۰ رأس میش آبستن دوقلو از نژاد لری-بختیاری انجام گردید. روش کار: تمامی پارامترهای خونی در میش‌های سالم و در روزهای ۱۴ و ۷ قبل از زایمان و روزهای ۷ و ۱۴ بعد از زایمان اندازه گیری شد. نتایج: میزان گلوکز سرم در میش‌های با آبستنی دوقلو در مقایسه با میش‌های با آبستنی تک قلو در قبل از زایمان و روز ۷ بعد از زایمان کمتر بود. مقادیر سرمی NEFA، BHBA و کلسترول در میش‌های آبستن دوقلو در دوره حوالی زایش بیشتر از میش‌های آبستن تک قلو بود. میزان کلسیم سرم در روزهای ۷ قبل و بعد از زایمان در میش‌های با آبستنی دوقلو کمتر از میش‌های آبستن تک قلو بود. نتیجه گیری نهایی: نتایج نشان داد که تغییرات در میزان NEFA و BHBA در دوره حوالی زایش در میش‌های آبستن دوقلو معنی‌دار ($p < 0.05$) هستند. این تفاوت‌های معنی‌دار می‌تواند ناشی از افزایش نیازهای متابولیک جنین‌ها در میش‌های آبستن دوقلو باشد.

واژه‌های کلیدی: متابولیت‌های انرژی، گوسفند لری-بختیاری، دوره حوالی زایش، آبستنی دوقلو

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