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Evaluation of The Efficiency of Glutaraldehyde Coagulation Test in Some Cattle Diseases: 2021-2022 Retrospective Study

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Abstract: Metabolic disorders occurring in negative energy balance (NEB) in cows cause diseases related to immunosuppression and economic losses. Although treatment, animal welfare, and herd immunity are possible with comprehensive clinical diagnostics combined with laboratory evaluation, the applicability and availability of these analytes in the farm setting are often limited. Therefore, the glutaraldehyde coagulation (GC) test, a fast, practical, and inexpensive test, help diagnose inflammatory diseases in cattle. This study aims to evaluate the effectiveness of the GC test in certain bovine diseases whose etiology is classified as infectious or non-infectious due to clinical and laboratory examinations. The animal material was 40 Holstein cows with various clinical findings brought to Harran University, Veterinary Faculty Animal Hospital for diagnosis and treatment. Following the anamnesis, physical examinations of all the cows were performed. As a result of the hemogram analysis of the venous blood samples (8-10 mL) along with the physical examination findings, the cows were divided into two subgroups, Infectious (n:14) and Non-Infectious (n:26); GC test was performed from the venous blood samples. In the physical examination, the respiratory rate, heart rate, and body temperature of the cows in the Infectious Group were higher than those of the Non-Infectious Group ($p<0.008$). WBC and granulocyte levels of the Infectious Group were higher than the Non-Infectious Group ($p<0.000$). The GC test result was 70 seconds in the Infectious Group and 165 seconds in the Non-Infectious Group ($p<0.001$). The optimal cut-off value was 100 seconds (sensitivity: 87%, specificity: 72.7%) for detecting the presence of an inflammatory process in the ROC analysis. As a result, it was concluded that the GC test might give results ranging from mild to severe even in some non-infectious diseases such as rumen alkalosis, left displacement of the abomasum and ulcer, and when evaluated along with clinical findings in a farm setting, it can provide helpful information for the diagnosis of the diseases.
Keywords: *Cattle, Diagnosis, Glutaraldehyde coagulation test, Infectious, Inflammatory.*

Bazı Sığır Hastalıklarında Gluteraldehit Koagulasyon Testinin Etkinliğinin Değerlendirilmesi: 2021-2022 Retrospektif Çalışma

Özet: İneklerde negatif enerji dengesinde (NED) oluşan metabolik hastalıklar, immunsupresyona bağlı hastalıklara sebep olup ekonomik kayıplara yol açmaktadır. Hastalıkların tedavisi, hayvan refahı ve sürünün korunması, kapsamlı klinik diagnostikler ile birlikte laboratuvar değerlendirilmesi ile mümkün olsa da çiftlik ortamında bu analitlerin uygulanabilirliği ve bulunabilirliği genellikle kısıtlıdır. Bu sebeple hızlı, pratik ve ucuz bir test olan gluteraldehit koagulasyon (GK) testi, sığırların yangısal hastalıklarının tanısında faydalıdır. Bu çalışmanın amacı, klinik ve laboratuvar muayeneleri sonucu hastalık etiyolojisi enfeksiyöz veya non-enfeksiyöz olarak sınıflandırılmış bazı sığır hastalıklarında GK testinin etkinliğini değerlendirmektir. Hayvan materyali Harran Üniversitesi Veteriner Fakültesi Hayvan Hastanesine çeşitli klinik bulgularla, tanı ve tedavi amacı ile getirilmiş toplam 40 adet Holstein inekti. Anamnez bilgisini takiben tüm ineklerin fiziksel muayeneleri gerçekleştirildi. Fiziksel muayene bulguları ile birlikte alınan venöz kan örneklerinin (8-10 mL) hemogram analizi sonucu, çalışmaya dahil edilen inekler Enfeksiyöz (n:14) ve non-Enfeksiyöz (n:26) olarak iki alt gruba ayrıldı, tüm ineklerin venöz kan örneklerinden GK testi gerçekleştirildi. Fiziksel muayenede Enfeksiyöz Grubu ineklerinin solunum sayısı, kalp ritmi ve vücut sıcaklığı Non-Enfeksiyöz Gruba göre daha yüksekti ($p<0.008$). Enfeksiyöz Grubun WBC ve granülosit düzeyleri Non-Enfeksiyöz Gruba göre daha yüksekti ($p<0.000$). GK testi sonucu Enfeksiyöz Grubunda 70 saniye iken Non-Enfeksiyöz Grubunda 165 saniyeydi ($p<0.001$). ROC analizinde yangısal süreç varlığının tespiti için optimal cut-off değeri 100 saniyeydi (sensitivite: %87, spesifite: %72.7). Sonuç olarak, GK testinin enfeksiyöz etiyolojide olmayan rumen alkalozu, abomazumun sola deplasmanı ve ülseri gibi bazı hastalıklarda bile hafif şiddetliden şiddetliye değişen sonuç verebileceği ve çiftlik ortamında klinik bulgularla birlikte değerlendirildiğinde hastalık tanısına yardımcı bilgi sağlayabileceği kanısına varıldı.
Anahtar Kelimeler: *Enfeksiyöz, Gluteraldehit koagulasyon testi, Sığır, Tanı, Yangısal.*

Introduction

It is a well-known fact that negative energy balance (NEB) and immunosuppression are the endemic insidious underlying causes of cattle diseases in herds and cause significant economic losses

(Abramowicz et al., 2019; Lei and Simoes, 2021; Mostert et al., 2018). Metabolic diseases such as fatty liver and ketosis occur in cows due to the development of NEB (Mostert et al., 2018). There is an increase in the amount of non-esterified fatty acids (NEFA) due to the formation of fatty liver, and these fatty acids are esterified to triglycerides as a result of the oxidation of hepatic fatty acids. It has been reported that ketosis, hypocalcemia, reticulo pericarditis traumatica (RPT), retention, metritis, mastitis, and increased risk of immunosuppression and decreased reproductive performance can be seen as a result of fatty liver (Lei and Simoes, 2021).

Immunosuppression, which develops with metabolic diseases, especially in the NEB period, causes many diseases and economic burdens (Liang et al., 2017; Zhang and Ametaj, 2020). Immunity should be boosted to prevent the occurrence of diseases in this period. It is well-known that the modern cattle industry is based on balanced nutrition, appropriate zoo-hygienic care, animal welfare, and herd protection (Abramowicz et al., 2019; Roche et al., 2013). The persistence of this is possible with comprehensive clinical diagnostics and laboratory evaluation. Applicability and availability of clinical assessment methods, particularly in the farm setting, are often limited, and in addition to this, economic concerns come forth. Thus, the diagnostic effectiveness and practicality of these methods used diagnosing of various diseases have been discussed recently (Ok et al., 2009; Overton et al., 2017). The glutaraldehyde coagulation (GC) test is a fast, practical, and inexpensive test used to diagnose of inflammatory diseases in cattle for years (Aslan and Ok, 1991; Metzner et al., 2007). The GC test primarily indicates increased serum fibrinogen and globulin concentrations. Glutaraldehyde reacts first-degree chemical reaction with the free amino groups in fibrinogen and immunoglobulin to form a clot. Thus, the GC test's clotting time provides an estimated amount of the protein content produced in response to the inflammatory process (Akyüz ve Aydın, 2022; Tschone et al., 2021, Tschone et al., 2022).

This study aims to evaluate the diagnostic efficacy of GC tests in some bovine diseases whose etiology is classified as infectious or non-infectious due toclinical examinations along with hemogram analysis.

Materials and Methods

Ethical approval: This study was conducted with the approval of the Harran University Animal Experiments Local Ethics Committee dated 17/10/2022, number 2022/008.

Animal Material: The animal material of this study consisted of 40 Holstein cows with various clinical findings; all were admitted to Harran University Veterinary Faculty Animal Hospital for diagnostic and/or treatment purposes between the dates 2021 and 2022. Following the anamnesis, physical examinations including measurement of respiratory rate, heart rate, rectal body temperature, and evaluation of palpable lymph nodes along with lung, heart, rumen, and intestinal auscultation were performed. As a result of the detailed clinical assessment, further diagnostic examinations were carried out to determine whether the existing disease was infectious or non-infectious.

Diagnosis of Health Issues: Diseases detected in cows of the Infectious Group were pneumonia (n:7) and enteritis (n:7). Diagnosis of pneumonia was made based on the presence of fever, nasal discharge, cough, and changed respiratory character (Demir and Bozukluhan, 2012). For the diagnosis of enteritis, clinical findings such as diarrhea and dehydration were considered (Bonadiman et al., 2018).

Diseases detected in cows of the Non-Infectious Group were abomasum ulcer (n:2), presence of bezoars (n:1), downer cow (n:1), Hoflund syndrome (n:1), simple indigestion (n:2), hepatic lipidosis (n:6), reticulo pericarditis traumatic (RPT) (n:7), rumen alkalosis (n:4), subcutaneous emphysema (n:1) and left displacement of the abomasum (LDA) (n:1). For the diagnosis of abomasum ulcer, the presence of clinical findings such as abdominal pain, melena, pale mucous membranes, indigestion, and low milk yield were considered (Radostitis et al., 2000). For the presence of bezoars, ultrasonographic imaging with cessation of defecation, loss of appetite, abdominal pain, decreased rumen motility, and succussion splash sound in the abdomen was considered (Abutarbush and Naylor, 2006). For the diagnosis of Downer cow syndrome, the inability to stand up within 24 hours and/or lie in the sternal position for more than 24 hours without showing signs of systemic disease, despite hypocalcemia treatment, were taken as the criterion (Dahlberg, 2012). For Hoflund syndrome, posterior functional stenosis with rumen dilatation and nutritional fullness, recurrent tympani, gradual weakness, and bradycardic symptoms were considered (Radostitis et al., 2007). For the diagnosis of simple indigestion, the presence of abrupt changes in the structure, amount, or frequency of diet in the anamnesis along with anorexia and decrease in milk yield, and abnormal rumen content findings such as a decrease in the number, size, and activity of protozoa and change in ruminal pH were considered (Constable, 2010). The criteria for the diagnosis of hepatic

lipidosis were increased liver size, rounding of liver borders, hyperechoid structure of the liver parenchyma towards the abdominal wall, and inadequate visualization of hepatic vessels as a result of ultrasonographic examination performed through the 10th intercostal space on the left side (Ok et al., 2013). For the diagnosis of RPT, fever, anorexia, increased heart and respiratory rate, ruminal stasis, frequently recurring tympani, abdominal tension, abdominal pain, groaning, dehydration and weakening along with tachycardia, venous engorgement, positive venous pulse, heart churning or rubbing sound, and the presence of edema in the submandibular region were considered (Roth and King, 1991). For the existence of rumen alkalosis, the presence of pH >7.2 with a decrease in the movement and number of rumen infusoria was accepted (Guzelbekteş and Şen, 2014). For the diagnosis of subacute emphysema, painless subcutaneous crepitation on palpation, dyspnea, and loss of appetite were considered (Muhammad et al., 2017). For LDA, clinical findings such as sudden onset anorexia, presence of ping sound on auscultation, low milk yield, moderate abdominal pain, and costal posterior enlargement of the left fossa paralumbalis were evaluated (Fouda et al., 2004). In addition, the suspicion of LDA was confirmed by visualization of the abomasum on ultrasonographic examination.

Hemogram Analysis: Hemogram analysis was carried out from venous blood samples (8-10 mL, in K_3EDTA tubes) taken by vena jugularis venepuncture from all cows included in the study, and the analysis was performed using an autoanalyzer (Sysmex Hematology Analyser, poch-100i, Japan) within 5-10 minutes after sampling. Within the scope of hemogram analysis, leukocyte (WBC), granulocyte, lymphocyte, and monocyte levels were evaluated.

Forming Subgroups: The results of physical examination and hemogram analysis were used to determine whether the present health issues were infectious or non-infectious and to establish subgroups. Thus, the cows included in the study were divided into two subgroups Infectious (n:14) and Non-Infectious (n:26) groups.

Performing Glutaraldehyde Coagulation Test: A glutaraldehyde coagulation (GC) test was performed on all the cows' venous blood samples, divided into subgroups. For this reason, we used a solution prepared from 50 mL of 25% glutaraldehyde, 1 g of Na_2EDTA, and 1 liter of 0.9% NaCl solution (saline). The components were mixed homogeneously and maintained in dark glass receptacles for optimal storage. For the test, 2 mL of blood was collected and mixed with 2 mL of the aforementioned glutaraldehyde solution in an anticoagulant collection tube (K_3-EDTA) (Doll et al., 1985). The GC was measured for up to 15 minutes or until coagulation (Doll et al., 1985). We used a digital chronometer to keep time. We interpreted the results as 0 – ≤ 3 minutes for severe, >3 – ≤ 6 minutes for moderate, >6 – ≤15 minutes for mild, and > 15 minutes for a non-detectable inflammatory process (Aslan ve Ok, 1991; Doll et al., 1985; Turgut, 2000). GC test results of all the cows included in the study are presented in Table 3.

Statistical Analysis: All data were evaluated using SPSS 25.00 (SPSS for Windows) statistical software. A one-sample Kolmogorov–Smirnov test was used to determine whether all data were parametric or nonparametric. Nonparametric data were presented as median (min-max) using the Mann–Whitney U, Kruskal-Wallis test. To investigate how accurately the present diagnostic glutaraldehyde coagulation test discriminates the presence of an inflammatory state and to determine optimal sensitivity, specificity, and cut-off values, receiver operating characteristic curve (ROC) analyses were performed. Statistical significance was considered at a $p<0.05$ for all data.

Results

In the physical examination, the respiratory rate, heart rate, and body temperature of the cows in the Infectious Group were higher than that of the Non-Infectious Group ($p<0.008$). Physical examination findings are presented in Table 1.

As a result of hemogram analysis, it was determined that WBC and granulocyte levels of the Infectious Group were higher than the Non-Infectious Group ($p<0.000$). There was no statistical difference between the groups regarding lymphocyte and monocyte levels. Hemogram analysis results are presented in Table 2.

As a result of the GC test, the coagulation time of the Infectious Group was determined as 70 seconds, while the duration of the Non-Infectious Group was 165 seconds ($p<0.001$) (Table 3). In addition, as a result of the receiver operating characteristic (ROC) analysis, it was determined that the optimal cut-off value was 100 seconds (sensitivity: 87%, specificity: 72.7%) for the determination of the presence of an inflammatory process. The ROC curve is presented in Figure ,1 and ROC analysis results are shown in Table 4.

Discussion and Conclusion

Studies have shown that the GC test is a helpful test that maintains its importance among diagnostic tools for a long time without losing its currency (Akyüz and Aydın, 2022; Aslan and Ok, 1991;

Metzner et al., 2007; Tschone et al., 2021, Tschone et al., 2022). The easily accessible, cheap, and fast results make the test frequently preferred in determining the presence of inflammation,

Table 1. Physical examination findings.

Parameters	Infectious Group (n: 14) median (min-max)	Non-Infectious Group (n:26) median (min-max)	p value
Respiratory rate (breath/min)	64 (35-80)	37 (22-72)	0.000
Heart rate (beats/min)	80 (64-108)	64 (48-84)	0.000
Body temperature (°C)	39.1 (37.9-40.2)	38.4 (37.1-40)	0.008

Table 2. Hemogram analysis results.

Parameters	Infectious Group (n: 14) median (min-max)	Non-Infectious Group (n:26) median (min-max)	p value
WBC ($x10^9$ cells per liter)	**15.65 (14.1-29.5)**	**9.9 (4.9-18.5)**	**0.000**
Granulocyte ($x10^9$ cells per liter)	**11.95 (11.3-24.9)**	**6.1 (2.2-12.4)**	**0.000**
Lymphocyte ($x10^9$ cells per liter)	2.6 (2.1-5)	2.3 (0.8-8.1)	0.745
Monocyte ($x10^9$ cells per liter)	0.65 (0.2-1.1)	0.5 (0.2-1.7)	0.582

Table 3. Intergroup glutaraldehyde coagulation test results.

Parameters	Infectious Group (n: 14) median (min-max)	Non-Infectious Group (n:26) median (min-max)	p value
Glutaraldehyde coagulation time (seconds)	70 (30-280)	165 (60-1020)	0.001

Table 4. Diagnostic ROC analysis result of the glutaraldehyde coagulation test.

Area Under Curve	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval		Cut-off	Sensitivity	Specificity
			Lower Bound	Upper Bound			
0.866	0.068	0.001	0.733	0.998	100	87%	72.7%

The test result variable(s): Glutaraldehyde coagulation test has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a]Under the nonparametric assumption

[b]Null hypothesis: true area = 0.5

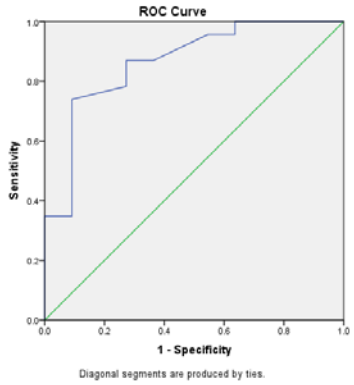

Figure 1. Diagnostic ROC curve of the glutaraldehyde coagulation test. Diagonal segments are produced by ties.

especially in a farm setting (Aslan and Ok, 1991; Turgut, 2000). In the present study, a significant difference was observed in GC test results between the Infectious and Non-infectious groups ($p<0.001$). Also, it was observed that the GC test is useful in understanding whether the diseases are of infectious etiology. According to the diagnostic ROC analysis, the cut-off value of the GC test in the differentiation of infectious and non-infectious diseases was determined as 100 seconds, considering the optimal sensitivity (87%) and specificity (72.7%) values (Table 4).

Respiratory system diseases of cattle are the most common diseases that harm the herd health and livestock industry (Peel, 2020). It is reported that the mortality of bovine respiratory system diseases in the United States causes an annual loss of 907 million United States dollars (USDA, 2017). The fact that respiratory system diseases cause high economic losses and are observed most frequently indicates that there is a need for up-to-date studies on the subject. A previous study reported that the GC test result was 2 minutes in cattle with pneumonia (Metzner et al., 2007). In a study of 30 calves in which the GC test was evaluated, it was reported that the coagulation time was less than 3 minutes (severe) in 18, between 3-6 minutes in 9 (moderate), and 6-15 minutes in 3 (mildly severe) out of 30 calves (Akyüz et al., 2022). The GC test time of the present study was 70 (35-90) seconds in cows of the Infectious Group was classified as severe, and the results were consistent with previous studies.

In cattle, especially calves, enteritis and diarrhea are important herd health problems, with the highest mortality causing significant economic loss following respiratory system diseases (Aygün and Yıldız, 2018;). In the literature review, we could not find a study in which the GC test was evaluated in cases of enteritis with an infectious etiology. However, it was reported that the GC test result ranged between 21-36 minutes (negative) in cases of enteritis due to alimentary simple indigestion etiology (Uhde et al., 2008). In the present study, the GC test result of cows in the Non-Infectious Group with simple indigestion was determined as 165 (70-200) seconds (severe). Compared with previous studies, the shortened GC time of the present study may be related to the amount and composition of feed consumed, and the time to hospital admission, which may affect the severity of the inflammation. In addition, due to the working principle of the GC test, a negative test result is a typical situation where the fibrinogen and globulin levels do not increase and/or slightly increase (Turgut, 2000). In the present study, the 70 (30-280) seconds (severe) GC time of the cattle with infectious enteritis was associated with increased amounts of globulin and fibrinogen during the infectious process compared with the alimentary enteritis cases. Investigation of the GC test in more cattle with enteritis of infectious etiology is recommended to demonstrate the diagnostic efficiency of the test.

Abomasum ulcer in cattle is an important condition that causes various clinical findings according to its grade, and diffuse peritonitis is a common finding. In cases of type-4 ulcers, the prognosis is unfavourable, and death usually occurs within 24-48 hours (Braun et al., 2019a, Braun et al., 2019b). In a study conducted in cattle with abdominal ulcers, GC test results were severe in 14% (<3 min), moderate in 17% (3-6 min), mild (6-15 min) in 67%, and negative in 2% (>15 min) (Braun et al., 2019 b). The GC time of the cattle with abomasum ulcers in the Non-Infectious Group of the present study was determined as 165 (104-230) seconds (severe). The higher values in the present study may be related to the grade of abomasal ulcer. It was reported that the severe GC test results in the previous study were observed in cows with third-grade abomasal ulcers (Braun et al., 2019a). Abomasal perforation was detected in two of the present cases, characterized by antemortem findings such as severe melena and pale mucous membranes and were referred to slaughter; thus, they were evaluated as grade 4. For this reason, more severe and widespread inflammation in grade 4 ulcer types causes a severe GC test result. Although the GC test results in cows with abomasum ulcer was defined as severe in the present study, it is recommended to investigate the diagnostic efficacy of the GC test in a study in which abomasum ulcers are classified according to their grade.

Reticuloperitonitis traumatica (RPT) is an important condition that causes different degrees of inflammation and complications in cattle due to sharp foreign bodies ingested with feed, penetrating the reticulum and damaging various tissues and organs (Braun et al., 2020). In a study, GC test results

were found to be severe in 80%, moderate in 15%, and mild in 5% of the group with adhesive RPT. In the same study, it was reported that in cows with non-adhesive RPT, 30% were severe, 45% were moderate, and 25% were mild (Akyüz and Aydın, 2022). In a study conducted in cases with acute and chronic RPT, it was reported that the GC time was 191 seconds in acute and 131 seconds in chronic cases (Ozba, 1996). In the present study, it was determined that the GC test result was 145 (33-360) seconds (severe) in cattle with RPT of the Non-Infectious Group (6 severe, 1 moderate). These findings may be related to the severe, acute, adhesive nature of RPT and the delayed admission time to the hospital.

Functional gastric stenosis (Hoflund syndrome) is a transitional disorder characterized by inhibition of gastric passage. Compression and/or damage of the various branches of the vagus nerve innervating the stomach prevent passage in the pylorus, ostium reticulomasi and/or cardia parts of the stomach and as a result, the disease occurs (Bilal, 2004; Gul and Issi, 2009). The results of the present study were in agreement with the previously reported results (Gül ve Issi, 2009). These findings may be related to the fact that denervation in the present case was associated with the presence of RPT (Bilal, 2004).

Although the presence of bezoar varies according to its localization, it can cause functional stenosis similar to Hoflund syndrome. In addition to stenosis, it can cause compression ischemia, and consequently necrosis, and gangrene in the affected region. Thus, the onset of an inflammatory process due to compression is expected (Cortés-Beltrán et al., 2022; Tschuor et al., 2010). Also, the results of the GC test may vary according to the severity of the inflammation in the region where the bezoars are located. The cows affected by the presence of bezoars in the present study had a GC time of 15 minutes (mild). The mild GC test result detected in the present study may be associated with ischemia and necrosis caused by the bezoar that initiates the inflammatory process (Tschuor et al., 2010).

Left displacement of the abomasum (LDA) is an important postpartum disease, especially in high-yielding dairy cows (LeBlanc et al., 2005). In a study conducted on 52 cows diagnosed with LDA, the GC test results were determined as moderately severe (6.5 (0.5-16) minutes) (Tschoner et al., 2021). In the present study, there was only 1 case with LDA, and the test was described as mild (10.5 minutes). The very different GC values in the previous study may indicate that the inflammatory process is related to the displacement's time of occurrence and severity. Since it was the first day of the cow's complaints in the present study, a mild GC test was considered as an expected result.

Although subcutaneous emphysema is mainly related to respiratory distress, it can also develop due to trauma, operative interventions, and gaseous gangrene (Rashid and Saqi, 2017). In the literature review, no study was found in which the GC test was evaluated in cows with subcutaneous emphysema. However, it was reported that the severity of inflammation and clinical findings that may occur in the cattle are highly variable depending on the etiology (Rashid and Saqi, 2017). GC test result was negative (17 min) in the cow with subcutaneous emphysema of the present study. Anamnestic data revealed that this case occurred due to traumatic reasons. During the physical examination, it was observed that the affected area was scanted and could not contribute significantly to generalized inflammation. Therefore, it was considered normal that the GC test did not result in a positive.

Causes such as sudden consumption of protein-rich feed in a short time, feeding with excessive amounts of urea, sudden ration changes, and providing large amounts of straw for a long time raise the rumen pH to 7.5 and above. This condition is called rumen alkalosis. (Vijayakumar et al., 2010). In the literature review, no study was found in which the GC test was evaluated in cows with ruminal alkalosis. Since the increase in rumen pH causes a decrease in the number of saccharolytic and amylolytic microorganisms and an increase in E. coli, Pseudomonas and Proteus group bacteria, the severe and moderate cases of the present study was associated with the inflammatory process due to overgrowth of these aforementioned bacteria (Guzelbekteş and Şen, 2014).

Especially in the first period of the postpartum period, the occurrence and prolongation of NEB state in high-yielding dairy cows increase the risk of hepatic lipidosis (Collins and Reid, 1980). Ketone bodies, which can reach pathological levels in the blood circulation as a result of NEB, cause generalized inflammation with metabolic acidosis (Ingvartsen, 2006; Lei and Simoes, 2021). Therefore, a positive GC test result is expected. In the Non-Infectious Group of the present study, 4 severe (128 (60-160) seconds) and 2 moderate (270 (180-360) seconds) cases were observed. These findings may be related to the presence of endotoxin, which enters the blood circulation from the rumen and contributes to the development of hepatic lipidosis and the activation of the immune system and acute phase response in cases of hepatic lipidosis (Ametaj, 2005).

This study has some limitations. Although the small sample size scenario is common in medical tests, a comprehensive study of small sample size properties of various methods for constructing the confidence/credible interval (CI) for the AUC has yet

to be included in the literature. As previously reported (Feng et al., 2017), it was observed that the larger the true AUC value and the smaller the sample size, the larger the discrepancy among the results of different approaches. Therefore, the major limitation of this study is the limited number of animals which may influence the results of the ROC-based diagnostic performance analyses. Hence, the authors recommend evaluating the promising results of this study with a larger number of animals with various diseases.

In the present study, the diagnostic efficacy of the GC test was evaluated along with clinical examinations and hemogram analysis results in infectious diseases such as pneumonia and enteritis and non-infectious diseases such as ulcer of the abomasum, presence of bezoars, downer cow, Hoflund syndrome, hepatic lipidosis, rumen alkalosis, and LDA. Conducting future studies on diseases that were evaluated in the present study, but for which the GC test has not been evaluated yet, may increase the effectiveness and prevalence of the GC test. Although the current study results are promising, there is a need for studies investigating the diagnostic and prognostic efficacy of GC tests with more animal material. As a result, it was observed that the GC test might give results ranging from mild to severe even in some non-infectious diseases such as rumen alkalosis, left displacement and, ulcer of the abomasum. It was concluded that, especially in the farm environment where comprehensive laboratory analyzes are difficult to perform, when evaluated along with the clinical findings, the GC test could provide helpful information for the diagnosis and prognosis of various diseases.

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

Permission was obtained for this study with the approval of the ethics committee of Harran University HADYEK 01-05, session number 2022/008 and dated 17.10.2022. In addition, the authors declared that they comply with the Research and Publication Ethics.

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