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A STUDY ON DIAGNOSTIC AND PROGNOSTIC ROLE OF PERIOSTIN IN RESPIRATORY SYSTEM DISEASE COMPLEX IN CALVES*

BUZAĞILARDA SOLUNUM SİSTEMİ HASTALIK KOMPLEKSİNDE PERİOSTİNİN DİAGNOSTİK VE PROGNOSTİK ROLÜ ÜZERİNE BİR ÇALIŞMA

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ABSTRACT

The purpose of this study was to compare the amounts of serum amyloid a, haptoglobin, fibrinogen, and periostin in calves with respiratory system disease complex before and after treatment. Three groups were used in the study: an acute group (n=10) made up of calves with acute respiratory system disease complex symptoms, a chronic group made up of calves with chronic respiratory system disease complex symptoms, and a control group made up of disease-free, healthy calves. Before and after therapy (day 0, 7 and 14), clinical examinations were performed and blood samples were taken from the acute and chronic groups. Calves in the control group only had one clinical evaluation and blood sample collection. Results showed that both the acute and chronic groups exhibited clinical improvement after treatment. Before treatment, the concentrations of fibrinogen, serum amyloid A, and haptoglobin in the acute and chronic groups were significantly higher than those in the control group ($p<0.001$). On days 7 and 14, the serum periostin concentrations of the acute group were lower than those of the chronic group and control group ($p<0.05$). However, no significant difference was observed in serum periostin concentrations before and after treatment in both the acute and chronic groups ($p>0.05$). Additionally, a positive correlation was found between the respiratory system disease complex scores and the concentrations of serum amyloid A, haptoglobin, and fibrinogen. However, there was no significant correlation between periostin concentrations and respiratory system disease complex scores, as well as between fibrinogen, haptoglobin, and serum amyloid A concentrations ($p>0.05$).Based on the findings, it can be concluded that haptoglobin, serum amyloid A, and fibrinogen values, rather than periostin, play an important role in supporting the diagnosis and prognosis of respiratory system disease complex in calves.

Keywords: Calves, diagnostic, periostin, prognostic, respiratory system.

ÖZ

Bu çalışmada buzağılarda Solunum Sistemi Hastalıkları Kompleks'in de serum amiloid A, haptoglobin, fibrinojen ve periostin konsantrasyonlarının tedavi öncesi ve tedavi sonrası değişimlerinin araştırılması amaçlandı. Çalışmada üç grup kullanılmıştır: Akut solunum sistemi hastalığı kompleksi semptomları olan buzağılardan oluşan bir akut grup (n=10), kronik solunum sistemi hastalığı kompleksi semptomları olan buzağılardan oluşan bir kronik grup ve hastalıksız, sağlıklı buzağılardan oluşan bir kontrol grubu. Tedaviden önce ve sonra (0, 7 ve 14. günler), akut ve kronik gruplardan klinik muayeneler yapıldı ve kan örnekleri alındı. Kontrol grubundaki buzağılardan sadece bir klinik değerlendirme ve kan örneği alınmıştır. Sonuçlar hem akut hem de kronik grupların tedaviden sonra klinik iyileşme gösterdiğini ortaya koymuştur. Tedavi öncesinde, akut ve kronik gruplardaki fibrinojen, serum amiloid A ve haptoglobin konsantrasyonları kontrol grubundakilerden anlamlı derecede yüksekti ($p<0.001$). 7. ve 14. günlerde, akut grubun serum periostin konsantrasyonları kronik grup ve kontrol grubundan daha düşüktü ($p<0.05$). Ancak, hem akut hem de kronik gruplarda tedavi öncesi ve sonrası serum periostin konsantrasyonlarında anlamlı bir fark gözlenmemiştir ($p>0.05$). Ayrıca, solunum sistemi hastalığı kompleksi skorları ile serum amiloid A, haptoglobin ve fibrihojen konsantrasyonları arasında pozitif bir korelasyon bulunmuştur. Ancak, periostin konsantrasyonları ile solunum sistemi hastalığı kompleksi skorları arasında ve ayrıca fibrinojen, haptoglobin ve serum amiloid A konsantrasyonları arasında anlamlı bir korelasyon bulunmamıştır ($p>0.05$).Bulgulara dayanarak, buzağılarda solunum sistemi hastalığı kompleksinin tanı ve prognozunu desteklemede periostin'den ziyade haptoglobin, serum amiloid A ve fibrinojen değerlerinin önemli bir rol oynadığı sonucuna varılabilir.

Anahtar kelimeler: Buzağı, diagnostik, periostin, prognostik, solunum sistemi

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INTRODUCTION

Cattle's lungs, compared to the size of their bodies, are very small, which may prevent them from performing the respiratory system's function to its full potential. This syndrome, which primarily affects beef cattle, has a considerable negative influence on feeding performance and increases the risk of respiratory illnesses in both the upper and lower respiratory tracts.[1] Hypercapnia, hypoxia, pulmonary hypertension, bacterial and viral pneumonias, and aspiration pneumonia are serious respiratory system disorders in newborn calves.[2] One of the most important health problems in the global cattle farming industry is respiratory system disease complex (RSDC), which causes morbidity and death in freshly weaned and recently brought animals.[3] According to reports, RSDC is a major contributor to increased morbidity and mortality in calves, especially in feedlots.[4] The livestock business is significantly impacted by the increasing mortality rates brought on by RSDC and the costs related to using drugs and other therapies to address these illnesses. Furthermore, RSDC has a negative effect on feeding effectiveness and carcass quality, which lowers profitability. The most expensive illness in cattle production, RSDC kills 30% to 34% of calves between the ages of 1 and 5 months, which accounts for the majority of calves' deaths. A recent study showed that RSDC could cost as much as $42.15 per affected calf.[5] In this context, understanding the pathophysiological mechanisms behind RSDC has become essential for the effective management of diagnostic and prognostic procedures in calves.

A breakdown in homeostasis brought on by tissue damage, infection, neoplastic development, or autoimmune illnesses causes the organism to exhibit the non-specific acute phase response.[6] Numerous researchers have examined changes in certain acute-phase proteins in calf respiratory system infections.[7-9] RSDC in calves has been shown to cause considerable modifications in hematological and biochemical parameters, as well as an increase in serum levels of haptoglobin (Hp) and serum amyloid A (SAA).[10]

In recent years, research in human medicine has been increasingly focusing on the importance of various biomarkers in addition to the acute phase response in respiratory system diseases. One of these biomarkers is periostin. Periostin is an extracellular matrix protein. It is secreted by bronchial epithelial cells in response to interleukin-13 (IL-13) in the lungs. This extracellular matrix protein, which contributes to fibrosis development in the heart and bone marrow, is also considered a potential marker for fibrosis development in the lungs. Studies have shown that the secretion of periostin in lung tissue is higher in patients with idiopathic pulmonary fibrosis, those with interstitial lung disease, compared to healthy individuals.[11] However, there is no existing research on how this biomarker behaves in calves with RSDC and its diagnostic, therapeutic, and prognostic significance in this context.

While the significance of periostin in various diseases has been investigated in human medicine, its research in veterinary medicine has primarily been conducted through experimental studies. Numerous molecules have been investigated as biomarkers in calves, with acute-phase proteins being among the most studied. To the best of our knowledge, this study is the first to investigate periostin in calves. In this study, serum perios tin levels in healthy calves and those displaying clinical symptoms of RSDC were determined. Periostin levels compared with well-known acute-phase proteins in calves with RSDC and its diagnostic and prognostic significance were elucidated.

MATERIAL AND METHOD

The study was conducted in 7 commercial farms within the boundaries of Aydın Province, with the approval from the Erciyes University Animal Experiments Local Ethics Committee (Decision no: 17/103). Holstein and Simmental breed calves aged between 7 and 60 days were used in the study. The calves were divided into three groups with equal numbers (n=10/group). In this study, a total of 20 calves comprising acute and chronic groups were selected based on clinical findings and medical history information. Both the acute and chronic group calves were evaluated for the following clinical parameters; Rectal temperature (ºC), lung auscultation/ percussion findings, cough, nasal discharge, eye score and ear score. Scoring was performed for these parameters to determine whether the calves were ill and to assess the severity of RSDC as described by McGuirk.[12] Calves displaying clinical symptoms such as fever persisting for 1-3 days, anorexia, respiratory distress, nasal discharge, and cough were assessed as having acute RSDC and formed the acute group. Calves that had previously received treatment for RSDC but still exhibited clinical symptoms such as anorexia, depression, growth retardation, cough, and wheezing, even after 14 days, were considered as having chronic RSDC as described by Mc Guirk.[12] The control group consisted of calves that did not exhibit any clinical symptoms.

The owners of the sick calves were questioned regarding the onset of the fever, hunger, and respiratory issues as well as the presence of any other symptoms including coughing and nasal discharge. They were also questioned about whether any other animals in the vicinity of the calves were exhibiting symptoms of respiratory issues. It was questioned whether the calves had received anti-parasitic medication, were immunized against the RSDC, had undergone any prior treatments, and whether the farm had enough ventilation.

In the present study, clinical examinations were conducted, and findings were recorded for healthy calves (control=10) and calves displaying symptoms of respiratory system disease (acute and chronic=20). Lung auscultation and percussion, lymph node examinations, and clinical observations were performed. Rectal temperatures above 39.5°C were regarded as high fever as a calf's normal body temperature normally ranges from 38.8 to 39.5°C when they are between 19 and 60 days old. Calves normal respiration rates are between 30 and 45 breaths per minute, hence respiratory rates above 45 were regarded as elevated respiratory rates. The heart rate in young, healthy calves normally ranges from 90 to 110 beats per minute.[13]

Heart rates below 90 beats per minute were categorized as bradycardic, while rates exceeding 110 beats per minute were categorized as tachycardic. Abnormal lung sounds were recorded through lung auscultation and percussion. Additionally, nasal discharge examinations

were conducted in calves, and the nature of any discharge was assessed. Mucous membranes were examined for color changes, and lymph nodes were palpated, with findings noted.

In the acute group (n=10), tulathromycin (Draxxin® - Zoetis, 2.5 mg/kg body weight, SC. single dose) and flunixin meglumine (Flumed® - Alke, 2.2 mg/kg body weight, intramuscularly, for 3 days) were administered along with supportive treatment using vitamin C (Provet Vitamin-C - Provet® 10 ml, intramuscularly, for 3 days). In the chronic group (n=10), tulathromycin (Draxxin® - Zoetis, 2.5 mg/kg body weight, SC. single dose), flunixin meglumine (Flumed® - Alke, 2.2 mg/kg body weight, intramuscularly, for 3 days), bromhexine (Mukolit - Provet® 0.4 mg/kg body weight, intramuscularly, for 3 days) for mucolytic purposes, and supportive treatment with vitamin C (Provet Vitamin-C - Provet® 10 ml, intramuscularly, for 3 days) were administered. The dosage of the medications was carried out in line with each drug prospectus.

Blood samples were collected from the control group animals once and from the diseased groups at day 0 (pre-treatment), as well as on days 7 and 14 after treatment, through the jugular veins Clot activator-coated plastic tubes (5 ml) were used for blood collection (BD Vacutainer®) then the sera were separated by centrifugation at 3000 rpm for 10 minutes. Serum samples were transferred to Eppendorf tubes. The tubes were labeled and stored at -20°C until the analysis day.

The sera were diluted in accordance with the manufacturer's procedure at appropriate ratios before being placed in Enzyme-Linked Immuno Sorbent Assay (ELISA) microplates. Haptoglobin, Periostin, Serum Amyloid A (SAA), and Fibrinogen values in the serum were measured using the ELISA method. To enhance the reliability of the study, standards were run in duplicate. Hp values from serum samples were measured using the Sun red® Bovine Haptoglobin ELISA Kit (Catalog Number: 201-04-0121, Shanghai, China), SAA values using the Mybiosource® Bovine Serum Amyloid A ELISA Kit (Catalog Number: MBS778656, San Diego, California, USA), Fibrinogen values using the Mybiosource® Bovine Fibrinogen ELISA Kit (Catalog Number: MBS1602414, San Diego, California, USA), and Periostin values using the Mybiosource® Bovine Periostin (POSTN) ELISA Kit (Catalog Number: MBS2610037, San Diego, California, USA). The tests were performed following the appropriate test procedure using the Sandwich ELISA method, and the results were read at 450 nm on a Biotek® ELx800 ELISA device.

Statistical Analysis

The statistical analysis of the data was conducted using the SPSS for Windows, version 25.0. The normality of the obtained data was assessed using the Shapiro-Wilk test. It has been confirmed that our data exhibits a normal distribution. Data collected over time for repeated measures analysis of variance. When determining the effect of the intervention, if statistical significance was observed, indicating the source of the difference among groups or subgroups, post-hoc multiple comparisons were carried out employing the Bonferroni test to account for P-value correction. The data are presented as mean ± standard error ($\bar{X} \pm S_{\bar{X}}$). The association between the RSDC Score and serum analyses was investigated using Pearson correlation analysis. A significance level of $P<0.05$ was considered statistically significant.

RESULTS

Pre-Treatment Clinical Findings

In the control group, all findings were determined to be normal, and physiological measurements (body temperature, heart rate, and respiration) were within normal values during the examinations of the calves. Additionally, it was observed that lymph nodes were normal, there was no cough, nasal or ocular discharge, and the calves had a good appetite. The clinical findings of the sick groups are presented in Table 1. Clinical examinations of the calves in the sick groups revealed that they had a poor appetite, their fur was wrinkled and dull, they appeared depressed, and were not responsive to their surroundings. Abdominal respiration was generally observed as the predominant breathing pattern during the examinations. Day 0: The body temperature in the acute group was higher compared to the control and chronic groups ($p<0.001$). Respiratory rate was higher in both acute and chronic groups compared to the control group ($p<0.01$). Despite no statistical difference, the heart rate was numerically higher in the acute group. In calves of the acute group, symptoms consistent with pneumonia were observed, including high fever, rapid and shallow respiration, open-mouth breathing, dilated nostrils, forward positioning of the head, ears drooping, occasional cough, crackling sounds in various lung areas during auscultation, wheezing during expiration, and increased resistance in vesicular sounds. Percussion examinations revealed dull areas, particularly in the cranio-ventral regions of the lungs in some calves, while emphysematous findings were also observed in the dorsal regions of others. Clinical examination of calves in the acute group also revealed nasal discharge ranging from serous to mucopurulent and serous ocular discharge in some individuals. While most calves had hyperemic nasal mucosa and conjunctivae, some calves exhibited cyanotic signs.

The chronic group was made up of calves with RSDC who still showed persistent clinical indications after 14 days of treatment or that had never received any treatment at all. These calves' clinical examinations revealed that they had no nasal discharge, an occasional dry cough, shallow breathing, apprehension when moving, distinct bronchial sounds in different lung areas, poor appetite, emaciation, tangled fur, rapid and shallow breathing, slightly elevated or normal body temperatures, and in some calves, cyanotic symptoms.

Post-Treatment of Clinical Findings

When the groups compared: before treatment; the body temperature value of the acute group was found to be significantly higher than that of the chronic and control groups ($p<0.001$). Furthermore, there were no statistically significant difference between body temperature values of the control and chronic group. After treatment on day 7, the body temperature values of the acute group were still significantly higher than those of the control group ($p<0.05$) but not chronic group. On day 14

Table 1. Comparison of body temperature (°C), heart rate (beats/min) and respiratory rates (min) between the acute, chronic and control groups according to group *time interaction.

	Body Temperature (°C)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	38.19±0.43^{b}	38.19±0.43^{b}	38.19±0.43			
Acute (n= 10)	39.91±0.07^{a}	38.24±0.02^{a}	38.18±0.02	<0.001	<0.001	<0.001
Chronic (n= 10)	38.36±0.04^{b}	38.09±0.05^{ab}	38.19±0.04			
	Respiratory Rate (min)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	45.20±0.64^{a}	45.20±0.64^{a}	45.20±0.64^{a}			
Acute (n= 10)	61.40±0.56^{b}	55.80±0.53^{b}	55.20±1.24^{b}	<0.001	<0.001	<0.01
Chronic (n= 10)	55.30±1.24^{c}	54.40±1.56^{b}	52.20±1.94^{b}			
	Heart Rate (beats/min)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	95.80±0.64	95.80±0.64	95.80±0.64			
Acute (n= 10)	98.30±1.08	92.70±0.53	91.80±1.07	>0.05	<0.001	<0.05
Chronic (n= 10)	96.50±1.61	93.00±1.51	93.10±1.72			

Data are expressed as mean ± standard error ($\bar{X} \pm S_{\bar{x}}$). Different letters indicate statistical significance. p<0.05 was considered statistically significant.

after treatment, there was no statistically significant difference in body the temperature values among the acute, chronic, and control groups.

When comparisons were made between the groups: before treatment, the Respiratory Rate value of the acute group was significantly higher than that of the control and the chronic groups (p<0.001). The Respiratory Rate value of the chronic group was significantly higher than that of the control group, but lower than that of the acute group (p<0.001). After treatment on day 7, the Respiratory Rate values of both the acute and chronic groups were significantly higher than those of the control group (p<0.001). There was no statistically significant difference between the Respiratory Rate values of the chronic and acute groups. On day 14 after treatment, the Respiratory Rate values of both the acute and chronic groups were still significantly higher than those of the control group (p<0.001). There was no statistically significant difference in Respiratory Rate values between the chronic and acute groups.

When comparisons were made between the groups; no statistically significant difference was found among the acute, chronic, and control groups before treatment and on days 7 and 14 after treatment (p>0.05).

Laboratory Findings

Table 2shows the concentrations of Periostin, Fibrinogen, Haptoglobin, and Serum Amyloid A before (day 0) and after treatment (days 7 and 14).

Periostin: Before treatment (day 0), there was no statistically significant difference among the groups in terms of the Periostin variable. However, after treatment, it was observed that Periostin concentrations measured on days 7 and 14 in the acute group were statistically significantly lower than those in the control group (p<0.05). There was no statistically significant difference in Periostin concentrations among the other groups on days 7 and 14.

Fibrinogen: When the data were evaluated between the groups: On day 0, the Fb value of the chronic group was found to be significantly higher than both the acute and control groups(p<0.001). Additionally, the Fb value of the acute group was significantly higher than that of the control group. After treatment on day 7 and 14, Fb values in both the acute and chronic groups were significantly higher than the control group (p<0.001). However, there was no statistical difference between Fb values of the acute and chronic groups on day 7.

Haptoglobin: When the data were compared between the groups, it was discovered that the chronic group's Hp value was considerably greater than those of the acute and control groups prior to treatment (p<0.001). The Hp value of the acute group was significantly higher than that of the control group. On day 7 and 14 after treatment, the Hp values in the acute and chronic groups were significantly higher than those in the control group (p<0.001), and the Hp values between the acute and chronic groups were not statistically different.

Serum Amyloid A: Both the acute and chronic groups exhibited significantly higher SAA values than the control group prior to treatment (p<0.001) when the data were examined with regard to the groups. Furthermore, before treatment, the SAA levels in the acute group were considerably greater than those in the chronic group (p<0.001). In addition, SAA values in the acute and chronic groups were substantially higher than in the control group on days 7 and 14 following treatment (p<0.05) than in the control group.

Correlation analysis

In the acute group, a very strong correlation (r= 0.932, p<0.001) was observed between the RSDC scores of the calves and their SAA concentrations, indicating a highly significant positive relationship. The RSDC scores of the calves in the acute group also showed a good correla-

Table 2. Serum Periostin, Haptoglobin, Fibrinogen and Serum Amyloid A concentrations obtained throughout the study in the Acute, Chronic and Control group according to group*time interaction.

	Periostin (ng/ml)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	1.60±0.22	1.60±0.22^{a}	1.60±0.22^{a}			
Acute (n= 10)	1.00±0.14	0.71±0.22^{b}	0.92±0.12^{b}	>0.05	>0.05	<0.05
Chronic (n= 10)	1.19±0.24	1.22±0.17^{ab}	1.30±0.19^{ab}			
	Fibrinogen (mg/ml)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	1.45±0.08^{a}	1.45±0.08^{a}	1.45±0.08^{a}			
Acute (n= 10)	2.77±0.06^{b}	2.72±0.14^{b}	2.37±0.11^{b}	<0.001	<0.001	<0.001
Chronic (n= 10)	3.48±0.07^{c}	2.62±0.19^{b}	2.38±0.16^{b}			
	Haptoglobin (mg/ml)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	0.08±0.15^{a}	0.08±0.15^{a}	0.08±0.15^{a}			
Acute (n= 10)	0.60±0.02^{b}	0.45±0.03^{b}	0.37±0.02^{b}	<0.001	<0.001	<0.001
Chronic (n= 10)	0.77±0.04^{c}	0.54±0.03^{b}	0.32±0.02^{b}			
	Serum Amyloid A (µg/ml)			P_{group}	P_{time}	$P_{group*time}$
Groups	**0. Day**	**7. Day**	**14. Day**			
Control (n= 10)	12.00±0.28^{a}	12.00±0.28^{a}	12.00±0.28^{a}			
Acute (n= 10)	31.55±1.54^{b}	15.79±0.65^{b}	14.76±0.51^{b}	<0.001	<0.001	<0.001
Chronic (n= 10)	18.77±0.57^{c}	13.86±0.47^{a}	14.17±0.62^{b}			

Data are expressed as mean ± standard error ($\bar{X} \pm S_{\bar{X}}$). Different letters indicate statistical significance. P<0.05 was considered statistically significant.

tion (r= 0.775, p<0.001) with their Hp concentrations, indicating a statistically significant positive relationship. However, there was a weak correlation (r= 0.365, p<0.05) observed between the RSDC scores and Fb concentrations, which was statistically significant and positive. However, there was no statistically significant correlation observed between Periostin concentration and RSDC scores. In the acute group, the calves showed a moderate level of correlation (r= 0.444, p<0.02) between Hp and Fb concentrations, which was statistically significant and positive. There was also a good correlation (r= 0.629, p<0.001) observed between Hp and SAA concentrations in the acute group, which was statistically significant and positive. However, in the acute group, there was no statistically significant correlation observed between the serum Periostin concentration and Fb, Hp, and SAA concentrations (Table 3).

In the chronic group, there was a very strong correlation (r= 0.833, p<0.001) observed between the RSDC scores of the calves and their SAA concentrations, indicating a highly significant positive relationship. The RSDC scores of the calves in the chronic group also showed a very good correlation (r=0.801, p<0.001) with their Hp concentrations, indicating a statistically significant and strong positive relationship. Additionally, there was a good correlation (r=0.621, p<0.001) observed between the RSDC scores and Fb concentrations in the chronic group, which was statistically significant and positive. However, there was no statistically significant correlation observed between Periostin concentration

Table 3. Acute Group Correlation Analysis

		Fb	Hp	Periostin	SAA	RSDC Score
	Correlation coefficient	1	0.444*	-0.251	0.306	0.365*
Fb	P value		0.014	0.181	0.100	0.047
	Correlation coefficient	0.444*	1	0.142	0.629**	0.775
Hp	P value	0.014		0.455	0.000	0.000
	Correlation coefficient	-0.251	0.142	1	0.111	0.132
Periostin	P value	0.181	0.455		0.559	0.487
	Correlation coefficient	0.306	0.629**	0.111	1	0.932**
SAA	P value	0.100	0.000	0.559		0.000
	Correlation coefficient	0.365*	0.775**	0.132	0.932**	1
RSDC Score	P value	0.047	0.000	0.487	0.000	

*p<0.05 and **p<0.01 are considered statistically significant. Fb: Fibrinojen, Hp: Haptoglobin, SAA: Serum Amyloid A

and the total RSDC scores in the chronic group. In the chronic group, the calves showed a moderate level of correlation (r=0.553, p<0.01) between Hp and Fb concentrations, which was statistically significant and positive. There was also a good correlation (r=0.680, p<0.001) observed between Hp and SAA concentrations in the chronic group, which was statistically significant and positive. On the other hand, in the chronic group, there was no statistically significant correlation observed between the serum Periostin concentration and Fb, Hp, or SAA concentrations (Table 4).

Table 4. Chronic Group Correlation Analysis

		Fb	Hp	Periostin	SAA	RSDC Score
	Correlation coefficient	1	0.553**	0.077	0.323	0.621**
Fb	p value		0.002	0.686	0.082	0.000
	Correlation coefficient	0.553**	1	0.114	0.680**	0.801**
Hp	p value	0.002		0.549	0.000	0.000
	Correlation coefficient	-0.077	0.114	1	0.231	-0.112
Periostin	p value	0.686	0.549		0.219	0.554
	Correlation coefficient	0.323	0.680**	-0.231	1	0.833**
SAA	p value	0.082	0.000	0.219		0.000
	Correlation coefficient	0.621**	0.801**	-0.112	0.833**	1
RSDC Score	p value	0.000	0.000	0.554	0.000	

*p<0.05 and **p<0.01 are considered statistically significant. Fb: Fibrinojen, Hp: Haptoglobin, SAA: Serum Amyloid A

DISCUSSION

The present study was conducted in 7 farms with a high number of animals where RSDC was expected to be prevalent. As a matter of fact, from the anamnesis information, it was determined that the calves in the acute and chronic groups were housed in crowded and poorly ventilated barns. Additionally, none of the calves used in the present study had been vaccinated against RSDC. No external mineral and vitamin supplements or antiparasitic drugs had been used. Research has shown that stress factors, such as poor welfare standards can facilitate the development of RSDC in calves and the medical history data from the current study is consistent with these findings.[14,15] In the present study, it was observed that the breeders of the calves in the RSDC groups did not appropriately manage the herd under the right circumstances. The fact that the animals were often bought and sold and that new animals, namely from animal markets, were included into the herd without going through RSDC agent checks or quarantine processes, was also noticed. The probability of disease transmission was also increased in this situation, which was brought on by unrestrained animal circulation, making it simpler for illnesses to spread.

Acute-phase proteins are thought to be crucial markers for assessing the severity of an animal's infection and distinguishing whether the illness is acute or chronic.[16] In the current investigation, it was found that there were statistically significant differences (p<0.001) between the serum SAA, Hp, and Fb parameters of the diseased calves (acute and chronic group) and those of the control group calves (p<0.001). SAA, Hp, and Fb concentrations in calves with RSDC were higher compared to healthy calves in the present study and the obtained data were similar to previous studies on this topic.[17-19] On the other hand, in a study on calves showing clinical symptoms of RSDC, no significant changes were observed in the serum Hp and SAA concentrations at the beginning of the treatment process: But, a significant increase was reported from the 5th day of treatment.[20]An increase in Hp levels is reported to be proportional to the severity of the infection.[18]In the present study, on Day 0, the Hp values in the chronic group was significantly higher than the same values in the acute and control groups (p<0.001). These findings are consistent with previous similar studies.[19] Hp is commonly defined as an acute-phase protein by most researchers, but it is also suggested to increase in subacute and chronic cases.[19] In the present study, before treatment, SAA values in both acute and chronic groups were found to be significantly higher than those in the control group (p<0.001). The results obtained in this study are similar to previous research.[16]Although SAA is reported to be an important Acute Phase Protein (AFP) in horses, it is also reported to be an effective AFP in diagnosing infections in ruminants. SAA increases 2-5 hours after inflammatory stimulation and reaches its peak level within 24 hours. Due to this characteristic, it is reported to be effective in the early diagnosis of acute cases.[18]

In the present study, before treatment, Fb (Fibrinogen) value in the chronic group was significantly higher than both the acute and control group values (p<0.001). Furthermore, Fb value in the acute group was significantly higher than that in the control group. (p<0.001). Indeed, it is reported that Fibrinogen starts to rise 24-48 hours after infection and reaches its highest level in approxi-

mately 7-10 days. It can increase 2-5 times higher than normal plasma concentration, and after the inflammation subsides, it returns to normal levels within 2 weeks.[21]In our study, the chronic group fibrinogen value was still high on the 14th day after treatment, and it is thought that calves in this group may have secondary infections. Findings observed in the present study are in line with the results of previous studies.[10,20,22-26] These results obtained in the present study confirm that SAA and Hp values are suitable markers for the early detection of RSDC in field conditions, especially in cattle.[9,27]

In both patient groups, there was a significant decrease in serum Hp concentrations on the 7th and 14th days compared to the baseline ($p<0.001$). Despite this decrease, it was observed that even on the 7th day after treatment, serum Hp concentration was still higher than that in the control group. This finding suggests that Hp is an important biomarker in calves with RSDC.[28] In the present study, significant clinical improvement was observed in both patient groups in the days following treatment. This clinical improvement is consistent with previous similar studies.[29] In the current study, there was no significant decrease in Hp levels parallel to clinical improvement. The fact that Hp levels remained high after recovery in cattle is suggested to be due to the mixed infection nature of RSDC. The long-term increase in Hp levels in the patient groups in this study is compatible with the findings of similar studies.[30]

After inflammatory events, an increase in SAA levels has been reported.[27] In this study, it was also found that the SAA concentrations of the patient groups were significantly higher than those of the control group ($p<0.001$). It was determined that SAA values in the acute group significantly decreased on the 7th day after treatment compared to their pre-treatment values. Indeed, studies on cattle with natural or experimental RSDC have shown increased SAA values similar to those reported in this study.[22,29,30]

The serum Fibrinogen concentration of the calves in the chronic group was found to be significantly higher than that of the acute and control groups ($p<0.001$). The serum Fibrinogen value of the acute group was also found to be higher than that of the control group ($p<0.001$).

The results obtained regarding Fibrinogen values in this study are consistent with the findings of similar studies.[31]Fibrinogen is a positive acute-phase protein that reacts slowly after infection. In our study, the fact that Fibrinogen values in the chronic group were higher than those in the acute group suggests that the infection in the calves in the chronic group is still ongoing and severe. It is understood from our study that Fibrinogen levels decreased in both the acute and chronic groups with treatment. However, even on the 7th and 14th days after treatment, Fibrinogen values in the diseased calves were still higher than those in the control group. It is believed that more than 14 days is required for the Fibrinogen value in the diseased calves to return to normal. In chronic cases, Fibrinogen remains at a high concentration as long as the disease persists.[32]

In this study, it was understood that tulathromycin was successful in the treatment of both acute RSDC and chronic RSDC, which is consistent with earlier studies showing tulathromycin's efficacy in treating RSDC in cattle.[23]It can be concluded that, in the present investigation, acute-phase proteins Hp, SAA, and fibrinogen concentrations in serum significantly increased in cases of respiratory system illnesses in calves. It was also discovered that these parameters temporarily restored to normal following treatment.

Numerous research has been carried out to diagnose early and begin therapy in time in order to minimize the financial losses brought on by RSDC in calves. Periostin has been linked to a number of illnesses in recent years, including bone development, cancer, non-small cell lung cancer, breast, bladder, head/neck, oral, and pancreatic tumors, myocardial infarction recovery, and bone marrow fibrosis, as well as conditions like asthma and allergies in human medicine.[11,33-40] However, in the present study, the severity of clinical symptoms and the course of the disease were not correlated with serum Periostin levels, which were not significantly changed in calves with RSDC.

In the present study, on the 7th and 14th days after treatment, the Periostin values of the acute group were significantly lower than the control group values ($p<0.05$). The reduction in the Periostin level in the acute group could not be explained, butan increase or decrease in its level should not be disregarded, as it may also be an indicator of bone development in growing animals. In the present study, the calves in the control group were selected to be healthy and not to have previously experienced the disease, so they were composed of calves aged between 7-15 days. On the other hand, the diseased groups (acute and chronic groups) were composed of calves aged between 19-60 days. The calves in the control group were younger than the calves in both the chronic and acute groups. It is believed that higher Periostin values obtained from control animals might be related to age differences. On the other hand, no significant relationship was found between Periostin levels and clinical symptoms.

In human medicine, Periostin is considered as a marker for the development of fibrosis in the lungs. According to a study, patients with idiopathic pulmonary fibrosis produce more Periostin from their lung tissue than patients with interstitial lung disease or healthy individuals.[11] The absence of elevated periostin levels in the acute and chronic groups of calves in the present investigation may indicate that lung fibrosis did not manifest.

To the best of our knowledge, no studies in veterinary medicine have looked into the serum level of Periostin up until this point. However, immunohistochemically, the importance of Periostin has been investigated in sheep infected with Fasciola hepatica and in dogs with dermatitis.[41,42] Additionally, using protein analysis, it has been indicated that Periostin secretion increases in cattle during the pathogenesis of ketosis and hypocalcemia.[43] In pigs, Periostin levels have also been studied using PCR.[44]

CONCLUSION

Future research will clarify if this Periostin value will serve as a reference value or not. Periostin, however, cannot be employed as a biomarker in calves exhibiting clinical symptoms of RSDC, based on the information gathered in the present study. Moreover, it should be noted that the limited number of animals used in the present study can be considered as a weakness of the

study. To fully understand the diagnostic and prognostic importance of Periostin in calves, further detailed studies are needed in more animals and different diseases.

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