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Blood Metabolic Profile in Barki Ewes during Transition Period

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ABSTRACT: This technical research article investigated the changes in the hemato-biochemical profile in Barki ewes during the transition period. A total of 15 healthy pregnant Barki ewes (age, 4.9 ± 0.7 years old; weight, 38.5 ± 4.9 Kg) were randomly selected for the current study. Blood samples were collected from the selected ewes via jugular vein puncture during the transition period at five different time points; 4 and 2 weeks prior the estimated date of delivery (EDD), at the parturition time, then at 2 and 4 weeks post-partum. Red blood cells (RBCs) count and packed cell volume (PCV) were significantly (P<0.05) decreased 2 weeks before the EDD and at the parturition time. Mean corpuscular volume (MCV) was significantly (P<0.05) decreased 4 weeks post-partum. The neutrophil count and neutrophil/lymphocyte ratio were significantly (P < 0.05) increased 2 weeks post-partum. The level of β-hydroxy butyric acid (BHBA) and concentration of non-esterified fatty acid (NEFA) in the serum were significantly (P<0.05) increased 2 weeks before EDD, at the parturition time, and 2 weeks post-partum. Both cholesterol and triglyceride levels increased significantly (P<0.05) 2 weeks before EDD and at the parturition time. Leptin level was significantly (P<0.05) decreased at 2 weeks and 4 weeks post-partum. Glucose level increased significantly (P<0.05) at the parturition time then decreased significantly (P<0.05) at 2 weeks post-partum, after which it increased again 4 weeks post-partum. Total protein level was significantly (P<0.05) increased 2 weeks post-partum. The level of both calcium and inorganic phosphorus was significantly (P<0.05) decreased at the parturition time and 2 weeks post-partum. The results revealed that Barki ewes showed profound physiological alterations during the transition period which are not necessarily indicative of a disease, but reflect physiological variations. Therefore, metabolic profile test was needed to determine the nutritional status of Barki ewes, and to take the possible preventive measures that increase ewes' productivity and predict health disorders.

Keywords: Hemato-biochemical profile; Transition period; Barki sheep.

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INTRODUCTION

Transition period in ewes, 4 weeks around the time of lambing, is physiologically stressful and is considered as a crucial stage in the production cycle of sheep (Pastrana et al., 1991). During that period, significant metabolic and immunological challenges occur to meet the requirements of the animal, as approximately 75 – 80% of the fetal growth occurs in the last 4-6 weeks of pregnancy, with consequent significant productive and reproductive disorders (Sormunen-Cristian and Jauhiainen, 2001; Overton and Waldron, 2004; Balıkcı et al., 2007).

The stress of parturition, the onset of lactation, and the high turnover of fluids, salts and soluble organic materials, are the main stressful conditions for ewes at the transition period (Sormunen-Cristian and Jauhiainen, 2001; Guo et al. 2007; Constable et al., 2017). Thus, minerals' metabolism particularly calcium and phosphorus, undergoes substantial changes to maintain colostrum and milk synthesis (Yokus et al., 2004). During peri-partum period and lactation, the maternal stores are in charge for providing energy needed for production, reducing the feed intake with a negative energy balance, mobilizing the body fat, and increasing both non-esterified fatty acid (NEFA) and ketone bodies in plasma (Maas and Pearson 2009; Rukkwamsuk 2010). This information is very crucial to guarantee the metabolic and nutritional needs of ewes during early lactation (Antunović et al., 2002). Therefore, blood metabolic profile, a rapid diagnostic test, helps to monitor the nutritional and metabolic states of transitional ewes and mitigate the resulting metabolic and hormonal alterations (Van Saun, 2000).

Leptin, the hormone of energy expenditure, acts on regions of the brain involved in the regulation of energy metabolism. It participates in the co-ordination of metabolism during transition period because of its role in regulating food intake and energy deposition. The low level and/or absence of leptin in blood at transition period signals to the central nervous system that a state of energy insufficiency prevails in the periphery. Thus, many adaptations are shared, including depressed reproductive and immune functions and increased metabolic efficiency (Vernon et al., 2002).

Barki sheep, named after the Libyan province Barka, plays a vital role in the livelihood of peoples in North-Western Coastal Zone of Egypt, which is the home tract of this breed in the country. This breed extends from the eastern provinces of Libya to the west of Alexandria in Egypt. It dominates the north western desert of Egypt with population of 470,000 heads and is known to adapt well to the harsh desert conditions and scarce vegetation, including poor feeding, and heat stress (Ahmed, 2008).

The data regarding blood metabolic profile and significance of monitoring the serum leptin in Barki ewes during transition period are still lacking. Most of the available data describing the metabolism during the transition period was carried out in other sheep breeds and not in Barki ewes. Thus, more studies are required to capture the dynamic changes in the transition period in such breed. Hence, the present study was designed to gain detailed information on the changes in the hemato-biochemical profile of the healthy Barki ewes during the transition period.

MATERIALS AND METHODS

Animals

A total of fifteen healthy pregnant Barki ewes (age, 4.9 ± 0.7 years old; weight, 38.5 ± 4.9 Kg) were selected for the current study. The selected ewes were raised in semi-open shaded pens at Mariut Research Station, Desert Research Center, Alexandria, Egypt in February 2018. The investigated ewes were subjected to thorough clinical examination according to the standard protocols (Constable et al., 2017). Accordingly, all ewes were selected to fulfill the following criteria: they were considered clinically healthy after clinical examination, were free from any nutritional and metabolic disorders or any evidence of other systemic diseases. All studied ewes were regularly dewormed twice a year, prior to spring turn out and again in the fall, according to an effective worming program. The last deworming was carried out 3 months before the start of the study using Doramectin (Dectomax Injectable solution 1 % m/v, Zoetis, Zoetis South Africa Ltd., 6th Floor, North Wing, 90 Rivonia Road, Sandton, 2196) at a dose rate of 200 µg kg⁻¹ body weight as a subcutaneous injection. The pregnancy status of the selected ewes was assessed using abdominal ultrasonography. The ewes were fed green fodder (green herbage, grass and remnant of plant, berseem and darawa), when available in addition to a concentrate ration (cottonseed cake, maize, wheat or rice bran, calcium carbonate, and sodium chloride). At two months of pregnancy, each ewe received 250 gm of concentrate twice a day, and increased up to 375 gm twice a day during the last 4 weeks of pregnancy according to National Research Council (NRC, 1985), while water was always available *ad libitum*.

All investigation procedures were performed in accordance with the guidelines of Mansoura University, Mansoura, Egypt and approved by Animal Welfare and Ethical Committee, Faculty of Veterinary Medicine, Mansoura University, code No. R/13.

Blood samples

Three blood samples (5 mL each) were collected from investigated ewes at each of the five different time points; 4 and 2 weeks prior the estimated date of delivery (EDD), then at the parturition time, then 2 and 4 weeks post-partum via jugular vein puncture. The first blood sample was collected in EDTA containing vacutainer tube to assess the complete blood picture using automatic blood cell counter (Exigo–Veterinary Hematology system, Boule Medical AB, Sweden).The second blood sample was collected in sodium fluoride containing vacutainer tube and centrifuged at 3000 rpm for 15 minutes to separate the plasma for determination of the concentration of glucose by spectrophotometer using a commercial test kits (Chronolab chemicals, Barcelona, Spain). The third blood sample was collected in a plain tube and centrifuged at 3000 rpm for 15 minutes to separate the serum, which was kept frozen at - 20 ℃ for subsequent biochemical analysis. The serum biochemical parameters were spectrophotometerically analyzed using commercial test kits according to standard protocol of supplier; cholesterol and triglycerides (Chronolab chemicals, Barcelona, Spain); β-hydroxylbutyrate (BHBA) (Ben Chemicals, Pakistan); total protein, albumin, calcium, inorganic phosphorus, and magnesium (BioMed, Egypt). Leptin was analyzed using sheep leptin ELIZA test kits supplied by SinoGeneclon (SinoGeneclon, Hangzhou, China). The serum NEFA was determined chemically according to the standard method (Schuster, 1979).

STATISTICAL ANALYSIS

Statistical analyses were carried out using a statistical software program (SPSS, version 21, Inc., Chicago, USA). Normal distribution of variables was tested with the Shapiro Wilks test. Data were normally distributed; therefore, mean and standard deviation were statistically analyzed and presented. Repeated measures ANOVA was used to check the assumption of sphericity using Mauchly's test of sphericity, where the P-value was > 0.05, indicating that sphericity was met. Thus, the Sphericity Assumed in tests of within-in-subjects effects was used to assess the statistical significant effect of time for the different five time points. For this test, the results were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Clinically, the investigated ewes demonstrated normal laboring of a single lamb for each and didn't express any detectable clinical alterations throughout the study period and remained clinically healthy.

In the investigated Barki ewes, red blood cells (RBCs) count and packed cell volume (PCV) were significantly ($P < 0.05$) decreased at 2 weeks before the EDD as well as at the parturition time when compared with other time points (Table 1). These findings may be caused by the stress related to parturition and lactation (Tharwat et al., 2015; Manat et al., 2016). On the other side, mean corpuscular volume (MCV) was significantly ($P < 0.05$) decreased at 4 weeks post-partum in comparison with other time points (Table 1), indicating a state of iron deficiency (microcytosis), where the cell undergoes an extra division due to insufficient hemoglobin concentration (Tharwat et al., 2015). Neutrophil count and neutrophil/lymphocyte ratio were significantly ($P < 0.05$) increased at 2 weeks post-partum when compared with other time points (Table 1), which may be attributed to lambing and lactation stress. Such stress stimulates the secretion of adrenocorticotropic hormone, which in turn induces the adrenal cortex to produce glucocorticoids that participate in the mobilization of granulocytes from body pool into peripheral circulation. These findings were in agreement with those previously reported in goats (Adenkola et al., 2011; Tharwat et al., 2015) and dairy cattle (El-Ghoul et al., 2000). Meanwhile, the white blood cells (WBCs) count, hemoglobin (Hb) level, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes count, monocytes count, eosinophils count, and band cells count showed non-significant changes among the five different time points (Table 1).

The level of BHBA and concentration of NEFA increased significantly at 2 weeks before EDD, at the parturition time, and 2 weeks post-partum when compared to 4 weeks before EDD then returned to the basal level at 4 weeks post-partum (Table 2). This may be attributed to the disturbance of carbohydrate and fat metabolism that occurs at transition period, with a subsequent hypoglycemia and tissue lipolysis, releasing long chain fatty acids which are converted by the liver into ketone bodies (LeBlanc, 2006). In the period of early lactation, increased lipolysis occurs at

high rates with the help of insulin resistance. Thus, the net quantity of NEFA is substantially higher than the amount that can be converted in the liver (Doepel et al., 2002; Roche et al., 2013). Furthermore, dry matter intake decreased gradually in studied ewes during the last days of pregnancy with the associated negative energy balance and body fat mobilization, resulting in an increase in the level of ketone bodies, including BHBA as well as in the concentration of NEFA in plasma (Bertics et al., 1992; Maas and Pearson 2009; Rukkwamsuk 2010; Manat et al., 2016). Moreover, the increased concentration of NEFA, at early lactation period, is beneficial for the animals to maximize milk synthesis with less glucose consumption (Mathews et al., 2016).

Table 1. Complete blood count (Mean ± SD) in Barki ewes (n =15) during transition period

| | Before EDD | | At parturition time | Post-partum | | P - value |
	4 Weeks	2 Weeks		2 Weeks	4 Weeks	
WBCs (×10 ^{9}/L)	9.6 ± 1.5	9.9 ± 2.2	10.6 ± 3.6	9.7 ± 1.4	9.4 ± 1.3	**0.693**
RBCs (× 10 ^{12}/L)	8.9 ± 0.5	7.0 ± 0.9 **	7.1 ± 0.6 **	8.9 ± 0.7	8.5 ± 0.9	**0.003**
Hb (g/dl)	10.3 ± 0.7	9.9 ± 0.6	10.4 ± 0.7	9.8 ± 1.0	10.0 ± 0.8	**0.184**
PCV%	31. 5 ± 1.5	28.4 ± 2.0 **	26.1 ± 3.5 **	31.3 ± 2.1	32.5 ± 2.2	**0.001**
MCV (fL)	34.4 ± 2.0	34.6 ± 2.3	35.5 ± 2.0	34.4 ± 1.8	32.0 ± 2.4 **	**0.001**
MCH (pg)	11.2 ± 0.3	11.2 ± 0.6	11.2 ± 0.5	11.0 ± 0.5	11.1 ± 0.4	**0.817**
MCHC (g/dl)	32.9 ± 0.9	32.3 ± 1.0	32.2 ± 1.0	32.1 ± 1.4	32.3 ± 1.2	**0.337**
Lymphocyte (× 10 ^{9}/L)	7.1 ± 1.2	7.2 ± 1.6	6.3 ± 1.9	6.3 ± 1.6	6.3 ± 1.5	**0.255**
Monocyte (× 10 ^{9}/L)	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	**0.331**
Neutrophil (× 10 ^{9}/L)	2.1 ± 1.0	2.3 ± 1.1	2.6 ± 1.1	3.7 ± 1.7 **	1.9 ± 0.7	**0.001**
Eosinophil (× 10 ^{9}/L)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.4	0.3 ± 0.1	0.2 ± 0.0	**0.341**
Band cells	0.2 ± 0.0	0.6 ± 0.7	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	**0.431**

EDD, expected date of delivery; WBCs, white blood cells; RBCs, red blood cells; Hb, hemoglobin; PCV, paced cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 2. Blood metabolic profile parameters (Mean ± SD) in Barki ewes (n =15) during the transition period

| | Before EDD | | At parturition time | Post-partum | | P - value |
	4 Weeks	2 Weeks		2 Weeks	4 Weeks	
BHBA (mmol/l)	0.8 ± 0.1	1.4 ± 0.5 *	1.4 ± 0.3 *	1.3 ± 0.3 *	0.8 ± 0.2	**0.021**
NEFA (mmol/l)	1.6 ± 0.3	2.1 ± 0.1 *	2.2 ± 0.1 *	2.1 ± 0.1 *	1.7 ± 0.2	**0.035**
Cholesterol (mmol/l)	2.8 ± 0.2	3.3 ± 0.1 *	3.4 ± 0.1 *	2.8 ± 0.1	2.8 ± 0.0	**0.027**
Triglyceride (mmol/l)	0.7 ± 0.1	1.0 ± 0.1 **	0.9 ± 0.1 **	0.4 ± 0.2	0.4 ± 0.2	**0.001**
Leptin (ug/l)	17.0 ± 1.9	16.6 ± 2.2	16.1 ± 1.9	12.4 ± 1.1 **	12.3 ± 1.4 **	**0.001**
Glucose (mmol/l)	3.6 ± 0.6	4.2 ± 0.1	5.1 ± 1.3 *	3.2 ± 0.1 *	4.2 ± 0.1	**0.021**
Total protein (g/l)	61.6 ± 5.7	63.3 ± 6.3	65.7 ± 4.9	77.8 ± 6.4 *	74.6 ± 7.4 *	**0.015**
Albumin (mmol/L)	30.5 ± 2.3	31.3 ± 2.6	33.6 ± 5.3	38.2 ± 6.1 *	39.6 ± 4.3 *	**0.012**
Calcium (mmol/L)	2.3 ± 0.4	2.2 ± 0.5	1.2 ± 0.2 *	1.1 ± 0.2 *	2.2 ± 0.5	**0.028**
Inorganic phosphorous (mmol/L)	2.4 ± 0.1	2.6 ± 0.3	1.8 ± 0.2 **	1.7 ± 0.2 **	2.8 ± 0.2	**0.005**
Magnesium (mmol/L)	1.4 ± 0.7	1.2 ± 0.6	1.2 ± 0.7	1.1 ± 0.9	1.2 ± 0.9	**0.128**

EDD, expected date of delivery; BHBA, β-hydroxybutyric acid; NEFA, non-esterified fatty acid.

The level of both cholesterol and triglyceride increased 2 weeks before EDD and at the parturition time when compared with 4 weeks before EDD, then returned to the basal level at 2 weeks and 4 weeks post-partum (Table 2). These results reflect their increased hepatic synthesis as a result of an increase in the activities of both lipoprotein lipase and hepatic lipase, which are responsible for the catabolism of the very low density lipoprotein (Watson et al., 1993).

The leptin level showed a significant (P<0.05) decrease at both 2 weeks and 4 weeks post-partum compared to other time points (Table 2). This may be attributed to the energetic cost of lactation by delivering milk, together with negative energy balance as previously reported (Block et al., 2001). Therefore, undernourished animals alleviate the level of leptin, while increasing the level of cortisol, which contributes to metabolic adaptations and feeding behavior (Vernon et al., 2002; Chilliard et al., 2005).

Plasma glucose level increased significantly (P<0.05) at the parturition time, then decreased significantly (P<0.05) 2 weeks post-partum when compared with other 3 time points (Table 2). The increased glucose level at the parturition time may be attributed to the metabolic changes towards gluconeogenesis as a result of the hormonal changes that occur at such time, promoting gluconeogenesis and glycogenolysis (Vazquez-Annon et al., 1994). On the other side, the decreased glucose level at 2 weeks post-partum can be attributed to the high energy demands needed for lactation (Mohammadi et al., 2016). The glucose level increased again 4 weeks post-partum and returned to the basal level, which could be attributed to the recovery of feed intake and the decreased state of negative energy balance (Cal-Pereyra et al., 2015).

Total protein level increased significantly (P<0.05) at both 2 weeks and 4 weeks post-partum when compared with other time points (Table 2). In late pregnant ewes, the total protein level tends to decrease as all fetal protein was synthesized from amino acids derived from dam and the growth of fetus increases exponentially, reaching a highest level, especially the muscles (Jainudeen and Hafez, 2000). However, at early lactation period in studied ewes, total protein level increased significantly (P<0.05) due to the increase in immunoglobulin needed for synthesis of colostrum (Shetaewi and Daghash, 1993; Tharwat et al. 2012). In the studied Barki ewes, albumin level tends to decrease during the peri-partum period in comparison with those recorded at the parturition time and at both 2 and 4 weeks post-partum (Table 2), suggesting a case of altered hepatocellular function and fatty liver in response to negative energy balance (Nehra et al., 2001; Tharwat et al., 2015). Moreover, a proportion of the presenting albumin in the circulating blood is correlated with calcium level. Thus, the decreased calcium level in the studied ewes may be another explanation for the low albumin level (Goff, 2000).

Calcium and inorganic phosphorus levels decreased significantly (P<0.05) at the parturition time and at 2 weeks post-partum when compared with other time points (Table 2), indicating profound physiological changes in both elements, but are not necessarily indicative of a disease. The decreased calcium level at transition period is a physiological state that reflects the onset of colostrum/milk production. Furthermore, the absorption of both calcium and phosphorous may be decreased from the intestine due to a fatty liver and a decrease in the number of 1,25-dihydroxyvitamin D receptors (Goff, 2000). However, the magnesium level showed non-significant changes among the five different time points (Table 2).

CONCLUSION

Barki ewes showed profound physiological alterations in certain analytes during transition period particularly β-hydroxy butyric acid (BHBA), non-esterified fatty acid (NEFA), cholesterol, triglyceride, leptin, glucose, total protein, albumin, calcium, and inorganic phosphorus, reflecting physiological variations. The results indicated that the metabolic profile test was required to determine the nutritional status of Barki ewes, and to take the potential preventive measures that increase ewes' productivity and predict health disorders.

CONFLICT OF INTEREST

None of the authors of this paper have a financial or personal relationship with other people or organizations which could inappropriately influence or biased the content of the paper.

STATEMENT OF ANIMAL RIGHTS

All Institutional and National Guidelines for the care and use of animals were followed.

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