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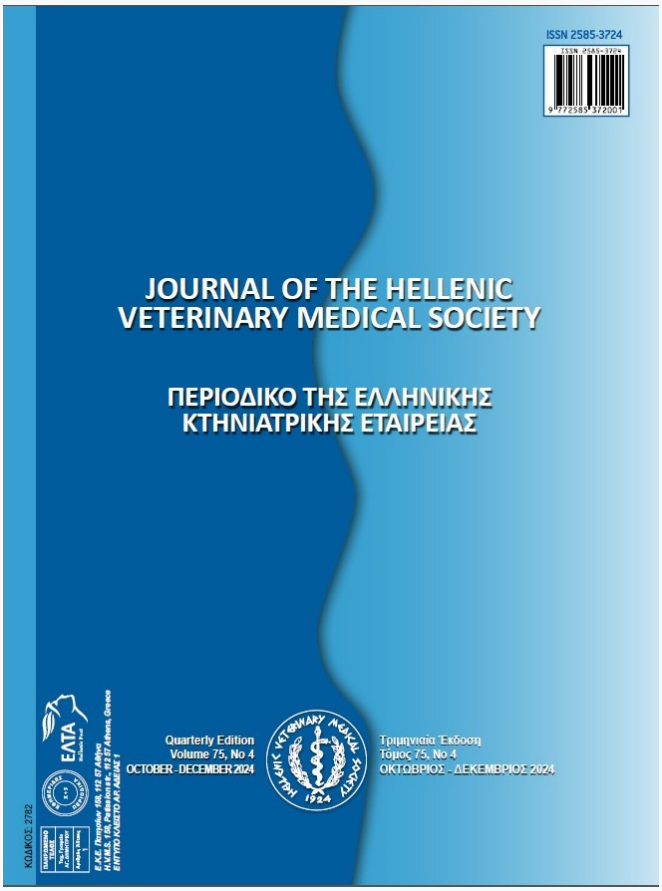

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Research article
Ερευνητικό άρθρο

Investigation of the relationship between apelin hormone response and some physiological parameters in enzootic bovine leukosis virus infected cattle

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ABSTRACT: Apelin is a hormone with lymphangiogenesis potential, associated with tumor growth and lymph node metastasis, and is reported to stimulate tumor vascularization. This study aimed to investigate the response of the apelin hormone in cattle with and without Enzootic Bovine Leukosis (EBL) infection, based on physiological parameters such as breed, age, and body condition score (BCS). A total of 120 Simmental and Montafon cattle of varying ages (<2, 2-8, and >8) and BCS categories (<2, 3-3.5, and ≥4) were included in the study. Serum apelin hormone levels were measured using the ELISA technique in blood samples collected from the neck vein (*Vena jugularis*) of the animals. The analysis of mean serum apelin (ng/mL) values revealed that, among EBL-positive cattle, the highest value was 2.77 ng/mL in Simmental cattle aged 2-8 years with a BCS of 3-3.5, while a value of 2.69 ng/mL was observed in Montafon cattle aged >8 years with a BCS of 3-3.5. For EBL-negative cattle, the highest mean serum apelin value was 2.24 ng/mL, recorded in Simmental cattle aged 2-8 years with a BCS of 3-3.5, whereas it was 1.45 ng/mL in Montafon cattle aged >8 years with the same BCS. In conclusion, this study demonstrated for the first time the effect of apelin hormone levels in cattle with or without EBL, depending on physiological parameters such as age, breed, and BCS. It is suggested that the apelin hormone, which is involved in neoplastic lymphocyte cell infiltration in the lymphoreticular tissues of cattle, and plays a role in regulating tumor growth in EBL, a malignant tumor disease, may contribute to eradication programs. Moreover, the evaluated parameters may prove valuable for disease prevention and control strategies.

Keywords: Enzootic Bovine Leukosis; apelin; bovine; physiological parameters

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INTRODUCTION

Enzootic bovine leukosis (EBL) is a disease caused by the bovine leukemia virus (BLV), an oncovirus belonging to the Retroviridae family that affects cattle, leading to significant economic losses worldwide (Camargos et al., 2002). BLV, also known as bovine lymphosarcoma, primarily targets the bone marrow, skin, lymph node, blood cells (lymphocytes) and thymus, causing lymphocyte proliferation and tumoral formations (Murakami et al., 2013). In animals with EBL, there is a decline in body condition score, live weight, and production parameters (Murtaugh et al., 1991). Clinical signs in cattle include fever, anorexia, weight loss, increased hearth rate, elevated respiratory frequency, swelling of all superficial lymph nodes, and firm tumoral formations in the prescapular, mandibular, and inguinal lymph nodes. BLV infection is challenging to control, as most infected cattle (approximately 70%) are asymptomatic or subclinically infected (Juliarena et al., 2017). Permanent lymphocytosis occurs in 30% of BLV-infected cattle, while malignant B-cell lymphosarcoma tumors may develop after a prolonged latent form (1-8 years) in 1-5% of cases (Aida et al., 2013; Pandey et al., 2017). The EBL virus is reportedly absent from semen and is not considered a transmission route for artificial insemination (Monkey, 1986). However, semen may act as a transmission medium if contaminated with blood during collection via massage technique (Lucas et al., 1980). Age plays a crucial role in the seroprevalence of the disease, with BLV commonly occurring between 5 and 8 years of age. The seroprevalence rate increases with age, as animals face prolonged exposure to infected cattle (Maezawa et al., 1998).

Apelin is a hormone secreted from adipose tissue, derived from a 77 amino-acids precursor (Tatemoto et al., 2001). Among its isoforms, apelin-13 exhibits biological activity that is 8 times stronger than apelin-17 and 60 times stronger than apelin-36 (Tatemoto et al., 1998). The Apelin/APJ system plays a crucial role in various physiological processes, including the endocrine stress response (Taheri et al., 2002), cardiovascular functions (Szokodi et al., 2002), regulation of blood pressure (Tatemoto et al., 2001), angiogenesis (Zhang et al., 2016), thermoregulation (Reaux et al., 2001), and energy metabolism regulation (Bertrand et al., 2015). Apelin and APJ are expressed in endothelial cells of newly developing blood vessels during angiogenesis (Kidoya et al., 2008). Apelin promotes tumor growth by stimulating tumor vascularization, which has lymphangiogenic potential and is associated with tumor progression and lymph node metastasis (Sorli et al., 2006; Sorli et al., 2007). Recognized as a therapeutic agent, apelin plays a significant role in tumor initiation and progression, making it a potential target for the treatment of tumors and cancers (Masoumi et al., 2020).

The findings of this study are expected to contribute to research on Enzootic bovine leukosis (EBL), a disease for which no treatment or vaccine currently exists. Moreover, no existing studies have examined the apelin hormone response in cattle with EBL concerning breed, age and body condition score. The current study aimed to investigate the relationship between EBL infection and the apelin hormone response in cattle, alongside some physiological parameters.

MATERIALS AND METHOD

Selection of the animals and sampling

This study was approved by the Veterinary Control Institute Animal Care and Use Committee Ethics Committee (Decision No: 08/2022). The study was carried out in cattle farms located in the Marmara region of Türkiye, which were raised under intensive conditions and whose records were followed regularly. To categorize the groups based on body condition score (BCS), palpation of the waist region was performed as described by Khan (1993). In accordance with the principles outlined by Thompson and Meyer (2006) scoring was conducted based on muscle and fat accumulation in the waist region, as follows: BCS <2 (thin), BCS 3-3.5 (normal), and BCS ≥4 (obese). The animal material for the study consisted 120 Simmental and Montafon cattle of varying ages (<2, 2-8, and >8) and body condition scores (≤2, 3-3.5 and ≥4). Those with questionable ELISA test results, age information, and body condition scores were not included in the study. Detailed information on the animal material and sample numbers is presented in Table 1.

During the research period, care was taken to ensure that the environment and feed factor (Table 2) remained consistent. The feed content in this study was analyzed using standard AOAC methods.

Collection of serum samples:

A 5 ml blood sample was collected from the neck vein (*vena jugularis*) of the cattle in the study groups into tubes without anticoagulant (VACUETTE® TUBE 9 ml Z Serum Clot Activator). After centrifuging the blood samples at 3000 rpm for 10 minutes in a refrigerated centrifuge (NF 1200R, NUVE, Ankara,

Table 1. Number of cattle included in the study across different ages and body condition scores

BCS	Age																	
	<2						2-8						>8					
	Montafon			Simmental			Montafon			Simmental			Montafon			Simmental		
	Neg	Pos	Total	Neg	Pos	Total	Neg	Pos	Total	Neg	Pos	Total	Neg	Pos	Total	Neg	Pos	Total
≤2	3	5	8	2	3	5	3	5	8	2	3	5	3	5	8	2	4	6
3-3.5	3	4	7	5	3	8	3	4	7	4	6	10	3	4	7	3	5	8
≥4	3	2	5	4	3	7	2	3	5	3	2	5	2	3	5	3	3	6
Total	9	11	20	11	9	20	8	12	20	9	11	20	8	12	20	8	12	20

*BCS: Body Condition Score, Neg: Negative, Pos: Positive

Table 2. Nutrient composition of feed used in the study (%)

Ingredient	% DM
Alfalfa haylage	31,35
Corn Silage	26,7
Soybean meal (30% CP)	15,2
Barley Straw	26,3
Mineral and vitamin mixture*	0,15
Sodium bicarbonate	0,3
Nutrient composition	
CP %	12,3
NDF	39,95
ADF	26,98
NFC	33,12
Ca	0,72
P	0,46
Energy (Mcal/kg)	1,41

*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

TÜRKİYE) in the laboratory, the obtained blood serums were transferred to sterile tubes and stored at -80°C until analysis.

Antibody ELISA test

The Enzootic Bovine Leukosis Virus (BLV) Antibody ELISA Test Kit (IDEXX, Lot SN 19090, France) was used to detect BLV antibodies in the serum samples obtained in this study. The test was performed following the manufacturer's instructions, and the optical density (OD) values were measured using an ELISA reader at a wavelength of 450 nm. According to the test procedure, the degree of positivity of each serum was determined, and any sample with a result equal to or greater than a plus (+) value was considered positive.

Measurement of apelin hormone levels in serum:

The minimum detectable concentration of the apelin hormone kit used for measuring of apelin levels in the blood serums obtained in this study was reported as 16pg/ml. The race-specific Bovine Apelin (AP) ELISA Kit (Apelin, SinoGeneClon, Product code: SG-60825, CHINA) was used in accordance with the procedure described in the manufacturer's catalog, with a detection limit of < 35 pg/ml, an intra-assay coefficient of 8.0% and an inter-assay coefficient of 10.0%.

Statistical analysis:

In this study, apelin hormone levels in the serum of Simental and Montafon cattle breeds during lactation and pregnancy were analyzed as the dependent variable; while gender, body score, and breed were analyzed as fixed effects. To investigate the effect of gender, body score and breed on apelin hormone levels in serum during lactation and pregnancy, a normal distribution test was first applied to the apelin measurements. Logarithmic transformation was applied to the data as a result of which the Shapiro-Wilk test turned out to be significant. It was determined by the Levene test (P=0.33), which showed a normal distribution, and the group variances were homogeneous. Then, analyses were performed according to the experiment

pattern of coincidence plots in factorial order (2x3x3) using a single-variable procedure in linear models generalized to logarithmic apelin measurements, and the interaction effects of body score and breed, gender and body score, race and body score, gender and breed factors were also investigated. Tukey multiple comparison tests was applied to compare the mean differences of Apelin hormone. IBM SPSS v25 statistical package program was used for all analyses in this study. All significant differences were evaluated by testing at the $P < 0.05$ level.

RESULTS

In the current study, cattle were categorized based on body condition scores (BCS) and age groups: <2 years, 2-8 years, and >8 years. BCS classifications were defined as low (≤2), optimal (3-3.5), and high (≥4). A total of 120 female cattle were included in the study, with 20 cattle in each age group (<2 years, 2-8 years, and >8 years) for each BCS category. The cattle were also evaluated for Enzootic Bovine Leukosis (EBL) infection status, as detailed in Table 3. As a result of the analyzes, when the mean serum Apelin (ng/mL) values for the BCS*EBL*Age*Race interaction were examined, the highest values for EBL positive cattle was 2.77 ng/mL in Simmental breed aged 2-8 years with BCS 3- 3.5, while it was 2.69 ng/mL in Montafon breed with BCS 3-3.5 and aged >8 years. For EBL negative cattle, the highest mean serum apelin value was 2.24 ng/mL in Simmental breed aged 2-8 years with BCS 3-3.5, and 1.45 ng/mL in Montafon breed aged >8 years with BCS 3-3.5. When analyzed for the BCS*EBL*Age* Breed interaction, the highest apelin hormone levels were observed in EBL positive Montafon and Simmental cattle aged ≥8 years, regardless of BCS, and in Simmental cattle aged 2-8 years with BCS 3-3.5 and EBL-positive status ($p<0.01$) (Table 4).

Table 3. The comparisons of apelin (ng/mL) levels based on physiological parameters and EBL status

Parameters		n	Apelin	±	SEM
Race	**Montofon**	60	1,479b	±	0,05
	Simental	60	1,925a	±	0,05
BCS	**≤2**	40	1,551b	±	0,062
	3-3.5	47	1,863a	±	0,055
	≥4	33	1,692ab	±	0,066
Age	**< 2**	40	1,449c	±	0,061
	2-8	40	1,625b	±	0,062
	≥8	40	2,031a	±	0,061
EBL +/-	**Positive**	67	2,111a	±	0,047
	Negative	53	1,292b	±	0,053

Source of variation (P-values)	
Race	0,003
BCS	<0,01
Age	<0,01
EBL	<0,01

Table 4. Interaction of apelin hormone level (ng/mL) according to enzootic bovine leukosis, age, races and body condition score.

BCS	EBL+						EBL-					
	Age						Age					
	<2		2-8		8<		<2		2-8		8 <	
	Montafon	Simental	Montafon	Simental	Montafon	Simental	Montafon	Simental	Montafon	Simental	Montafon	Simental
≤2	1,71±0,165gjnrt	1,713±0,214r	2,132±0,165jn	1,31±0,214dm	2,232±0,165hj	2,433±0,185h	0,97±0,214cefg	0,590±0,262adf	0,847±0,214ce	1,785±0,262ad	0,99±0,214c	1,895±0,262a
3-3.5	2,218±0,185jnrt	2,19±0,214fr	2,043±0,214ejn	2,773±0,151hk	2,693±0,185hj	2,626±0,165h	0,643±0,214eg	1,674±0,165adf	0,803±0,214be	1,385±0,185ad	1,453±0,185ab	1,86±0,214a
≥4	1,72±0,262rt	1,81±0,214fr	0,567±0,214p	2,695±0,262dhk	2,433±0,185hj	2,707±0,214h	1,007±0,214befg	1,142±0,185df	0,92±0,262be	2,243±0,214ad	1,24±0,262abc	1,817±0,214a

Source of variation (P-values)	
BCS*Age*Race	<0,01
BCS*EBL*Race	0,016
BCS*EBL*Age	0,029
Age*Race*EBL	0,024
BCS*EBL*Age*Race	<0,01

DISCUSSION

Enzootic Bovine Leukosis is a malignant tumoral disease characterized by neoplastic lymphocyte infiltrations in the lymphoreticular tissues of various organs and systems. The clinical symptoms very depending on the organs where the lesions are located (Barlet et al., 2020). The disease manifests in five forms: enzootic viral leukosis in adult cattle, sporadic juvenile leukosis in calves under six 6 months old, sporadic thymic leukosis in young cattle under two years old, sporadic skin leukosis in young cattle, and benign lymphocytosis (Donnik et al., 2021). EBL-infected animals are persistently infected and spread the disease without displaying clinical symptoms (Asfaw et al., 2005). The absence of a vaccine or treatment makes the disease one of the major livestock problems globally. Pregnancy and lactation involve physiological and hormonal changes that can influence disease transmission. The disease can be transmitted to the fetus via blood, and to offspring through infected milk and colostrum after birth. The secretion of the apelin hormone, produced by adipose tissue, varies with physiological conditions such as body mass index, pregnancy, and lactation, resulting in fluctuating levels in circulation (Hughes & Watson, 2018; Lérias et al., 2014). Apelin is a hormone with a well-defined role in tumor progression and is easily measurable in blood serum (Zhang et al., 2016). Apelin/APJ system has been reported to inhibit tumor growth and regulate tumor formation in an autocrine manner through its role in tumor angiogenesis. Endothelial cell-derived Apelin is also known to regulate tumor growth via the Apelin/APJ axis (Hu et al., 2021). It was determined that BCS*EBL*Age*Breed was effective on the mean serum apelin hormone level in cattle with positive EBL disease with different body condition scores, which is the target of our current study ($p<0.05$). In addition, the results of our current research are similar to the results of studies in humans and different animals in which serum apelin levels are examined in tumor patients (Picalt et al., 2014; Feng et al., 2016; Podgórska et al., 2019).

CONCLUSION

In conclusion, we believe that the apelin hormone may serve a useful biomarker for evaluating tumor progression in cattle infected with EBL, a disease characterized by tumoral formations in lymph nodes. However, further and more comprehensive studies are necessary.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Authors' Contribution Statements: Design and idea stage of the article AS, BB, Apelin analysis part BB, SB, GCK, GBÖ, MO; in statistical analysis; SK, ET, VA, HK; In the fieldwork, AS, BB, SB, SK, ŞA, GCK, ASB, VA has taken part. All authors read and approved the final manuscript.

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