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The Relationship of Blood Asprosin Levels and Biochemical Parameters in Pregnant Cows

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Abstract: In this study, it was aimed to determine asprosin levels in pregnant and non-pregnant cows and to determine the relationship between some biochemical parameters and asprosin levels. For this purpose, 60 Simmental cows were divided into two groups as pregnant (n=30) and non-pregnant cows on the postpartum 15th day (n=30), and asprosin levels and biochemical parameters were measured by taking blood from the cows once. Measured biochemical parameters were Alkaline phosphatase (ALP), Amylase, Creatine kinase myocardial band (CK-MB), Creatine kinase N-acetyl cysteine (CK-NAC), Gamma glutamyl transferase (GGT), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT)), Direct bilirubin, Total bilirubin , Calcium (Ca), Choline (CHO), Creatine, Glucose, Total protein, Urea, Albumin, Iron, Phosphorus, Lactate dehydrogenase (LDH-P), High-density lipoprotein cholesterol (HDL-C)), Low-density lipoprotein (LDL), Triglyceride, Magnesium (Mg) and Unsaturated iron binding capacity (UIBC). Asprosin levels were measured using commercial ELISA kits and biochemical parameters were measured in an autoanalyzer. Asprosin levels and biochemical parameters were compared both within and between groups and the results were evaluated. As a result of the data obtained, the amount of direct bilirubin and total bilirubin in non-pregnant cows was found to be higher than that of pregnant cows, while the amounts of CHO, HDL-C, LDL and asprosin were found to be lower. When asprosin and biochemical parameters of pregnant cows were compared, a positive correlation was found between asprosin and albumin, direct bilirubin, total bilirubin and creatinine levels. In non-pregnant cows, there was a negative correlation between asprosin and amylase and phosphorus, and a positive correlation between asprosin and creatine and glucose. As a result, it was concluded that asprosin levels are higher in pregnant cows and there may be negative or positive correlations between this hormone and biochemical parameters. However, it would be beneficial to carry out more comprehensive studies on the subject in order to reach more definite conclusions.

Gebe İneklerde Kan Asprosin Düzeylerinin Biyokimyasal Parametrelerle İlişkisi

Anahtar Kelimeler
İnek,

Öz: Bu çalışmada, gebe ve gebe olmayan ineklerde asprosin düzeylerinin tespit edilmesi, bazı biyokimyasal parametreler ile asprosin düzeyleri arasındaki ilişkinin belirlenmesi amaçlanmıştır. Bu amaçla 60 adet Simental ırkı inek, gebe olan (n=30) ve postpartum 15. günde (n=30) gebe

Asprosin, Biyokimyasal parametreler, Gebelik, Simental.

olmayan inekler olmak üzere iki gruba ayrıldı ve ineklerden bir kez kan alınarak asprosin düzeyleri ve biyokimyasal parametreler ölçüldü. Ölçülen biyokimyasal parametreler Alkalen fosfataz (ALP), Amilaz, Kreatin kinaz miyokardial band (CK-MB), Kreatin kinaz N-asetil sistein (CK-NAC), Gama glutamil transferaz (GGT), Aspartat Aminotransferaz (AST), Alanin aminotransferaz (ALT), Direkt bilirubin, Total bilirubin, Kalsiyum (Ca), Kolin (CHO), Kreatin, Glikoz, Total protein, Üre, Albümin, Demir, Fosfor, Laktat dehidrogenaz (LDH-P), Yüksek yoğunluklu lipoprotein kolesterol (HDL-C), Düşük yoğunluklu lipoprotein (LDL), Trigliserit, Magnezyum (Mg) ve Doymamış demir bağlama kapasitesi (UIBC)'dir. Asprosin düzeyleri ticari ELİSA kitleri kullanılarak, biyokimyasal parametreler ise otoanalizörde ölçüldü. Asprosin düzeyleri ve biyokimyasal parametreler hem grup içi hem de gruplar arası karşılaştırılarak sonuçlar değerlendirildi. Elde edilen veriler ışığında gebe olmayan ineklerin direkt biluribin ve total biluribin miktarı gebe ineklerden yüksek, CHO, HDL-C, LDL ve asprosin miktarları ise düşük bulundu. Gebe ineklerin asprosin ile biyokimyasal parametreleri karşılaştırıldığında ise asprosin ile albümin, direkt biluribin, total biluribin ve kreatin miktarları arasında pozitif yönde korelasyon bulundu. Gebe olmayan ineklerde ise asprosin ile amilaz ve fosfor arasında negatif korelasyon, asprosin ile kreatin ve glikoz arasında pozitif yönde korelasyon gözlendi. Sonuç olarak, gebe ineklerde asprosin düzeylerinin daha yüksek olduğu ve bu hormonla biyokimyasal parametreler arasında negatif veya pozitif yönde korelasyonlar olabileceği kanaatine varıldı. Ancak daha kesin sonuçlara varılabilmesi için konu ile ilgili daha kapsamlı çalışmaların yapılması faydalı olacaktır.

1. INTRODUCTION

Evaluation of blood parameters in cows refers to the analysis of blood biochemical components helpful in diagnosing and preventing metabolic and nutritional problems [1]. Monitoring the metabolic profile is more important when animals are sensitive to metabolic changes, such as in early lactation, considering the herd characteristics, geographical location and physiological condition of the animals [2].

Asprosin is a new peptide hormone released from white adipose tissue. Asprosin, encoded by two exons (exon 65 and exon 66) of the fibrillin 1 (FBN1) gene, was first described by Romere et al. [3] in a study on neonatal progeroid syndrome (NPS) patients. Asprosin, which release during periods of fasting, activates the G protein-cAMP-PKA pathway and increases glucose secretion in the liver [4-6]. Studies on humans and mice prove that recombinant asprosin injection is associated with insulin resistance due to elevation of blood glucose and insulin [4]. Therefore, many studies have focused on the association of asprosin with obesity and reported that asprosin concentrations increase in obese humans and mice [7-9]. Recent studies have reported that asprosin plays an essential role in metabolism and metabolic diseases [6]. In studies conducted with subjects with type 2 diabetes (T2DM), it has been reported that asprosin levels are higher than the control group and asprosin levels are positively correlated with insulin resistance in subjects with T2DM [10-12]. Polycystic ovary syndrome (PCOS) is a common metabolic and reproductive disorder associated with insulin resistance [4]. According to studies conducted on subjects with polycystic ovary syndrome, it has been reported that asprosin levels are higher than in the control group, and it is a candidate molecule for the determination of this disease in the future [4-13]. In addition to performing a glucogenic function, asprosin has been reported as a potential appetite stimulating hormone in the treatment of both obesity and diabetes [6]. Excessive weight gain and gestational diabetes during pregnancy cause pregnancy pathologies. Insulin resistance (IR), high blood glucose levels and hormonal disorders cause incorrect programming of the infant's energy metabolism [14]. When the asprosin level is examined in pathological pregnancies such as gestational diabetes, preeclampsia, severe preeclampsia, intrauterine growth retardation and fetal macrosomia, it is reported that there is a statistically significant increase compared to the control group, and a significant decrease is observed in those with intrauterine growth retardation [15]. As a result of these studies, asprosin is thought to be not only a potential biomarker for early diagnosis of type 2 diabetes mellitus and other metabolic disorders, but also a new therapeutic agent to counter the perinatal programming of childhood obesity [16].

There are not many publications in ruminants about Asprosin, which has been frequently examined in human medicine, especially in diabetes-related publications recently. It is thought that it will be useful to follow the data of this hormone, especially in monitoring the parameters related to the metabolic status of pregnant cows. Therefore in this study, it was aimed to determine the relationship between asprosin and biochemical parameters in pregnant and non-pregnant cows both within themselves and between groups, and to determine the relationship between them in the postpartum and pregnancy period. At the same time, it is aimed to evaluate the possibility of asprosin being a marker in the problems that may occur by determining the relationship with the changes in biochemical parameters in these processes.

2. MATERIAL AND METHOD

In this study, 60 simmental breed 2 and 4 years old cows in a farm located in Bingöl province in the eastern part of Turkey. The animals were fed year-round in semi-open pastures and a free-roaming barn with concentrate feed containing barley and a ration containing dry meadow grass, corn silage, alfalfa and hay. The age, lactation number, lactation period, daily milk yield, and previous disease information of the cows used as material were obtained.

The cows included in the study were divided into two groups as the cows that were pregnant in the 2nd and 4th months of pregnancy (n=30) Group I and the cows that completed the normal gestation period (n=30) as Group II. Cows in Group II were selected from clinically healthy cows with body condition scores varying from 3.5 to 3.8 and on the 15th postpartum day. Group 2 was formed as a control group by selecting clinically healthy animals on the 15th postnatal day. Blood samples were taken from the cows once in 10 ml tubes, then their serums were removed and the serums were kept at -80 ˚C.

Biochemical parameters and asprosin levels were measured in the collected blood serum.

Approval for the research was obtained from Bingöl University Experimental Animals Ethics Committee (Date and Number: 15/03/2022-E.54083).

2.1. Biochemical Analyzes

ALP, CK-MB, CK-NAC, GGT, AST, ALT, Direct bilirubin, Total bilirubin, Ca, CHO, Creatine, Glucose, Total protein, Urea, Albumin, Iron, Phosphorus, LDH-P, HDL-C, LDL , Triglyceride, Mg and UIBC analyzes were performed using an autoanalyzer (SIEMENS, ADVIA 2400, USA) [17]. These analyzes were performed in Fırat University Veterinary Faculty Animal Hospital Diagnostic Laboratory.

2.2. Measuring Asprosin Levels

Asprosin levels in the blood serum were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Sunred Bovine Asprosin Kit, Shanghai) and reading the levels on an ELISA reader (Bio Tek Instruments, USA) as described [7].

2.3. Statistical Analysis

First of all, descriptive statistics of the data obtained at the end of the research were calculated. It was analyzed and evaluated whether the data were normally distributed and whether they met the parametric test assumptions for all the examined features. Then, the independent student t test was used for comparisons between groups for all parameters. The Pearson method was used to calculate the correlation coefficients between Asprosin and other parameters within each group and overall [18]. SPSS program was used in all statistical analyzes [19].

3. RESULTS

As a result of the comparison of the biochemical parameters of the cows in group I and group II (ALP, amylase, CK-MB, CK-NAC, GGT, AST, ALT, albumin) between the groups, no statistically significant difference was found (P>0.05), (Table 1).

Table 1. Comparison of biochemical parameters between groups I.

	Group I			Group II			P
	n	Mean	Std. Error of Mean	n	Mean	Std. Error of Mean	
ALP (U/L)	30	61.17	7.33	30	47.93	4.20	-
Amylase (U/L)	30	136.93	11.71	30	131.73	9.37	-
CK-MB (U/L)	30	125.47	10.85	30	129.37	10.57	-
CK-NAC (U/L)	30	207.07	13.38	30	667.45	322.91	-
GGT (U/L)	30	23.80	1.01	30	24.37	.89	-
AST (U/L)	30	77.89	5.78	30	132.04	29.81	-
ALT (U/L)	30	24.47	2.27	30	27.77	3.46	-
Albumin (g/dl)	30	3.09	.084	30	2.95	.046	-

P, independent student t test

As a result of the comparison of other biochemical parameters (direct bilirubin, total bilirubin, Ca, CHO, creatine, glucose, total protein, urea, iron, phosphorus, LDH-P, HDL-C, LDL, triglyceride, magnesium, UIBC, asprosin) between the groups, the amount of direct bilirubin (0.1119 mg/dl) and total bilirubin (0.2787 mg/dl) of cows in Group II was higher than Group I, the amount of CHO (93.300 mg/dl), HDL-C (49.7333 mg/dl), LDL (29.6333 mg/dl) and asprosin (0.887 mg/dl) amounts were found to be lower than Group I (P<0.005), (Table 2).

Table 2. Comparison of biochemical parameters between groups.

	Group I (n=30)	Group II (n=30)	P
Iron (ug/dl)	94.0690	84.0333	-
Phosphorus (mg/dl)	4.2818	4.5843	-
LDH-P (U/L)	2828.0667	3263.4667	-
HDL-C (mg/dl)	60.8667	49.7333	.022
LDL (mg/dl)	54.7667	29.6333	.000
Triglyceride (mg/dl)	15.0667	11.4333	-
Mg (mg/dl)	2.4333	5.5500	-
UIBC (ug/dl)	203.2000	193.2000	-
Asprosin (mg/dl)	2.1783	.8887	.000
Direct Bilirubin (mg/dl)	.0713	.1119	.028
Total bilirubin (mg/dl)	.1253	.2787	.010
Ca (mg/dl)	10.0500	10.2967	-
CHO (mg/dl)	118.4000	93.3000	.002
Creatine (mg/dl)	1.3313	1.1633	-
Glucose (mg/dl)	54.1000	45.6552	-
Total protein (g/dl)	6.5833	6.9800	-
Urea (mg/dl)	36.6333	38.5172	-

P, independent student t test

When the asprosin values and biochemical parameters of the animals in Group I were compared, a positive correlation was found between asprosin and albumin

(0.398), direct bilirubin (0.367), total bilirubin (0.459) and creatine (0.522) (P<0.05), (Table 3).

Table 3. Correlation of asprosin with other biochemical parameters in pregnant animals.

Asprosin	1
UIBC	-,061
MG	-,050
Triglyceride	-,094
LDL	,160
HDLC	,195
LDHP	,029
Phosphorus	-,183
Iron	,085
Urea	,220
Total protein	,224
Glucose	-,099
Creatine	,522(**)
CHO	,245
CA	,249
T biluribin	,459(*)
D biluribin	,367(*)
Albumin	,398(*)
ALT	,012
AST	,351
GGT	,018
CKNAC	-,157
CKBM	-,036
Amylase	-,044
ALP	,005
	Asprosin

P < 0.05* significant positive correlation
P < 0.01** significant positive correlation

When the asprosin values and biochemical parameters of the animals in Group II were compared, weak negative correlation (P<0.05) between asprosin and amylase, weak positive correlation (P<0.05) between asprosin and creatine and glucose, moderate negative correlation between asprosin and phosphorus was determined (P<0.01), (Table 4).

Table 4. Correlation of asprosin with other biochemical parameters in non-pregnant animals.

Asprosin	
-,260	**ALP**
-,395(*)	**Amylase**
,123	**CKBM**
,140	**CKNAC**
-.225	**GGT**
,148	**AST**
,155	**ALT**
-,115	**Albumin**
,091	**D biluribin**
,131	**T biluribin**
,285	**CA**
-,161	**CHO**
,444(*)	**Creatine**
,444(*)	**Glucose**
-,108	**Total protein**
-,116	**Urea**
,070	**Iron**
-,629(**)	**Phosphorus**
,118	**LDHP**
,009	**HDLC**
,239	**LDL**
-,280	**Triglyceride**
,190	**MG**
-,150	**UIBC**
1	**Asprosin**

P < 0.05* significant positive correlation
P < 0.01** significant positive correlation

4. DISCUSSION AND CONCLUSION

Determination of the blood biochemical profile in cows is widely used to reveal the causes of diseases or low productivity [20]. It has been reported that blood biochemical parameters are important in the evaluation of the health status of animals, and the determination of these parameters is a standard rule for diagnosing various pathophysiological and metabolic disorders in cattle [21]. Tainturier et al. [22], it is stated that while the activities of AST and GGT show irregular and small changes from time to time during pregnancy and early lactation, ALT activity decreases significantly in the seventh and eighth months of pregnancy and at the beginning of lactation. Stojević et al. [23] reported that there are significant differences in AST, ALT and GGT activities in dairy cows in lactation and dry period according to the lactation stage. In a different study, a significant negative correlation was found between daily milk yield and blood ALT concentration [24]. Prodanovic et al. [25] reported that there was a significant positive correlation between urea nitrogen and lipid content in the liver ten days after calving, and that total protein and albumin concentrations were significantly lower than in the dry period. In a study conducted in sheep, it was stated that serum urea, total bilirubin, direct bilirubin, total protein, albumin, ALT and CK concentrations increased significantly during pregnancy, and serum glucose, creatinine, Ca, ALP and phosphorus concentrations were higher after birth than before lambing [26]. In the study of Sevinç et al. [27], total protein, albumin, glucose, creatinine, inorganic phosphorus, magnesium, AST, ALT, calcium, total and direct bilirubin and urea values were statistically significantly higher in cows followed from the seventh month of pregnancy to the second month of lactation significant differences are reported. In the present study, there was no statistically significant difference as a result of the comparison of ALP, amylase, CK-MB, CK-NAC, GGT, AST, ALT, and albumin between cows in the 2nd and 4th months of pregnancy and the cows on the 15th day after calving. The direct bilirubin and total bilirubin amounts of the cows in Group II were higher than the pregnant cows, and the CHO, HDL-C and LDL amounts were lower than the pregnant cows. It was seen that the obtained findings were mostly in agreement with our source literature. It was concluded that some of the differences were due to factors such as feeding, race and lactation period.

Asprosin is a recently discovered hormone that is released from white adipose tissue during fasting and stimulates hepatic glucose and insulin release by stimulating the feeling of starvation after crossing the blood-brain barrier. In humans, a genetic deficiency in asprosin causes a syndrome characterized by anorexia and extreme weakness. Asprosin rises with fasting and falls acutely with food intake. In the case of intravenous administration of asprosin, it has been reported that it activates the hunger center in the hypothalamus and causes appetite stimulation [3]. It has been reported that the serum asprosin level is increased in obese humans and mice, and neutralization of asprosin through antibody treatment regulates food intake, reduces body weight, and increases

insulin sensitivity in mice [5]. Studies have shown that asprosin levels are significantly higher in women with T2DM and positively correlated with insulin resistance in women with polycystic ovary syndrome (PCOS) [4-28]. In other studies with results similar to this study, it is stated that asprosin level is relatively high in patients with T2DM [10-29]. There are not many publications on the levels of the hormone asprosin in cattle. In a study conducted to determine the relationship between asprosin and PCOS [30] heifer ovaries collected from the slaughterhouse were used as material and it was shown that asprosin receptors were expressed in the follicles of the collected ovaries. In another study, it was found that the hormone asprosin is present in the blood serum of cows at a measurable level and is higher in cows with postpartum disease than in healthy cows [31]. In the present study, asprosin level was found to be higher in pregnant cows compared to cows on postpartum 15th day as a result of comparison between groups. As a result of the comparison of the groups within themselves, there was a positive correlation between asprosin and albumin, direct bilirubin, total bilirubin, and creatinine in pregnant cows, a weak negative correlation between asprosin and amylase, a weak positive correlation between asprosin and creatine and glucose, a moderate negative correlation between asprosin and phosphorus in cows on the postpartum 15th day was found.

As a result, when the levels of asprosin and biochemical parameters in pregnant and non-pregnant cows were compared both within themselves and between the groups, it was found that asprosin levels were higher in pregnant cows and there might be negative or positive correlations between this hormone and biochemical parameters. However, it was concluded that it would be beneficial to carry out more comprehensive studies to evaluate the possibility of asprosin being a marker in the problems that may occur by determining the relationship between changes in biochemical parameters.

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