



The Effect of Change in Body Condition Score at Different Stages of Pregnancy on Serum Apelin-36 Hormone Levels in Simmental Cattle

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Abstract: Apelin is a hormone secreted from adipose tissue that has a role in many physiological processes and intrauterine growth, especially energy metabolism in the body, originating from a preproapelin of 77 amino acids. In this study, it was aimed to investigate the effect of Apelin on hormone response due to changes in Body Condition Score (BCS) in Simmental breed cows in different pregnancy periods. The study material consisted of 90 Simmental cattle aged 3-8 years with a clinically healthy appearance which were selected by random sampling at different pregnancy periods and BCS. Cows that made up the study material were divided into three groups according to their pregnancy period. The first group consisted of cattle in the first 3 months of pregnancy (n = 30), the second group consisted of cattle in 3-7 months of pregnancy (n = 30) and the third group consisted of cattle in the last 2 months of pregnancy (dry period) (n = 30). In blood serum samples taken from the jugular Vena of animals, the level of the hormone Apelin-36 was determined by ELISA technique. Serum Apelin-36 levels in the first 3 months of pregnancy, 3-7 months of pregnancy and last 2 months of pregnancy groups were determined as 0.66 ± 0.009 , 0.52 ± 0.009 and 0.65 ± 0.009 ng mL⁻¹, respectively. It was found that the effect of pregnancy status on serum Apelin-36 levels in cattle with BCS was found to be significant ($p < 0.01$). As a result, it was concluded that the pregnancy status and BCS significantly affect the serum Apelin-36 level.

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INTRODUCTION

Energy balance, simply, is the difference between energy consumed and sum of energy used for maintenance and production. The negative difference indicates body reserves being mobilized whereas the

positive difference indicates body reserves being replenished or increased. Negative Energy Balance (NEB) is accepted as natural event in dairy cattle because, dry matter intake decrease during the close-up period and increases more slowly than milk yield after parturition. Body Condition Score (BCS) is one of the most

frequently used methods in estimating the intensity and duration of the NEB^[1]. In other words, BCS used to assess the subcutaneous fat level, body energy and fat reserves owned by a cow^[2]. Thus, it is of critical importance in protecting against reproductive and metabolic diseases that lead to significant economic problems in Herd Management^[3].

The BCS techniques are based on visual, tactile, or combined assessment of the amount of body condition (fat) carried by the cow. Cows are normally scored on a five-point scale (1: cachectic, 2: weak, 3: medium, 4: fat, 5: obese)^[4].

Apelin is a peptide hormone identified as a ligand for the G-protein coupled receptor (APJ) receptor and secreted from adipose tissue^[5]. Apelin hormone has several apelin isoforms such as Apelin-12,13, 17 and Apelin-36^[6]. Apelin/APJ system, endocrine stress response^[7], food intake^[7, 8], body-fluid homeostasis^[7], cardiovascular functions^[9], blood pressure regulation^[10], angiogenesis^[11], thermoregulation^[12] and energy metabolism regulation^[13], it is effective in important physiological processes.

As a result of clinical studies, it is reported that Apelin hormone has a therapeutic potential in clinical use as a potential serum biomarker in the early diagnosis of different diseases (cancer, diabetes, cardiovascular) and follow-up of metabolic processes. This study aimed to examine the effect of BCS change on serum Apelin-36 hormone level depending on some physiological parameters (first 3 months of pregnancy, 3-7 months of pregnancy and last 2 months of pregnancy).

MATERIALS AND METHODS

Animal selection and creation of groups: In the study, 90 head of Simental cattle between 3 and 8 years old raised in Turkey's Balikesir city were used. These animals were randomly divided into 3 separate groups according to their BCS and pregnancy status (Table 1).

Before starting the current study, ethics committee approval was obtained from the Local Ethics Committee for the study (Decision date and number: 04.09.2020/65). Animals that were included in the study groups and animals that were clinically healthy were included and care was taken to ensure that the environment, feed and age factors were the same throughout the study period. The analysis of the feed content in this research was analyzed according to standard AOAC methods. The cows in lactation were fed with feeds of 18% crude protein, 12% crude cellulose, 3.5% crude fat and 2600 kcal/metabolic energy level. Dry cows were fed with feeds of 15% crude protein, 12% crude cellulose, 3% crude fat and 2500 kcal/metabolic energy level.

Table 1: Distribution of cattle comprising the study material by groups

Pregnancy status	Body condition scores	N
First 3 months of pregnancy	Poor (BCS<2.5)	10
	Normal (BCS = 2.5-3.0)	10
	Fat (BCS>3)	10
	Total	30
3-7 months of pregnancy	Poor (BCS<2.75)	10
	Normal (BCS = 2.75-3.25)	10
	Fat (BCS>3.25)	10
	Total	30
Last 2 months of pregnancy (dry period)	Poor (BCS<3.25)	10
	Normal (BCS = 3.25-3.75)	10
	Fat (BCS>3.75)	10
	Total	30

Collection of serum samples: The 10 mL blood samples were collected from the neck vein (vena jugularis) of the cattle forming the study groups into anti-coagulant-free tubes (VACUETTE@TUBE 9 mL Z Serum Clot Activator). Blood collected by taking it in a laboratory cooled centrifuge (NF 1200R, NÜVE, Ankara, Turkey) 3000 rpm at 10 min after centrifuging the blood serums are separated. The separated serums were transferred to sterile tubes and kept in freezers set to -80°C until laboratory analysis was carried out.

Measurement of Apelin-36 hormone levels in serum: The minimum detectable concentration of the Apelin hormone kit used in the measurement of Apelin levels in the blood serums obtained as a result of the study is reported as 16 pg mL⁻¹. The race-specific Bovine Apelin (AP) ELISA Kit Bovine Apelin (AP)ELISA Kit (Apelin, SinoGeneClon, Product code: SG-60825, CHINA) was studied in accordance with the procedure described in the manufacturer's catalog using the determination of 35-1600 pg mL⁻¹, the intra assay coefficient of 8.0% and the inter-assay coefficient of 10.0%.

Statistical analysis: In the analysis of the data obtained in the experiment, SPSS package program was used and the General Linear Model (GLM) procedure was applied^[14]. Multiple comparison tests were analyzed with Duncan. The following mathematical model was used to determine the effect of period (first 3 months of pregnancy, 3-7 months of pregnancy, last 2 months of pregnancy) and BCS on the level of the Apelin hormone. This model with statistical notation; it is expressed as:

$$Y_{ijk} = \mu + a_i + b_j + (a*b)_{ij} + e_{ijk}$$

Where:

- a_i = Effect of different periods of pregnancy
- b_j = Effect of BCS
- (a*b)_{ij} = Interaction
- e_{ijk} = The error due to chance

RESULTS

It was concluded that the mean serum Apelin-36 level increased with the increase in BCS (p<0.01). In other

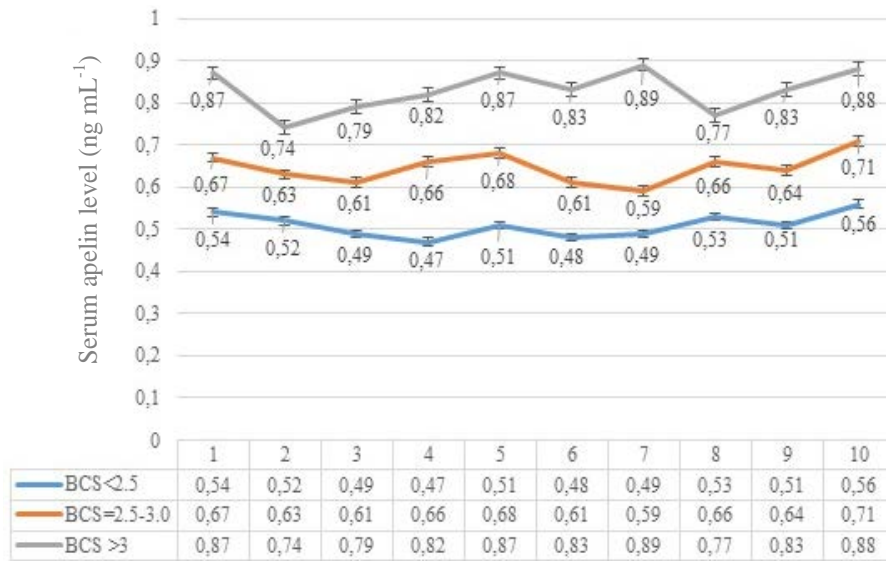


Fig. 1: Serum Apelin-36 hormone levels in cows according to BCS in the first 3 months of pregnancy (ng mL⁻¹)

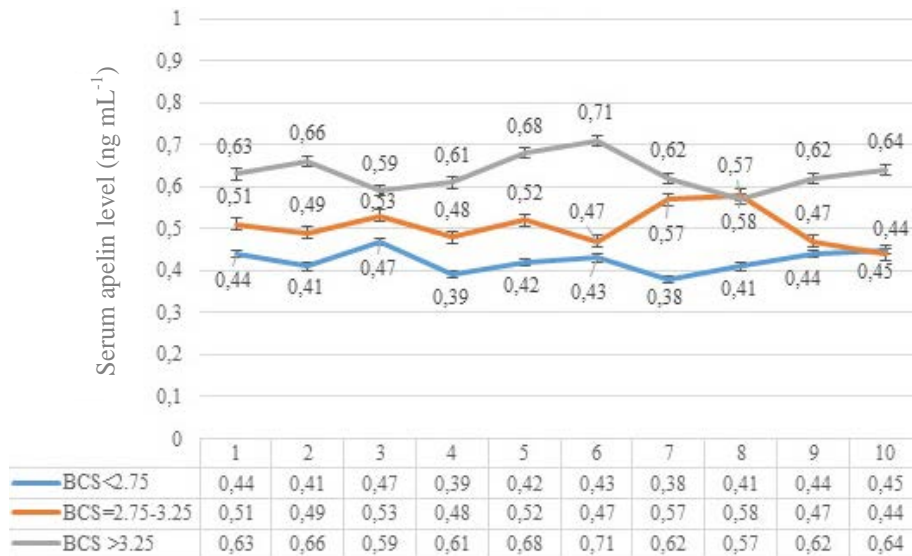


Fig. 2: Serum Apelin-36 hormone levels in cows according to BCS in the 3-7 months of pregnancy (ng mL⁻¹)

words, this hormone level was found to be lowest in cows with poor condition, medium level in cows with normal condition and highest in fat cows.

Mean serum Apelin-36 levels of cows in the first 3 months of pregnancy were determined as 0.66 ng mL⁻¹. The mean serum Apelin-36 levels of cows in the first 3 months of pregnancy were determined as 0.51 ng mL⁻¹ in the BCS < 2.5 group; 0.65 ng mL⁻¹ in the BCS = 2.5-3.0 group; 0.83 ng mL⁻¹ in the BCS > 3.0 group (Fig. 1 and Table 2).

In the same way, mean serum Apelin-36 levels of cows in the 3-7 months of pregnancy were determined as 0.52 ng mL⁻¹. The mean Apelin-36 levels of cows in the 3-7 months of pregnancy were determined as 0.42 ng mL⁻¹ in the BCS < 2.75 group; 0.51 ng mL⁻¹ in the BCS = 2.75-3.25 group; 0.63 ng mL⁻¹ in the BCS > 3.25 group (Fig. 2). The lowest mean serum Apelin-36 level for the dry period was found in BCS < 3.25 groups 0.49 ng mL⁻¹ while the highest mean serum Apelin-36 level was 0.75 ng mL⁻¹ in the BCS > 3.75 group (Fig. 3).

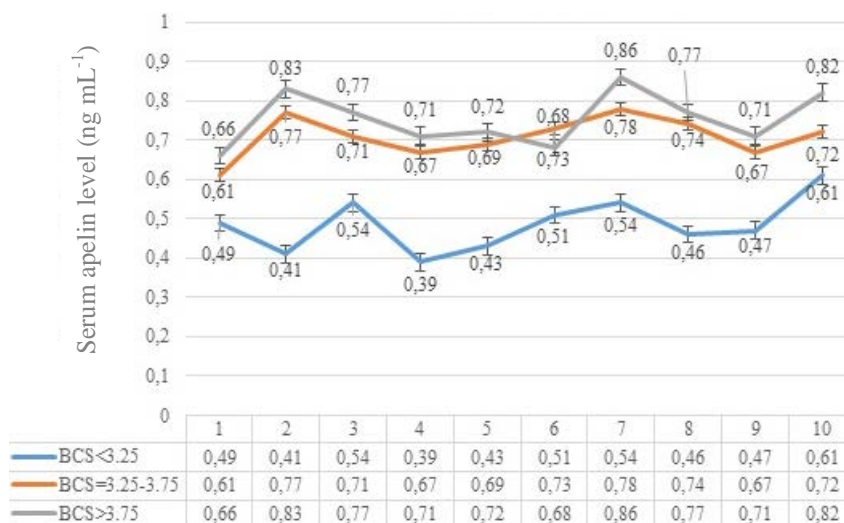


Fig. 3: Serum Apelin-36 hormone levels in cows according to BCS in the last 2 months of pregnancy (ng mL⁻¹)

Table 2: Mean serum Apelin-36 hormone levels of study groups (ng mL⁻¹)

Status	Body condition scores	Mean
First 3 months of pregnancy	BCS < 2.5	0.51±0.015
	BCS = 2.5-3.0	0.65±0.015
	BCS > 3	0.83±0.015
	Total	0.66±0.009 ^a
3-7 months of pregnancy	BCS < 2.75	0.42±0.015
	BCS = 2.75-3.25	0.51±0.015
	BCS > 3.25	0.63±0.015
	Total	0.52±0.009 ^b
Dry period (last 2 months of pregnancy)	BCS < 3.25	0.49±0.015
	BCS = 3.25-3.75	0.71±0.015
	BCS > 3.75	0.75±0.015
	Total	0.65±0.009 ^a
P		
Status	**	
Body condition scores	**	
Status*Body condition scores	**	

**p<0.01

DISCUSSION

Pregnancy is an important process in which the response to the development of the fetus, lactation period and physiological and hormonal changes are experienced with pregnancy. Some adipokines such as Apelin, play a role in the growth, function and lactogenic regulation of the mammary gland^[15, 16]. The hormone Apelin, which originates from adipose tissue, is secreted from adipose tissue varies according to many physiological periods such as body mass index, pregnancy, lactation and is released into the circulation accordingly^[17]. A significant increase in Apelin expression has been reported during pregnancy and lactation. It has been reported that high levels of apelin in the fetus and placenta may have a role in intrauterine growth^[16, 18].

As a result of the study, it was determined that the effect of in different pregnancy period cows on the mean level of serum Apelin-36 was important in with different BCS belonging to the Simmental breed (p<0.01).

CONCLUSION

In conclusion, we anticipate that due to the role of Apelin-36 in metabolic processes, it may be an early indicator in the diagnosis and follow-up of metabolic diseases and a biomarker in taking precautions. However, it is believed that conducting research on a large number of animals and different cattle breeds is important in terms of shedding light on physiological mechanisms and strategies that can be developed.

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