Vol. 18, No. 3, Autumn, 2022, 114-125
DOI: 10.22055/IVJ.2022.340124.2462
Journal Homepage: http://www.ivj.ir/

Original Article

Evaluation of metabolic profile at mating, gestation, and early lactation in Gray Shirazi ewes

Hasan Rahij Torfi[1], Seyedeh Missagh Jalali[2*], Meisam Makki[3], Ali-dad Boostani[4] and Mohamad-Rahim Hajikolaie[5]

[1] PhD Student, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Assisstant Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Assisstant Professor, Department of Animal Science, Fars Agricultural and Natural Resources Research and Education Center, Iran
[5] Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: 30.04.2022 Accepted: 07.09.2022

Abstract

This study was aimed to investigate the status of energy balance during mating, pregnancy and after lambing and its relationship with reproductive outcomes in Gray Shirazi sheep. Thirty healthy Gray Shirazi ewes that were kept in industrial conditions were randomly selected. During lambing, the ewes were examined for the rate of multiplication as well as abortions and the weight of lambs at birth. Blood sampling was performed during mating, on the last two to four weeks of pregnancy, and on one to two weeks after delivery. Serum levels of insulin-like growth factor-1 (IGF-1), insulin, non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA), and progesterone were assessed. There was a significant rise in IGF-1 level in late pregnancy compared to that in early lactation. In addition, the concentration of BHBA was significantly increased during pregnancy and postpartum compared to the mating time. Maximum BHBA and NEFA concentrations at the end of pregnancy and postpartum were in twin and singleton pregnancies, respectively. In addition, the highest BHBA concentrations were accompanied by the lowest BCS of ewes. Moreover, there was a significant direct correlation between lamb weight and NEFA, BHBA, and progesterone. In conclusion, serum indicators of energy balance, particularly insulin and BHBA, are largely influenced by reproductive stages, especially pregnancy and the number of lambs in Gray Shirazi ewes. Accurate identification of these changes is essential in diagnosing abnormal conditions and metabolic and nutritional disorders in this breed.

Keywords: Metabolic profile, Mating, Gestation, Lactation, Gray Shirazi ewes

Introduction

Today, metabolic and production related diseases in small ruminants have increased significantly due to the increasing use of productivity enhancement systems along with genetic modifications (Hernández et al., 2020). Metabolic disorders affect herd health and inflict huge economic losses on the livestock industry. Therefore,

* **Corresponding Author**: Seyedeh Missagh Jalali, Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: mi.jalali@scu.ac.ir

continuous monitoring of all factors affecting metabolism, including diet type, health status of the herd, as well as food intake seems necessary. Such information can be accessed, to a large extent, by measuring and interpreting some blood parameters and metabolites (Calamari et al., 2016). The chemical composition of blood plasma can reflect the metabolic status of various organs and tissues and can be applied as a valuable means for identifying tissue damage and organ dysfunction, as well as nutritional or metabolic imbalance (Braun et al., 2010). In fact, the metabolic profile allows the study of dietary quality and composition and the identification of possible nutritional disorders even before it affects herd production (Antunovic et al., 2015; Antunovic et al., 2011; Cabiddu et al., 2020; Macrae et al., 2006).

Twinning is one of the desirable features in ewes and makes it economical to rear different breeds of this species. Various factors affect the reproductive efficiency of ewes and increase the lambing rate, most of which are related to the conditions of ewes around mating (Meikle et al., 2018; Perry et al., 2007), including ewe weight at mating, feeding status, mother's age, climate and season (Akhtar et al., 2012; Gaskins et al., 2005).

Although initial reproductive events (ovulation, fertilization, and early pregnancy) require less energy than late pregnancy and lactation, there is still a high sensitivity to energy and nutrition fluctuations in the early stages and particular attention to this stage. It can play a significant role in the final reproductive efficiency and prevention of many metabolic disorders in the recent stages of pregnancy (Meikle et al., 2018).

Negative energy balance effects in ewes include increasing the period of seasonal anestrus, reducing fertility, and increasing embryo death (Diskin & Morris, 2008; Kakar et al., 2005).

Poor nutrition leads to a decrease in the number of large ovarian follicles and delayed ovulation in ewes (Meikle et al., 2018). The relationship between BCS and seasonal anestrus length and ovulation rate was also studied and it was found that ewes with moderate BCS had shorter anestrus and more multiple ovulation rates than sheep with low BCS (Forcada & Abecia, 2006). Therefore, the resumption of the ovarian cycle after lambing or the beginning of the breeding season depends on the body reserves of females.

Another critical stage during the sheep and goat breeding period is the peripartum period which includes the end of pregnancy and the beginning of lactation. During this period, the animal undergoes severe changes like limited food intake, delivery, onset of lactation, appetite fluctuations, dietary changes (from pregnancy to lactation) and hormonal fluctuations (Hernández et al., 2020).

Currently, the most ordinary metabolic diseases of ewes in the peripartum period are hypocalcemia, hypomagnesemia, and gestational intoxication, all of which are due to failure to accommodate nutritional needs at the end of pregnancy or early lactation (Castillo et al., 1999).

Small ruminants with hyperketonemia are more affected by peripartum problems, immunosuppression and following infectious diseases (mastitis, metritis, lameness, etc.) and will be less resistant to parasitic diseases of the gastrointestinal tract (Barbagianni et al., 2015).

There are some serum factors that reflect the state of energy metabolism. Negative energy balance in ruminants is identified by decreased blood glucose, insulin and insulin-like growth factor (IGF-1) and increased non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) (Castillo et al., 1999; Sosa et al., 2010).

Considering the importance of sheep breeding industry in the country on one hand and the significant economic effects of diseases and metabolic disorders with energy imbalance and also with the aim of increasing production and breeding of small ruminants, measuring and examining metabolic profile indicators to diagnose possible disorders and managerial and control amounts are inevitable. In this regard, it is necessary to study and identify the values and changes in serum indices of a metabolic profile, especially in the native breeds and breeding conditions of the country.

Gray Shirazi is one of the most famous breeds of skin sheep in the world, bred in Fars province. However, with increasing demand for meat, the activity of most sheep farmers has changed to produce meat. This breed is more adapted to the climate and weather of the region than other breeds and has lower maintenance costs. Therefore, breeding this livestock provides an appropriate background and potential for entrepreneurship and job creation (Karimi et al., 2021).

Despite some limited studies, there is still insufficient information on the state of metabolism, energy reserves and nutritional lacks and its relationship with reproductive efficiency in sheep, especially in terms of breeding and local breeds in the region.

Hence, this study was aimed to investigate the status of some indicators related to energy balance during mating, second half of pregnancy and after lambing and its relationship with reproductive outcomes in Gray Shirazi sheep.

Materials and Methods

Animal selection

Thirty healthy Gray Shirazi ewes that were kept in industrial conditions and receive a daily ration with a specific composition were randomly selected. Their body condition score (BCS) was recorded by the method of Russell et al. (1969) on a scale of 1 to 5 based on the measurement of fat and muscle mass at the transverse and longitudinal appendages of the vertebrae as well as on the ribs (Russel et al., 1969).

All ewes had ear plates so that the data could be recorded accurately. The synchronization program used in all ewes was the utilization of the effect of ram and also the injection of a single dose of intramuscular PGF2α, which was repeated about 8 days later if estrus was not observed. Colored neck straps were also used to easily identify the ewes in the herd. During lambing, the ewes were examined for the rate of multiplication as well as abortions and the weight of lambs at birth, and the results were recorded.

Sampling

Sampling was performed in three steps. The first blood sampling was done during mating (ramming), the second step was on the last 2 to 4 weeks of pregnancy and the third step was one to two weeks after delivery. Blood samples were collected in clot activator tubes and after separating the serum by centrifugation for 10 minutes at 3,000 rpm, the serum samples were stored in -20° C freezer until testing.

Hormonal analysis

Serum levels of insulin-like growth factor-1 (IGF-1) was assessed by ELISA method using a species specific kit (Bioassay Technology Laboratory, China). Insulin levels were also analyzed via ELISA method (Monobind, Germany).

Samples collected on the second and third stages were also subjected to progesterone assessment which was performed by ELISA method (Monobind, Germany).

Assessment of NEFA and BHBA

Serum concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHB) were evaluated photometrically (Randox Laboratories, UK) by a biochemistry autoanalyzer (BT-1500, Biotechnica, Italy).

Statistical analysis

Statistical analysis was performed using SPSS statistical program version 22 (SPSS Inc., Chicago, IL, USA). The results are expressed as means ± standard error (SE) for different groups. Data were analyzed statistically using Repeated measures analysis of variance (ANOVA), One-way ANOVA, Tukey's post hoc tests, Independent samples T-test, Paired T-test and Pearson correlation test. P< 0.05 was considered statistically significant.

Results

Metabolic status in various reproductive stages in ewes

Comparison of serum indicators of energy balance in different sampling stages revealed a significant rise in IGF-1 level in late pregnancy compared to that in early lactation sampling (p<0.05) (Table 1). In addition, the concentration of BHBA was significantly increased during pregnancy and postpartum compared to the mating time (p<0.05). Progesterone was significantly increased in the second stage sampling compared to the first stage, as expected (p<0.05).

There were not any significant changes in the insulin concentration in time (p>0.05). However, the minimum serum insulin was observed in late pregnancy sampling and the maximum was observed during mating.

Metabolic status in different pregnancy types in ewes

Non-pregnant, singleton, and twin pregnant ewes were compared in terms of serum indicators of energy balance (Table 2). The lowest insulin concentration was in twin pregnancies, at late pregnancy and after lambing, although no significant difference was observed (p>0.05).

Table 1. Mean±SE of serum indicators of energy balance in various sampling stages in ewes

	First sampling (Mating)	Second sampling (Pregnancy)	Third sampling (Lactation)
Insulin (μIU/ml)	17.072±2.637	10.455±1.541	11.869±1.059
IGF-1 (ng/ml)	92.560±13.914 ^{A}	127.484±23.307 ^{A}	46.938±7.086 ^{B}
BHBA (mmol/l)	0.285±0.021 ^{A}	0.345±0.022 ^{B}	0.362±0.031 ^{B}
NEFA (mmol/l)	0.628±0.152	0.524±0.165	0.652±0.131
Progesteron (ng/ml)	5.82±0.68 ^{A}	10.57±1.75 ^{B}	NA

* Different superscript letters in each row represent significant difference between groups (p<0.05).
* NA: not assessed; IGF-1: insulin-like growth factor-1; BHBA: beta-hydroxybutyric acid; NEFA: non-esterified fatty acids.

Table 2. Mean±SE of serum indicators of energy balance in different pregnancy types in ewes

	Sampling stage	Non-pregnant	Singleton-pregnancy	Twin-pregnancy
Insulin (μIU/ml)	1st	11.927±1.296	22.892±5.186	14.691±5.934
	2nd	12.665±3.099	10.185±1.638	7.679±1.458
	3rd	13.350±2.156	12.746±1.603	9.683±2.557
IGF-1 (ng/ml)	1st	81.614± 16.801	96.213± 21.784	104.393 ±35.569
	2nd	102.782±20.168	163.326±40.520	120.547±52.432
	3rd	51.310±11.886	51.734±9.170	22.696±4.651
BHBA (mmol/l)	1st	0.315± 0.029	0.244± 0.036	0.321 ±0.046
	2nd	0.310±0.022 ^{A}	0.335±0.028 ^{AB}	0.452±0.085 ^{B}
	3rd	0.252±0.017 ^{A}	0.458±0.035 ^{B}	0.372±0.131 ^{AB}
NEFA (mmol/l)	1st	1.049± 0.380 ^{A}	0.477± 0.146 ^{AB}	0.280 ±0.132 ^{B}
	2nd	0.267±0.112	0.630±0.303	0.416±0.156
	3rd	0.228±0.524 ^{A}	0.967±0.196 ^{B}	0.522±0.219 ^{AB}
Progesteron (ng/ml)	1st	5.770± 1.306 ^{A}	5.288±0.808 ^{A}	7.329±1.792 ^{B}
	2nd	4.464±1.572 ^{A}	15.411±2.768 ^{B}	12.643±4.379 ^{B}
	3rd	NA	NA	NA

* Different superscript letters in each row represent significant difference between groups (p<0.05).
* NA: not assessed; IGF-1: insulin-like growth factor-1; BHBA: beta-hydroxybutyric acid; NEFA: non-esterified fatty acids.

In addition, maximum BHBA concentration at the end of pregnancy and postpartum were in twin and singleton pregnancies, respectively, which were significantly different from non-pregnant ewes (p<0.05).

Regarding NEFA in the first sampling, at the mating time, the lowest amounts were in twin pregnancies and the highest were in the non-pregnant group (p<0.05). In contrast, at the end of pregnancy and after delivery, the minimum and maximum levels of NEFA were observed in the non-pregnant and singleton groups, respectively which were significantly different (p<0.05). Progesterone levels were also significantly higher in the twin cases at mating and in both pregnant groups (singletons and twins) at late pregnancies compared to the non-pregnant group (p<0.05).

Effect of parity on metabolic status in ewes

Serum indicators of energy balance were compared based on parity (Table 3). Parity had a considerable effect on insulin levels, so that it was the highest in the fourth lambing during mating time and in contrast in the late pregnant and early lactating ewes was the highest in the first lambing (p<0.05). In addition, parity had a significant effect on serum progesterone levels so that in both stages of sampling the highest values were observed in the fourth, third, second lambing, respectively, compared to the first pregnancy (p<0.05).

Table 3. Mean±SE of serum indicators of energy balance in different groups of ewes based on parity

	Sampling stage	Primiparous (1st)	Multiparous (2nd)	Multiparous (3rd)	Multiparous (4th)
Insulin (µIU/ml)	1st	6.127±0.000 A	17.633±5.199 AB	15.635±3.000 AB	21.255±6.937 B
	2nd	24.308±8.209 A	7.388±0.885 B	11.997±1.953 B	8.442±1.855 B
	3rd	15.704±6.338	13.345±1.718	11.797±1.997	12.183±2.787
IGF-1 (ng/ml)	1st	36.170±4.022	67.736±11.105	115.949±23.481	93.460±27.420
	2nd	185.964±101.405	83.964±14.089	160.890±40.943	117.736±37.393
	3rd	33.740±20.299	37.651±7.872	59.760±12.565	36.563±5.973
BHBA (mmol/l)	1st	0.417±0.032	0.279±0.036	0.278±0.033	0.267±0.050
	2nd	0.270±0.070	0.360±0.045	0.353±0.040	0.331±0.167
	3rd	0.250±0.010	0.306±0.056	0.411±0.055	0.365±0.048
NEFA (mmol/l)	1st	0.890±0.340	0.832±0.302	0.650±0.305	0.267±0.713
	2nd	0.745±0.475	0.215±0.067	0.595±0.309	0.361±0.141
	3rd	0.180±0.140	0.599±0.235	0.745±0.203	0.438±0.890
Progesteron (ng/ml)	1st	4.572±1.154 A	6.847±5.251 B	6.007±1.044 B	6.609±1.441 B
	2nd	6.836±0.062 A	7.186±2.464 B	12.632±2.427 B	13.393±4.947 B
	3rd	NA	NA	NA	NA

* Different superscript letters in each row represent significant difference between groups (p<0.05).
* NA: not assessed; IGF-1: insulin-like growth factor-1; BHBA: beta-hydroxybutyric acid; NEFA: non-esterified fatty acids.

Metabolic status in relation to ewe BCS

Ewes were divided in five groups based on their BCS and metabolic markers were analyzed and compared between groups (Table 4). Although there was no significant difference between groups in terms of insulin level, lower body scores were associated with the minimum levels of insulin at all stages of sampling ($p>0.05$). In addition, the highest BHBA concentrations were accompanied by the lowest BCS of ewes (BCS=2) in all stages which was significantly higher than that of BCS groups of 3.5 and 4 ($p<0.05$). Regarding NEFA in postpartum sampling, the concentration of this analyte in ewes with a BCS of 2 was significantly higher than other scores ($p<0.05$). However, in other stages of sampling, such a relationship was not established and the highest concentrations of NEFA were observed at higher body scores, although no significant difference was observed ($p>0.05$). Progesterone concentrations in late pregnancy were also significantly higher in ewes with a BCS of 2 than other body scores ($p<0.05$).

Correlation between metabolic status of ewe and lamb weight

There was a significant direct correlation between lamb weight and NEFA and BHBA concentration in the third stage of sampling ($p<0.05$) (Table 5). In addition, progesterone was also directly and significantly correlated with lamb weight in late pregnancy ($p<0.05$).

Table 4. Mean±SE of serum indicators of energy balance in different groups based on ewe BCS

	Sampling stage	Ewe BCS				
		2	2.5	3	3.5	4
Insulin (µIU/ml)	1st	7.324±0.07	11.972±4.155	22.681±7.539	17.023±4.040	14.965±3.548
	2nd	7.887±1.120	4.302±1.120	8.653±1.710	12.028±2.270	13.190±4.080
	3rd	8.943±2.958	12.042±4.789	13.511±2.238	14.231±2.545	10.722±1.838
IGF-1 (ng/ml)	1st	54.038±3.173	117.465±4.377	95.742±29.211	73.928±14.627	119.484±37.427
	2nd	75.170±37.477	232.500±79.079	120.734±50.340	141.895±41.465	117.254±29.039
	3rd	46.507±27.785	62.981	35.702±16.958	37.974±18.952	67.423±55.814
BHBA (mmol/l)	1st	0.357±0.062	0.225±0.025	0.279±0.055	0.254±0.031	0.331±0.042
	2nd	0.520±0.270 ^{A}	0.330±0.010 ^{AB}	0.354±0.030 ^{AB}	0.321±0.036 ^{B}	0.345±0.22 ^{AB}
	3rd	0.710±0.160 ^{A}	0.410±0.010 ^{AB}	0.379±0.653 ^{B}	0.329±0.424 ^{B}	0.292±0.190 ^{B}
NEFA (mmol/l)	1st	0.020±0.020	0.18	0.505±0.108	0.928±0.322	0.530±0.266
	2nd	0.335±0.105	0.365±0.155	0.328±0.748	0.734±0.366	0.212±0.113
	3rd	1.660±0.270 ^{A}	0.340±0.050 ^{B}	0.579±0.122 ^{AB}	0.533±0.206 ^{AB}	0.500±0.323 ^{AB}
P4 (ng/ml)	1st	4.864±0.846	8.528±2.442	5.149±1.829	6.503±1.071	5.033±1.313
	2nd	16.716±4.122 ^{A}	^{B} 4.453±3.949	14.988±4.824 ^{AB}	8.053±1.710 ^{B}	8.412±3.408 ^{B}
	3rd	NA	NA	NA	NA	NA

* Different superscript letters in each row represent significant difference between groups ($p<0.05$).
* BCS: body condition score; NA: not assessed; IGF-1: insulin-like growth factor-1; BHBA: beta-hydroxybutyric acid; NEFA: non-esterified fatty acids; P4: progesterone.

Table 5. Correlation between serum indicators of energy balance in different sampling stages in ewes with lamb weight

	Sampling stage	Lamb Weight (Kg)	
		Pearson correlation	Significance
Insulin (µIU/ml)	1st	0.295	0.823
	2nd	-0.194	0.305
	3rd	-0.109	0.575
IGF-1 (ng/ml)	1st	0.121	0.541
	2nd	0.160	0.400
	3rd	-0.111	0.575
BHBA (mmol/l)	1st	-0.314	0.091
	2nd	0.108	0.569
	3rd	0.517	0.003
NEFA (mmol/l)	1st	0.047	0.823
	2nd	0.217	0.250
	3rd	0.525	0.003
Progesteron (ng/ml)	1st	0.104	0.584
	2nd	0.551	0.002
	3rd	NA	NA

* Different superscript letters in each row represent significant difference between groups ($p<0.05$).
* NA: not assessed; IGF-1: insulin-like growth factor-1; BHBA: beta-hydroxybutyric acid; NEFA: non-esterified fatty acids.

Discussion

In the present study, the state of energy balance in different stages of pregnancy and its relationship with some reproductive indices in Gray Shirazi ewes were investigated.

According to the results of comparing hormonal values between different stages of pregnancy, the minimum serum insulin concentration was observed in late pregnancy sampling and the maximum was observed during mating.

In addition, the concentration of BHBA as one of the valuable indicators of negative energy balance was significantly increased during pregnancy and postpartum compared to the mating time. Progesterone was also assessed to evaluate the quality of the placenta during mating and late pregnancy, which was significantly increased in the second stage sampling compared to the first stage, as expected.

All these results confirm the increase in metabolism and energy demand at the end of pregnancy and after delivery (peripartum period) compared to pre-pregnancy and during mating.

One of the most accurate and sensitive indicators of energy balance in small ruminants is BHBA, high serum levels of which are associated with reduced milk production, increased clinical ketosis, and decreased fertility (Constable et al., 2016). In fact, BHBA testing can be used in small ruminants at the end of pregnancy in hypoglycemic conditions as a tool to diagnose subclinical pregnancy poisoning (Bani Ismail et al., 2008). In the study of Cal-Pereyra et al. (2015), the reduction of dietary energy in pregnant ewes from the 130th day of pregnancy with a significant decrease in glucose and a considerable increase in BHBA compared to the control group, these changes could be traced before the onset of pregnancy symptoms (Cal-Pereyra et al., 2015).

Taghipour et al. (2007) study on energy related biochemical metabolites status in Fat-Tailed Baloochi breed sheep revealed that serum concentration of BHBA is low at the beginning of pregnancy but reaches its peak during lambing and gradually decreases after delivery (Taghipour et al., 2010). In addition, in a study conducted by zakian et al. (2019) in Khuzestan also the serum concentration of BHBA in heavy pregnant Arabian ewes was higher than non-pregnant animals (Zakian et al., 2018). Similarly, in the study of Lotfollahzadeh et al. (2016), the amount of serum BHBA in pregnant Afshari sheep was more than non-pregnant (Lotfollahzadeh et al., 2016).

Small ruminants have shorter gestation periods and higher fertility and production than cows (so that the ratio of the baby's birth weight to the mother's weight is significantly higher). Therefore, the growth rate of the fetus increases per kilogram of maternal weight. On the other hand, during pregnancy, the digestive capacity of sheep and goats, to receive and digest forage does not meet the growing energy needs of animal. This is due to the restriction of rumen dilation and dry matter intake by the volume occupied by the uterus, as well as sudden hormonal changes as labor approaches. As a result, food intake decreases in the last two to three weeks of pregnancy compared to the initial stages, especially in fattening ewes. The mismatch between energy supply and demand at this time leads to a negative energy balance (NEB) (Cannas et al., 2016; Nielsen et al., 2015; Simões et al., 2021).

Postpartum dry matter intake is typically severely reduced and peaks only 30 to 40 days after lactation. This leads to a negative energy balance in the early stages of lambing, especially in high-yielding sheep with increased motility and catabolism of fat stores which increases the risk of hyperketonemia (Bouvier-Muller et al., 2016).

One of the consequences of the transition from pregnancy to lactation is the imbalance between oxidants and the antioxidant defense system and the occurrence of oxidative stress. Oxidative stress is associated with many negative effects on the health of ewes and fetuses, one of which is to disrupt the regulation of insulin secretion and increase the resistance of peripheral tissues to this hormone and prevent proper glucose metabolism and predispose the animal to future ketosis (Hernández et al., 2020).

In contrast to the above mentioned results, in the current study the highest level of IGF-1 was at the end of pregnancy and the lowest level after delivery which was inconsistent with other assessed energy balance markers. As a marker of energy balance, IGF-1 is usually in line with insulin levels and is influenced by this hormone in such a way that it starts to decline in cases of reduced feed intake (Rhoads et al., 2004; Salazar-Ortiz et al., 2014; Yang et al., 2019). However, inconsistent data has been documented regarding the concentrations and effects of this hormone in relation to pregnancy and energy balance (Kenyon et al., 2009; Stremming et al., 2021).

In terms of the effect of twinning on different indices, although there was no significant difference in the values of IGF-1 and insulin, some changes were observed in some stages of pregnancy. The lowest insulin concentration was in twin pregnancies, at late pregnancy and after lambing, although no significant difference was observed. In addition, maximum BHBA concentration at the end of pregnancy and postpartum were in twin and singleton pregnancies, respectively, which were significantly different from non-pregnant ewes. However, no significant difference was observed in BHBA concentration at mating. Also, in the first and third stage sampling, there was a direct correlation between BHBA level and the number of lambs. Regarding NEFA in the first sampling, at the mating time, the lowest amounts were in twin pregnancies and the highest were in the non-pregnant group. In contrast, at the end of pregnancy and after delivery, the minimum and maximum levels of NEFA were observed in the non-pregnant and singleton groups, respectively. Progesterone levels were also significantly higher in the twin cases at mating and in both pregnant groups (singletons and twins) at late pregnancies compared to the non-pregnant group. In addition, there was a direct correlation between progesterone concentration and the number of lambs in ewes sampled at late pregnancy. Consistent with the present study, Lotfollahzadeh et al. (2016) showed that the levels of BHBA in Afshari ewes in twin pregnancies were more than singletons when sampled in late pregnancy. In fact, in the last month of pregnancy in small ruminants, the growth rate of the fetus in twin pregnancies is four times higher and in triplets is about six times higher than in singleton pregnancies. This causes an extremely rapid increase in the nutritional needs of ewes in a short period of time (Lotfollahzadeh et al., 2016). Furthermore, according to NEFA variations in the current study, it seems that better metabolic status and energy balance at mating have been associated with increased reproductive efficiency and twinning. Nutrition during the period when ovarian follicles develop from the primordial pool (approximately 6 months before they ovulate in ewes) can influence ovulation rate in ewes and oocyte quality (Robinson et al., 2006). Undernutrition at this time reduces the number of follicles that emerge and therefore the number that is available to ovulate. There is now evidence that the reduction in ovulation rate can be prevented by improved nutrition (flushing) in the 10-day period prior (or even shorter) to mating (Robinson et al., 2006).

Parity had a considerable effect on insulin levels, so that it was the highest in the fourth lambing during mating time and in contrast in the late pregnant and early lactating ewes was the highest in the first lambing. In addition, parity had a significant effect on serum progesterone levels so that in both stages of sampling the highest values were observed in the fourth, third, second and first lambing, respectively. In line with these findings, Robles et al., (2018) stated that primiparous dams are associated with higher glucose tolerance, restricted intrauterine growth, less mature and smaller foals compared to multiparous dams with altered metabolism (Robles et al., 2018). A lower insulin level in nulliparous compared to multiparous ewes was also reported by Akbarinejad et al., (2014) (Akbarinejad et al., 2014).

In the present study, the ewes' BCS did not show any significant correlation or difference in IGF-1 concentration. Although insulin also did not significantly correlate with the BCS of ewes, lower body scores were associated with the minimum levels of insulin at all stages of sampling. In agreement with the latter mentioned results, the highest BHBA concentrations were accompanied by the lowest BCS of ewes (BCS=2) in all stages which was significantly higher than that of BCS groups of 3.5 and 4. There was also a significant inverse correlation between BCS and BHBA of ewes after delivery. Regarding NEFA, the data, especially in postpartum sampling, were consistent with the previous results, so that the concentration of NEFA in ewes with a BCS of 2 was significantly higher than other scores. However, in other stages of sampling, such a relationship was not established and the highest concentrations of NEFA were observed at higher body scores, although no significant difference was observed. Progesterone concentrations in late pregnancy were also significantly higher in ewes with a BCS of 2 than other body scores. In terms of body condition score overall, it seems that ewes with lower body scores at mating are more likely to have a negative energy balance due to lower energy reserves, especially at the end of pregnancy and postpartum and ewes with higher body scores are in a better condition in this respect. Findings of Cam et al. (2018) also showed that ewe BCS at the time of mating had a significant effect on reproductive efficiency so that the most outstanding performance was observed in BCS of 2.5 to four (Cam et al., 2018). Also in a study by Aktas et al. (2015) in Central Anatolian Merino sheep, ewes weighing more than 60 kg had more lambs born, fertility rate, multiplication ratio and higher lamb growth rate than the underweight groups. In addition, uterine function in the early stages of pregnancy is also affected by the energy status of ewes (Aktaş et al., 2015). In an experimental study by de Brun et al. (2016), good quality embryo transfer to poorly fed receiver ewes was associated with more significant embryonic mortality at 18 to 40 days of gestation than controls. Receptor animals with reduced energy in the previous study had lower serum insulin and progesterone levels in the early stages of the luteal phase, which affects embryonic development and can lead to termination of pregnancy (de Brun et al., 2016).

In the current study there was a significant correlation between lamb weight and NEFA and BHBA concentration in the third stage of sampling. In addition, progesterone was also directly and significantly correlated with lamb weight in late pregnancy. Thus, in general, although lambs with higher weights have stronger placenta, they also impose more energy demand and more metabolic changes on ewes (Hu et al., 1990; Stewart et al., 2019).

Serum indicators of energy balance, particularly insulin and BHBA, are largely influenced by reproductive stages, especially pregnancy and the number of lambs in Gray Shirazi ewes. Accurate identification of these changes is essential in diagnosing abnormal conditions and metabolic and nutritional disorders in this breed.

Acknowledgments

The authors would like to thank the staff of Fars Agricultural and Natural Resources Research and Education Center who helped in sample collection.

Conflict of interest

The authors declare that they have no known conflict of interest.

Funding

This research was carried out in the form of a thesis with the financial support of Shahid Chamran University of Ahvaz, Grant No. SCU.VC1400.199.

References

Akbarinejad, V., Niasari-Naslaji, A., Mahmoudzadeh, H., & Nikjou, D. (2014). Effect of parity on insulin and insulin like growth factor-1 (IGF-1) concentrations in sheep 18th Iranian Veterinary Congress.

Akhtar, M., Javed, K., Abdullah, M., Ahmad, N., & Elzo, M. (2012). Environmental factors affecting preweaning growth traits of Buchi sheep in Pakistan. *The Journal of Animal and Plant Science*, *22*(3), 529-536.

Aktaş, A., Dursun, Ş., Doğan, Ş., Kiyma, Z., Demirci, U., & Halıcı, I. (2015). Effects of ewe live weight and age on reproductive performance, lamb growth, and survival in Central Anatolian Merino sheep. *Archives Animal Breeding*, *58*(2), 451-459.

Antunovic, Z., Markovic, B., Šperanda, M., & Didara, M. (2015). Blood Metabolic profile and oxidative status of endangered Mediterranean sheep breeds during pregnancy. *Bulgarian Journal of Agricultural Science*, *21*(3), 655-661.

Antunovic, Z., Novoselec, J., Sauerwein, H., Speranda, M., Vegara, M., & Pavic, V. (2011). Blood metabolic profile and some of hormones concentration in ewes during different physiological status. *Bulgarian Journal of Agricultural Science*, *17*(5), 687-695.

Bani Ismail, Z., Al-Majali, A., Amireh, F., & Al-Rawashdeh, O. (2008). Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. *Veterinary clinical pathology*, *37*(4), 434-437.

Barbagianni, M., Spanos, S., Ioannidi, K., Vasileiou, N., Katsafadou, A., Valasi, I., Gouletsou, P., & Fthenakis, G. (2015). Increased incidence of peri-parturient problems in ewes with pregnancy toxaemia. *Small Ruminant Research*, *132*, 111-114.

Bouvier-Muller, J., Allain, C., Enjalbert, F., Tabouret, G., Portes, D., Caubet, C., Tasca, C., Foucras, G., & Rupp, R. (2016). Response to dietary-induced energy restriction in dairy sheep divergently selected for resistance or susceptibility to mastitis. *Journal of dairy science*, *99*(1), 480-492.

Braun, J., Trumel, C., & Bézille, P. (2010). Clinical biochemistry in sheep: A selected review. *Small Ruminant Research*, *92*(1-3), 10-18.

Cabiddu, A., Dattena, M., Decandia, M., Molle, G., Lopreiato, V., Minuti, A., & Trevisi, E. (2020). The effect of parity number on the metabolism, inflammation, and oxidative status of dairy sheep during the transition period. *Journal of Dairy Science*, *103*(9), 8564-8575.

Cal-Pereyra, L., González-Montaña, J., Benech, A., Acosta-Dibarrat, J., Martín, M., Perini, S., Abreu, M., Da Silva, S., & Rodríguez, P. (2015). Evaluation of three therapeutic alternatives for the early treatment of ovine pregnancy toxaemia. *Irish veterinary journal*, *18*(1), 1-7.

Calamari, L., Ferrari, A., Minuti, A., & Trevisi, E. (2016). Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on Fourier Transform mid-infrared spectroscopy: preliminary results. *BMC veterinary research*, *12*(1), 1-10.

Cam, M. A., Garipoglu, A. V., & Kirikci, K. (2018). Body condition status at mating affects gestation length, offspring yield and return rate in ewes. *Archives Animal Breeding*, *61*(2), 221-228.

Cannas, A., Thonney, M. L., & Lunesu, M. F. (2016). Feeding dairy sheep: Nutritional challenges and opportunities. DAIRY SHEEP ASSOCIATION OF NORTH AMERICA SYMPOSIUM.

Castillo, C., Hernandez, J., Lopez-Alonso, M., Miranda, M., & Benedito, J. (1999). Effect of physiological stage and nutritional management on some serum metabolite concentrations in Assaf ovine breed. *Archives Animal Breeding*, *42*(4), 377-386.

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2016). *Veterinary medicine-e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences.

de Brun, V., Meikle, A., Fernández-Foren, A., Forcada, F., Palacín, I., Menchaca, A., Sosa, C., & Abecia, J.-A. (2016). Failure to establish and maintain a pregnancy in undernourished recipient ewes is associated with a poor endocrine milieu in the early luteal phase. *Animal reproduction science*, *173*, 80-86.

Diskin, M., & Morris, D. (2008). Embryonic and early foetal losses in cattle and other ruminants. *Reproduction in Domestic Animals*, *43*, 260-267.

Forcada, F., & Abecia, J.-A. (2006). The effect of nutrition on the seasonality of reproduction in ewes. *Reproduction Nutrition Development*, *46*(4), 355-365.

Gaskins, C., Snowder, G., Westman, M., & Evans, M. (2005). Influence of body weight, age, and weight gain on fertility and prolificacy in four breeds of ewe lambs. *Journal of animal science*, *83*(7), 1680-1689.

Hernández, J., Benedito, J. L., & Castillo, C. (2020). Relevance of the study of metabolic profiles in sheep and goat flock. Present and future: A review. *Spanish Journal of Agricultural Research*, *18*(3), e06R01-e06R01.

Hu, G., Mccutcheon, S., Parker, W., & Walsh, P. (1990). Blood metabolite levels in late pregnant ewes as indicators of their nutritional status. *New Zealand Journal of Agricultural Research*, *33*(1), 63-68.

Kakar, M., Maddocks, S., Lorimer, M., Kleemann, D., Rudiger, S., Hartwich, K., & Walker, S. (2005). The effect of peri-conception nutrition on embryo quality in the superovulated ewe. *Theriogenology*, *64*(5), 1090-1103.

Karimi, A., Abarghuei, M. J., & Boostani, A. (2021). Influence of Crossbreeding of Ghezel Ram With Grey Shirazi Ewe on Growth Performance, Feed Efficiency and Carcass Traits of Fattening Lambs. Preprint from Research Square. DOI: 10.21203/rs.3.rs-642724/v1

Kenyon, P., Jenkinson, C., Blair, H., Breier, B., & Gluckman, P. (2009). Late-pregnancy nutrition differentially affects the birthweight of lambs born to ewes from divergently selected plasma IGF-1 lines. *New Zealand Journal of Agricultural Research*, *52*(1), 9-16.

Lotfollahzadeh, S., Zakian, A., Tehrani-Sharif, M., & Watson, D. G. (2016). Assessment the alterations of some biochemical parameters in Afshari sheep with possible metabolic disorders. *Small Ruminant Research*, *145*, 58-64.

Macrae, A., Whitaker, D., Burrough, E., Dowell, A., & Kelly, J. (2006). Use of metabolic profiles for the assessment of dietary adequacy in UK dairy herds. *Veterinary Record*, *159*(20), 655-661.

Meikle, A., Chilibroste, P., Carriquiry, M., Sosa, C., Abecia, J. A., de Brun, V., Soca, P., & Adrien, M. d. L. (2018). *Influences of nutrition and metabolism on reproduction of the female ruminant*. No. ART-2018-108136. 2018.

Nielsen, M., Nadeau, E., Markussen, B., Helander, C., Eknæs, M., & Nørgaard, P. (2015). Relationship between energy intake and chewing index of diets fed to pregnant ewes. *Small Ruminant Research*, *130*, 108-116.

Perry, G., Smith, M., Roberts, A., MacNeil, M., & Geary, T. (2007). Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *Journal of animal science*, *85*(3), 684-689.

Rhoads, R. P., Kim, J. W., Leury, B. J., Baumgard, L. H., Segoale, N., Frank, S. J., Bauman, D. E., & Boisclair, Y. R. (2004). Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. *The Journal of nutrition*, *134*(5), 1020-1027.

Robinson, J., Ashworth, C., Rooke, J., Mitchell, L., & McEvoy, T. (2006). Nutrition and fertility in ruminant livestock. *Animal Feed Science and Technology*, *126*(3-4), 259-276.

Robles, M., Dubois, C., Gautier, C., Dahirel, M., Guenon, I., Bouraima-Lelong, H., Viguié, C., Wimel, L., Couturier-Tarrade, A., & Chavatte-Palmer, P. (2018). Maternal parity affects placental development, growth and metabolism of foals until 1 year and a half. *Theriogenology*, *108*, 321-330.

Russel, A., Doney, F., & Gunn, R. (1969). Subjective assessment of fat in live sheep. *Journal of Agricultural Science*, *72*, 451-454.

Salazar-Ortiz, J., Monget, P., & Guillaume, D. (2014). The influence of nutrition on the insulin-like growth factor system and the concentrations of growth hormone, glucose, insulin, gonadotropins and progesterone in ovarian follicular fluid and plasma from adult female horses (Equus caballus). *Reproductive biology and endocrinology*, *12*(1), 1-12.

Simões, J., Abecia, J., Cannas, A., Delgadillo, J., Lacasta, D., Voigt, K., & Chemineau, P. (2021). Managing sheep and goats for sustainable high yield production. *Animal*, 100293.

Sosa, C., Gonzalez-Bulnes, A., Abecia, J., Forcada, F., & Meikle, A. (2010). Short-Term Undernutrition Affects Final Development of Ovulatory Follicles in Sheep Synchronized for Ovulation. *Reproduction in Domestic Animals*, *45*(6), 1033-1038.

Stewart, S., McGilchrist, P., Gardner, G., & Pethick, D. (2019). Feed deprivation in Merino and Terminal sired lambs:(1) the metabolic response under resting conditions. *Animal*, *13*(7), 1458-1467.

Stremming, J., Heard, S., White, A., Chang, E. I., Shaw, S. C., Wesolowski, S. R., Jonker, S. S., Rozance, P. J., & Brown, L. D. (2021). IGF-1 infusion to fetal sheep increases organ growth but not by stimulating nutrient transfer to the fetus. *American Journal of Physiology-Endocrinology and Metabolism*, *320*(3), E527-E538.

Taghipour, B., Seifi, H. A., Mohri, M., Farzaneh, N., & Naserian, A. (2010). Variations of energy related biochemical metabolites during periparturition period in fat-tailed baloochi breed sheep. *Iranian Journal of Veterinary Science and Technology*, *2*(2), 85-92.

Yang, C., Zhang, J., Ahmad, A. A., Bao, P., Guo, X., Long, R., Ding, X., & Yan, P. (2019). Dietary energy levels affect growth performance through growth hormone and insulin-like growth factor 1 in yak (Bos grunniens). *Animals*, *9*(2), 39.

Zakian, A., Haji-Hajikolaei, M., Tehrani-Sharif, M., Faramarzian, K., & Safaei, P. (2018). Evaluation of negative energy balance and some metabolic disorders on peri-partum period in Arabian ewes, Khuzestan. *Veterinary Journal*, *63*(3), 141-146.

Received: 30.04.2022
Accepted: 07.09.2022

DOI: 10.22055/IVJ.2022.340124.2462

بررسی پروفایل متابولیک در زمان جفت‌گیری، آبستنی و اوایل شیرواری در میش‌های نژاد کبوده شیراز

حسن رهیج طرفی[۱]، سیده میثاق جلالی[۲*]، میثم مکی[۳]، علی داد بوستانی[۴] و محمدرحیم حاجیکلائی[۵]

[۱] دانشجوی دکتری تخصصی، گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۲] دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۳] استادیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۴] استادیار مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی فارس، ایران
[۵] استاد گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

دریافت: ۱۴۰۱/۲/۱۰ پذیرش: ۱۴۰۱/۶/۱۶

چکیده

این مطالعه با هدف بررسی وضعیت تعادل انرژی در دوران جفت‌گیری، آبستنی و پس از بره‌زایی و ارتباط آن با پیامدهای تولیدمثلی در گوسفند کبوده شیراز انجام شد. ۳۰ راس میش کبوده شیراز سالم که در شرایط صنعتی نگهداری شده بودند به طور تصادفی انتخاب شدند. در طول بره‌زایی، میش‌ها از نظر میزان تکثیر و همچنین سقط جنین و وزن بره‌ها در بدو تولد بررسی شدند. خون‌گیری در طول جفت‌گیری، در دو تا چهار هفته آخر بارداری و یک تا دو هفته پس از زایمان انجام شد. سطح سرمی فاکتور رشد شبه انسولین ۱ (IGF-1)، انسولین، اسیدهای چرب غیر استریفیه (NEFA) و اسید بتا هیدروکسی بوتیریک (BHBA) و پروژسترون مورد ارزیابی قرار گرفت. افزایش قابل توجهی در سطح IGF-1 در اواخر آبستنی در مقایسه با اوایل شیرواری مشاهده شد. علاوه بر این، غلظت BHBA در دوران آبستنی و پس از زایمان نسبت به زمان جفت‌گیری به طور معنی‌داری افزایش یافت. حداکثر غلظت BHBA و NEFA در پایان آبستنی و پس از زایمان به ترتیب در آبستنی‌های دوقلو و تک قلو بود. علاوه بر این، بالاترین غلظت BHBA با کمترین BCS میش همراه بود. همچنین بین وزن بره و NEFA، BHBA و پروژسترون همبستگی مستقیم و معنی‌داری وجود داشت. در نتیجه، شاخص‌های سرمی تعادل انرژی، به ویژه انسولین و BHBA، تا حد زیادی تحت تأثیر مراحل تولید مثلی، به ویژه آبستنی و تعداد بره‌ها در میش‌های کبوده شیراز قرار دارند. شناسایی دقیق این تغییرات در تشخیص شرایط غیرطبیعی و اختلالات متابولیکی و تغذیه‌ای در این نژاد ضروری است.

کلمات کلیدی: پروفایل متابولیک، جفت‌گیری، آبستنی، شیرواری، میش کبوده شیراز

*نویسنده مسئول: سیده میثاق جلالی، دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
E-mail: mi.jalali@scu.ac.ir