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The Relationship of Mitochondrial-derived Peptide (MOTS-c) and Brain Mitochondrial Carrier Protein 1 (BMCP1) Response in Sheep with Some Physiological Parameters

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ABSTRACT

Background: Mitochondria are organelles called power plants of the cell, which are found in eukaryotic cells and are responsible for the production of energy needed by the cells through aerobic respiration. Mitochondrial dysfunction causes endocrinological, cardiovascular, metabolic and neurodegenerative diseases. The aim of this study was to examine the effects of body condition score (BCS), lactation, pregnancy, age and gender on Mitochondrial-Derived Peptide (MOTS-c) and Brain Mitochondrial Transporter Protein 1 (BMCP1) levels in Akkaraman sheep.

Methods: Sheep are classified into 3 categories according to BCS (BCS<2, BCS=3-3.5, BCS≥4). In creating groups in terms of BCS, it was determined by the palpation method applied to the waist area. The sheep included in the study were divided into 4 groups: early lactation, early pregnancy, late pregnancy (dry period) and age (female and male sheep).1st group, ewes in the first period of pregnancy (on the 100th day of the first pregnancy) (n = 30), 2nd group, ewes in the early lactation period (n = 30), 3rd group, ewes in the dry period (the last two months of pregnancy) (n = 30) and in the 4th group, <2 (young) (n=30), 2-8 (adult) (n=30), 8> (old) (n=30), male and female sheep (n=30) of different ages. =180) total of 270 animals. Blood samples were taken from the jugular vein of the sheep before the morning feeding. MOTSc and BMCP1 levels were determined in blood serum samples by ELISA method.

Result: In the study, serum MOTS-c and BMCP1 levels in sheep during lactation and pregnancy were determined to be lowest in the VKS<2 group and highest in the VKS ≥4 groups. Likewise, in age and gender groups, the highest level was detected in young lambs and yearlings in the VKS<2 group, while the highest level was detected in old sheep and rams in the VKS ≥4 group. As a result, it was determined that different age, gender, pregnancy and lactation periods affected serum MOTS-c and BMCP1 levels depending on the change in BCS (P<0.05). It was concluded that MOTS-c and BMCP1 may be useful parameters in the evaluation of mitochondrial function, energy metabolism and metabolic profile.

Key words: Brain mitochondrial transporter protein 1 (BMCP1), Mitochondrial-derived peptide (MOTS-c), Physiological parameters, Sheep.

INTRODUCTION

Mitochondria are the necessary organelles of the cell for apoptosis and cell signaling, along with the synthesis of ATP, heme, cholesterol and phospholipids through oxidative phosphorylation responsible for energy production (Pfanner *et al.*, 2019). Mitochondrial dysfunction causes endocrinological, cardiovascular, metabolic and neurodegenerative diseases (Nunnari and Suomalainen, 2012).

Uncoupling proteins (UCP) are regulators of mitochondrial membrane function in thermogenesis, oxidative stress, energy balance.Brain mitochondrial carrier protein 1 (BMCP1, SLC25A14, UCP5) is a mitochondrial decoupling protein, a member of the SLC25 family, that regulates mitochondrial function and oxidant production in neurons by catalyzing proton leaks across the inner mitochondrial uncoupling protein (Mizuno *et al.*, 2000).

Mitochondrial-derived peptide (MOTS-c, Mitochondrial open reading frame of the 12S rRNA type-c), called mitochondrial hormone, is a 16 amino acid peptide encoded as a short open reading frame (sORF) in mitochondrial DNA (mtDNA). MOTS-c binds and inhibits the folate chain and has a regulatory role in metabolic homeostasis by restarting purine biosynthesis and activating adenosine monophosphate-activated protein kinase (AMPK) (Lee *et al.*, 2015; Kim *et al.*, 2019).

Akaraman sheep breed, which is the subject of our study, is among the indigenous breeds of sheep specific to

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Türkiye country. It is a breed resistant to harsh climatic conditions and diseases due to its oily tail. In addition, it is easy to manage, has a docile feature and has a high ability to benefit from poor pastures. It has the ability to walk long distances and benefit from poor pastures (Özcan, 1990). Pregnancy and lactation periods are important periods in which intense physiological and hormonal changes are experienced and high energy needs are tried to be met (Bayraktar, 2020). There are no studies examining the level of MOTS-c, called Mitochondrial hormone, which allows the evaluation of mitochondrial changes in the blood and brain during lactation and pregnancy in sheep and the level of BMCP-1, which is concentrated in the hypothalamus. In this study, the effect of early and dry periods of pregnancy on serum MCP-1 levels in Akkaraman native sheep was examined.

MATERIALS AND METHODS

Ethics committee approval was obtained from the Local Ethics Committee before starting the current study (Decision date and number: 27.03.2019/01). The study was carried out in line with ethical principles and rules by protecting animal welfare and rights. The animal material of the study comprised a total of 270 Akkaraman sheep, which were randomly selected from a homogeneous flock and whose total body weight average was equal in each group.Sheep are classified into 3 categories according to BCS (BCS<2, BCS=3-3.5, BCS≥4) in the study. In creating groups in terms of BCS, it was determined by the palpation method applied to the waist area (Khan, 1993). In creating groups in terms of BCS, it was determined by the palpation method applied to the waist area. The sheep included in the study were divided into 4 groups: early lactation, early pregnancy, late pregnancy (dry period) and age (female and male sheep). 1st group, ewes in the first period of pregnancy (on the 100th day of the first pregnancy) (n = 30), 2nd group, ewes in the early lactation period (n = 30), 3rd group, ewes in the dry period (the last two months of pregnancy) (n = 30) and in the 4th group, <2 (young) (n=30), 2-8 (adult) (n=30), 8> (old) (n=30), male and female sheep (n=30) of different ages. =180) total of 270 animals. Blood samples were taken from the jugular vein of the sheep before the morning feeding. MOTSc and BMCP1 levels were determined in blood serum samples by ELISA method.The study was carried out in a sheep farm in Demirözü, Bayburt, which was raised under intensive conditions and whose records were followed regularly (N: 40° 9′ 45.1332″; M: 39° 53′ 34.2564″). Blood samples were taken from the vena jugularis before the morning feeding of the sheep. MOTS-c and BMCP1 levels were determined in blood serum samples by ELISA method. Sera obtained from blood samples were stored at -80°C. Serum MOTS-c and BMCP1 levels were determined by the ELISA method. During the research period, care was taken to ensure that the environment (Ambient temperature 20°C, relative humidity 75%) and feed factor (Table 1) were the same. Analysis of the content of the feeds in this study was analyzed according to standard AOAC methods (AOAC, 2005).

Collection of serum samples

Blood sample of 10 ml was taken from the neck vein (*vena jugularis*) of the sheep belonging to the study groups into tubes without anti-coagulant (VACUETTE ® TUBE 9 ml Z Serum Clot Activator). The blood taken into the tubes was centrifuged for 10 min at 3000 rpm in a refrigerated centrifuge (NF 1200R, NÜVE, Ankara, TURKEY) in the laboratory. The obtained serums were transferred to sterile Eppendorf tubes. It was stored in deep freezers set to -80°C until laboratory analyses were performed.

Measurement of serum MOTS-c level

The minimum detectable concentration of the kit used to measure the MOTS-c level in sheep serum samples from horses obtained as a result of the research was reported as 1 ng/mL. ELISA kit species-specific Sheep Mitochondrial Open Reading Frame Of The 12S rRNA-c (MOTS-c) ELISA Kit, Product code: SG-70775, CHINA), determination 6 ng/mL-200 ng/mL, intra assay coefficient 8.0%, inter the assay coefficient was used in accordance with the procedure specified in the manufacturer's catalogue, using 10.0%.

Measurement of serum BMCP 1 level

The minimum detectable concentration of the kit used to measure BMCP-1 level in sheep serum samples belonging to horses obtained as a result of the research was reported as 7.2 pg/ml. ELISA kit, species-specific Sheep brain mitochondrial carrier protein 1 (BMCP 1) ELISA Kit product code: SG-70775, CHINA), determination 37.5 pg/ml-1000 pg/ml, intra-assay coefficient % 8.0 inter-assay coefficient %in accordance with the procedure specified in the manufacturer's catalog was studied using 10.0.

Statistical analysis

The normal distributions of the data obtained as a result of the experiment (MOTSc, BMCP-1) were checked. Statistical analyses of the effects of diet and temperature on hormone levels are given below it was performed using the general linear model (GLM).

$$Yijk = \mu + DI + eijk$$

Here,
Yijk = Observation.
μ = Average.
DI = Interaction effect.
eijk = Experimental error effect.

RESULTS AND DISCUSSION

Mitochondria are an important organelle that plays a role in the production of energy and meeting the needs of the cell. That is why the mitochondrial organelles are called the power plants of the cell (Pfanner *et al.*, 2019). Pregnancy and lactation is an important process that involves physiological and hormonal changes (Bayraktar, 2020). Therefore, pregnancy and lactation periods are important physiological periods during which intense mitochondrial activity takes

place (Sormunen-Cristian, 2001). The mammary gland is the tissue in which rapid physiological changes occur with lactation, both during pregnancy and postpartum period. It is under the control of many hormones, as well as adipokines such as apelin, in meeting the metabolic energy needs required for energy metabolism in lactation (Kırbaş *et al.*, 2024).

Mitochondria-derived peptides (MDPs) are a set of peptides encoded by mitochondrial DNA and have similar functions to mitochondria. There are 3 types of MDP's, Humanin (Hashimoto *et al.*, 2001), MOTS-c (Lee *et al.*, 2015) and small humanin-like peptide 1-6 (SHLP 1-6). MOTS-c, which forms the basis of our research, regulates metabolic functions in the body and most importantly converts glucose into usable energy (Kim *et al.*, 2019). It also targets skeletal muscle and increases glucose metabolism (Lee *et al.*, 2015). Serum MOTS-c levels were highest in lactation and dry periods of pregnancy and lowest in sheep groups on the 100th day of pregnancy, respectively ($p<0.05$). Likewise, when the gender and age data are examined, in line with the data obtained, there is no study examining the serum MOTS-c level in sheep in our study.According to the results of the study, Age, Gender, BCS, Age * Gender, Age * BCS, Age * Gender * BCS interactions were found to be statistically significant (Table 2), ($p<0.05$). As a result of the study, BCS, Lactation Period and BCS*Lactation interactions were found to be statistically significant (Table 2) ,($p<0.05$). Mean serum MOTS-c levels were determined in the BCS=<2; groups during the highest dry period, while the BCS= 3-3.5 groups during the lowest early lactation period were determined (Table 2).When the findings obtained from the study were examined, the interactions of Age, Gender, BCS, Age * Gender, Age * BCS, Age * Gender * BCS were found to be statistically significant (Table 3), ($p <0.05$). When average serum MOTSc levels were examined in terms of gender and age, in female sheep and male sheep BCS=≥4 groups between 2-5 years of age; The lowest level was detected in BCS=<2 groups aged ≥8 years (Table 3).

BMCP1 and MOTS-c have an important role in the regulation of cellular and energy metabolism and metabolic homeostasis (Lee *et al.*, 2015). BMCP1 expression in mammary epithelial cells during lactation is regulated by insulin (Yonezawa *et al.*, 2009). The average serum BMCP

Table 1: Nutrient composition of feed used in the study (%).

Feed composition	Kangal
Barley	65.0
Wheat bran	7.0
Soybean meal	20.5
Dicalcium phosphate	1.0
Salt	0.5
Premix	0.5
Chemical composition %	
Dry matter	90.60
Crude protein	16.99
Crude ash	5.78
ADF	11.26
NDF	32.72
ME Kcal/kg	2620

1 kg vit.-min. Premix contains vitamin A, 7,000,000 IU; vitamin D3, 1,000,000; vitamin E, 30,000; Mn, 50,000 mg; Zn, 50,000 mg; Fe, 50,000; Cu, 10,000 mg; I, 8,000 mg; Co, 200 mg; Se, 150 mg; and Mg, 100 mg.

Table 2: Mean serum MOTSc values (ng/ml) and statistical comparisons (Mean±SH) of the study groups.

BCS/Lactation period	Early lactation	Early gestation	Late gestation (Dry period)
BCS=<2	1.57^{e}	1.88^{c}	2.17^{a}
BCS= 3-3.5	1.46^{g}	1.77^{d}	1.89^{c}
BCS=≥4	1.52^{f}	1.82^{d}	2.05^{b}
SEM		0.02	
Source of variation (*P*-values)			
BCS		0.00	
Lactation period		0.00	
BCS*Lactation period		0.00	
Main effect means (BCS)			
BCS=<2		1.87^{a}	
BCS= 3-3.5		1.71^{c}	
BCS=≥4		1.80^{b}	
SEM		0.01	
Main effect means (Period)			
Early lactation		1.51^{c}	
Early gestation		1.82^{b}	
Late gestation (Dry period)		2.04^{a}	
SEM		0.01	

*MOTS-c (Mitochondrial ORF of the 12S rRNA type-c), Means within a column showing different superscripts are significantly different ($p<0.05$): SEM = Standard error of the mean.

Table 3: Mean serum MOTSc values (ng/ml) and statistical comparisons (Mean±SH) of the study groups.

BCS/Gender	Female/Age Old			Male/Age Old		
	2-5	5-8	≥8	2-5	5-8	≥8
BCS= <2	1.61^{h}	1.55^{i}	1.48^{j}	1.69^{g}	1.61^{h}	1.57^{ih}
BCS= 3-3.5	1.92^{c}	1.84^{d}	1.77^{f}	1.98^{b}	1.92^{c}	1.78^{ef}
BCS= ≥4	1.97^{b}	1.90^{c}	1.84^{d}	2.06^{a}	1.97^{b}	1.82^{ed}
SEM			0.02			
Source of variation (*P*-values)						
Age			0.00			
Gender			0.00			
BCS			0.00			
Age * Gender			0.02			
Age * BCS			0.04			
Gender * BCS			0.21			
Age *Gender * BCS			0.02			
Main effect means (Period)						
Age= 2-5			1.87^{a}			
Age= 5-8			1.80^{b}			
Age= ≥8			1.71^{c}			
SEM			0.01			
Main effect means (BCS)						
BCS= <2			1.58^{c}			
BCS= 3-3.5			1.87^{b}			
BCS= ≥4			1.93^{a}			
SEM			0.01			
Main effect means (Gender)						
Female			1.76			
Male			1.82			
SEM			0.01			

*MOTS-c (Mitochondrial ORF of the 12S rRNA type-c), Means within a column showing different superscripts are significantly different (p<0.05): SEM = Standard error of the mean.

Table 4: Mean serum BMCP-1 mean values (pg/ml) and statistical comparisons of study groups (Mean±SE).

BCS/ Lactation period	Early lactation	Early gestation	Late gestation (Dry period)
BCS= <2	1.61^{e}	1.93^{c}	2.25^{a}
BCS= 3-3.5	1.50^{g}	1.81^{d}	1.96^{c}
BCS= ≥4	1.56^{f}	1.86^{d}	2.10^{b}
SEM		0.02	
Source of variation (*P*-values)			
BCS		0.00	
Lactation period		0.00	
BCS* Lactation period		0.00	
Main effect means (BCS)			
BCS= <2		1.93^{a}	
BCS= 3-3.5		1.76^{c}	
BCS= ≥4		1.84^{b}	
SEM		0.01	
Main effect means (Period)			
Early lactation		1.56^{c}	
Early gestation		1.87^{b}	
Late gestation (Dry period)		2.10^{a}	
SEM		0.01	

*BMCP1 (brain mitochondrial carrier protein 1), Means within a column showing different superscripts are significantly different (p<0.05): SEM = Standard error of the mean.

Table 5: Mean serum BMCP-1 mean values (pg/ml) and statistical comparisons of study groups (Mean±SE).

BCS/Gender	Female/Age Old			Male/Age Old		
	2-5	5-8	≥8	2-5	5-8	≥8
BCS=<2	1.57	1.51	1.45	1.63	1.57	1.50
BCS= 3-3.5	1.86	1.81	1.77	1.93	1.87	1.83
BCS=≥4	1.92	1.86	1.84	2.02	1.96	1.86
SEM			0.02			
Source of variation (*P*-values)						
Age			0.00			
Gender			0.00			
BCS			0.00			
Age * Gender			0.14			
Age * BCS			0.82			
Gender * BCS			0.65			
Age * Gender * BCS			0.36			
Main effect means (Age)						
Age=2-5			1.82[a]			
Age=5-8			1.76[b]			
Age=≥8			1.71[c]			
SEM			0.01			
Main effect means (BCS)						
BCS=<2			1.54[c]			
BCS= 3-3.5			1.84[b]			
BCS=≥4			1.91[a]			
SEM			0.01			
Main effect means (Gender)						
Female			1.73			
Male			1.79			
SEM			0.01			

*BMCP1 (brain mitochondrial carrier protein 1), Means within a column showing different superscripts are significantly different ($p<0.05$): SEM = Standard error of the mean.

1 levels of the study groups were found to be the highest in the late lactation period BCS ≤2 groups; the lowest level was found in the early lactation period BCS=3-3.5 group (Table 4). When the mean serum BMCP 1 levels of our study were examined, it was found to be statistically significant in terms of BCS, Lactation Period, BCS* lactation period (Table 4), ($p<0.05$). As a result of the study, when the average serum BMCP 1 levels were examined, the highest was determined in the BCS≥4 groups in the 2-5 age group, while the lowest was detected in the BCS≤2 groups in the 8≥ age group. In line with the findings, Age, Gender, BCS were found to be statistically significant ($p<0.05$), but the interaction between each other (Age*Gender*BC) was not found to be significant ($p>0.05$) (Table 5). In a study conducted by Kondou *et al.*, (2000), it was reported that BMCP1 mRNA expression in rats increased 2 times in the first week after birth and increased 3.2 times in the 10th week and 28th week. In our study, the BMCP1 level of the gestational period was found to be higher in the sheep in the dry period group than in the sheep on the 100th day of pregnancy ($p<0.05$). In line with the results obtained, there is no study examining the BMCP1 level in sheep, although it is limited, but it is consistent with the result of a similar study (Kondou *et al.*, 2000). As the reason for the high BMCP1 level in the dry period, we think that 70-80% of rapid fetal growth is due to increased energy needs due to the formation of the last 6 weeks of pregnancy.

CONCLUSION

In conclusion, this study showed that the effects of pregnancy, lactation, gender and age significantly affect serum BMCP1 and MOTS-c levels in Akkaraman sheep with different BCS ($p<0.05$). It was concluded that it will be a new, useful biomarker with prognostic importance that can be measured in serum in the evaluation and follow-up of BMCP1 and MOTS-c-related diseases due to its physiological role in mitochondrial function and energy and metabolic homeostasis.

Conflict of interest

The authors declare that there is no conflict of interest.

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