Evaluation of dysrhythmias and myocardial biomarkers in high and low-yielding dairy cows

Jafari Dehkordi, A.1*, Mohebbi, A.N.2, Balali Dehkordi, Sh.3

1Department of Large Animal Internal Medicine, Veterinary Faculty of Shahrekord University, Shahrekord, Iran
2Department of Veterinary Clinical Pathology, Veterinary Faculty of Shahrekord University, Shahrekord, Iran
3Graduated from the Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Key words:
dairy cows, dysrhythmias, myocardial biomarkers

Abstract:
BACKGROUND: Cardiovascular system is a very important organ that plays a vital role in tissue function. In farm animals, the growth and high milk production depends on function of cardiovascular system. OBJECTIVES: Cardiovascular health in high and low-yielding dairy cows was investigated. METHODS: Fifty 4-year-old high- yielding Holstein dairy cows and fifty 4-year-old low- yielding Holstein dairy cows were used in this study. Electrocardiogram was recorded by a base- apex lead, and blood samples were collected from the jugular vein for the measurement of cardiac biomarkers (CK (Creatine Kinase), CK-MB (Creatine Kinase-Myocardial Band), LDH (Lactate Dehydrogenase), and AST (Aspartate Aminotransferase) and troponin I). RESULTS: Cardiac dysrhythmias were detected more in low- yielding Holstein dairy cows (62%) compared to high- yielding Holstein dairy cows (46%). The cardiac dysrhythmias that were observed in low- yielding Holstein dairy cows included sinus arrhythmia (34.7%), wandering pacemaker (22.45%), sinus bradycardia (18.37%), sinus tachycardia (10.20%), atrial premature beat (2.04%), sinoatrial block (2.04%), atrial fibrillation (8.16%), and atrial tachycardia (2.04%). The cardiac dysrhythmias were observed in high- yielding Holstein dairy cows, including sinus arrhythmia (86.95%) and wandering pace maker (13.05%). Also, notched P wave was observed in high- and low- yielding Holstein dairy cows, 30% and 14% respectively. The amount of cardiac biomarkers in the low yielding cows was significantly higher than that of the high yielding cows. Further more, there was not any detectable significant difference of serum concentration of total CK between the high and low- yielding Holstein cows. CONCLUSIONS: Despite significant differences in cardiac biomarkers and based on the normal range of cardiac biomarkers in both groups, the increase in cardiac dysrhythmias in low- yielding Holstein dairy cows may be metabolic and electrolyte disorders.

Introduction

The primary function of the cardiovascular system is to ensure an adequate circulation of blood so that nutrients are delivered, waste products are removed, and a homeostatic milieu is maintained at the organ and cellular level. An inadequate circulation interferes with nutrient delivery and waste product removal, and ultimately leads to circulatory failure, the primary concept in diseases of
the cardiovascular system (Radostits et al., 2007).

ECG is the best way to measure and diagnose abnormal rhythm of the heart, particularly abnormal rhythm caused by damage to the conductive tissue that carries electrical signals, or abnormal rhythm caused by electrolyte imbalances. In a myocardial infarction (MI), the ECG can identify if the heart muscle has been damaged in specific areas, though not all areas of the heart are covered. The ECG device detects and amplifies the tiny electrical changes on the skin that are caused when the heart muscle depolarizes during each heartbeat (Smith, 2009; Rezakhani et al., 2010).

The electrocardiogram (ECG) is used primarily to detect cardiac arrhythmias in large animals. For this purpose, a single channel machine can be used, and the lead system chosen can be any that generates distinctive QRS and T complexes. Two leads commonly used for the diagnosis of cardiac arrhythmias are the base-apex lead I and the Y lead of the orthogonal lead system arrhythmias result from abnormalities of impulse generation or impulse conduction or a combination of both. A variety of mechanisms can cause abnormal impulse generation or conduction. Abnormal impulse generation occurs because of localized changes in ionic currents that flow across the membranes of single cells or groups of cells. Abnormal impulse generation can be seen as automatic (normal and abnormal) or triggered activity (Edwards, 1993; Rezakhani et al., 2004; Radostits et al., 2007; Smith, 2009).

Cardiac markers are biomarkers measured to evaluate heart function. They are often discussed in the context of myocardial infarction; however, there are other conditions that can lead to an elevation in cardiac marker level. Most of the early markers identified were enzymes, and as a result, the term "cardiac enzymes" is sometimes used. However, not all of the markers currently used are enzymes. For example, in formal usage, troponin would not be listed as a cardiac enzyme (Laterza et al., 2008; Leonardi et al., 2008).

Therefore, a number of tissue enzymes are valuable tools as diagnostic agents for heart disease such as: AST, LDH, CK, CK-MB, and troponin (Fredericks et al., 2001).

The serum concentration of cardiac troponin I provides an excellent cardiac biomarker in large animals, providing a sensitive and persistent indicator of cardiac injury. Troponin I, T, and C are components of the tropomyosin - troponin complex in cardiac and skeletal muscle, with cardiac troponin I and T having different amino acid sequences at the N-terminal and compared to skeletal muscle troponin I and T. This means that an immunosassay directed at the N-terminal end will be able to differentiate between cardiac and skeletal muscle isoforms and the site of injury (Roberts, 1998; Radostits et al., 2007; Serra et al., 2010).

Serum activities of cardiac isoenzymes of creatine kinase (creatine kinase isoenzyme) MB (CK-MB) and lactate dehydrogenase (isoenzymes 1 and 2) have been used in the past as indices of cardiac disease in large animals (Reimers et al., 1997; Radostits et al., 2007).

Cardiovascular system is a very important organ that plays a vital role in tissue function. In farm animals, the growth and high milk production depends on the function of cardiovascular system. Therefore, it is important to investigate cardiovascular health in high and low-yielding dairy cows based on determination of dysrhythmias and cardiac biomarkers in high and low-yielding dairy cows.

**Materials and Methods**

Fifty 4-year-old high-yielding Holstein dairy cows and fifty 4-year-old low-yielding Holstein dairy cows were used in this study (the animals were healthy without clinical signs of any organ abnormalities in the clinical examination). All work was performed in a large dairy farm (Zagros Dairy farm, Shahrekord, Iran).

The average 305-day milk yield of high-yielding and low-yielding Holstein dairy cows were 10600 and 6000 kg, respectively, with 3.5% fat and 3.25% protein (100 days in milk). Cows were fed according to their requirements for maintenance and milk production (NRC, 2001). The ration consisted of high quality roughages (maize silage and sugar beet pulp), soybean meal, concentrates (corn, barley), and mineral and vitamin supplement.

For ECG recording, each cow was kept in a stock and allowed to settle for 10 minutes. The base-apex lead was used to detect the arrhythmias; it was attached using positive, negative, and ground leads as
follows:

a) The positive electrode was attached to the skin over the left fifth intercostal space at the point of maximal intensity (PMI) of the apex beat; using lead I, this is the left arm electrode.

b) The negative electrode was attached to the skin of the left jugular furrow two thirds of the distance from the ramus of the mandible to the thoracic inlet; using lead I, this is the right arm.

c) The earth electrode was placed to the skin of the left flank.

After spraying the area with ethanol as a degreasing agent, we attached the electrodes to the specific position.

The ECG was recorded from 3 to 5 minutes (Animal in a relaxed state) while the cows kept in a standing position. The ECGs were obtained on a single channel machine (Kenz-ECG 110 Class I, Japan) with the paper speed 25mm/sec and calibration of 10 mm equal to 1mV. All of the ECGs were inspected by two of the authors independently, and finally both authors explained all dysrhythmias together. Blood samples were collected from the jugular vein and delivered to laboratory. The serum was separated and stored at -20°C until measurement of cardiac biomarkers.

The following step by-step approach can be used:

1. Identify all the QRS complexes. Each QRS complex should be followed by a T wave, and the QT interval should be similar for all QRS configurations, unless there is a marked change in heart rate. Identify the remaining complexes. Are P waves, "F" (flutter) waves, or "f" (fibrillation) waves present? Are there any artifacts?

2. Determine the atrial and ventricular rates. Are they identical? Is one too fast or too slow? This step determines whether there is a tachycardia or bradycardia.

3. Are the P-P and R-R intervals regular? Determine whether an irregular rhythm has underlying regularity that is interrupted by irregular intervals or whether the rhythm is consistently irregular. Second-degree AV block and atrial and ventricular premature beats are arrhythmias with underlying regularity, whereas atrial fibrillation, sinus arrhythmia, and sinus arrest are truly irregular rhythms.

4. Are P waves present? If so, is there a P wave preceding every QRS complex? If not, there are premature depolarizations, escape beats, or atrial fibrillation. Are all P waves followed by QRS complexes? If not, second degree AV block may be present. Is the resultant P-R interval constant? If not, there may be a first-degree AV block.

5. Are all P waves and QRS complexes identical or normal in contour? If not, this signifies more than one pacemaker, premature depolarizations, or escape beats (Radostits et al., 2007; Smith, 2009).

Blood samples were collected from the jugular vein after recording ECG. The blood serum and plasma were obtained by centrifugation at 2500g for 15 minutes. The serum and plasma samples were preserved at -20°C until analysis. The activity of AST, LDH, CK, CK-MB, and troponin I were determined. Serum aspartate aminotransferase (AST), creatine kinase (CK), and myocard originating creatine kinase (CK-MB) levels were measured spectrophotometrically by IFCC method using commercial test kits (pars azmoon kits Company, Iran) as instructed by producers. Serum lactate dehydrogenase (LDH) was measured spectrophotometrically by using commercial test kit (Darman kave kit Company, Iran) as instructed by producers. Concentration of troponin I was determined by ultra immunoassay with commercial test kit (Monobind Inc. Lake Forest, CA 92630, USA) [normal range, less than 1.3 ng/mL].

Data were analyzed by one-way ANOVA to determine significant difference. Probability of p<0.05 was considered to be statically significant.

Results

ECGs were recorded from fifty 4-year-old high-yielding Holstein dairy cows and fifty 4-year-old low-yielding Holstein dairy cows. The types and number of cardiac dysrhythmias in both groups are given in Table 1. The mean concentration of CK, CK-MB, LDH, AST, and troponin I in both groups are given in Table 2.

Cardiac dysrhythmias were detected more in the low-yielding Holstein dairy cows (62%) as compared to the high-yielding Holstein dairy cows (46%).

The cardiac dysrhythmias that were observed in the low-yielding Holstein dairy cows included sinus arrhythmia (34.7%) Figure 1, wandering pace maker
Figure 1. Sinus arrhythmia and wandering pace maker in high yielding Holstein cow.

Figure 2. Bradycardia in low yielding Holstein cow.

Figure 3. Atrial tachycardia in low yielding Holstein cow.

(22.45%) Figure 1, bradycardia (18.37%) Figure 2, tachycardia (10.20%) Fig. 3, atrial premature beat (2.04%) Figure 4, sinoatrial block (2.04%) Figure 5, atrial fibrillation (8.16%) Figure 6, and atrial tachycardia (2.04%) Figure 7. The cardiac dysrhythmias were observed in the high- yielding Holstein dairy cows included sinus arrhythmia (86.95%) and wandering pace maker (13.05%). Also, notched P wave was observed in both high- and low- yielding Holstein dairy cows, 30% and 14% respectively Figure 8. The results of this study have shown significant difference (p<0.05) between serum concentration of CK- MB, LDH, AST, and troponin I in the high and
low yielding cows. According to these results, the amount of cardiac biomarkers in the low yielding cows was higher than that of the high yielding cows. Furthermore, there was not any detectable significant difference of serum concentration of total CK between the high and low-yielding Holstein cows.

Discussion

Cardiac dysrhythmias or arrhythmias are defined as disturbances of impulse formation, disorders of impulse conduction, or both. In the present study, Cardiac dysrhythmias were detected more in the low-yielding Holstein dairy cows (62%) as compared to
the high- yielding Holstein dairy cows (46%). Eight types of dysrhythmias, either alone or in combination with another type, were observed in the low- yielding Holstein dairy cows, whereas two types of dysrhythmias determined in the high- yielding Holstein dairy cows.

Among biochemical parameters for the diagnosis of cardiac muscular disease, CK-MB, LDH, and AST have been used commonly. However, many disadvantageous of these parameters have been reported, and thus these parameters were replaced with newly developed cardiac markers such as cardiac troponin I (CTnI) and cardiac troponin T (CTnT) (McLaurin et al., 1997; Leonardi et al., 2008; O'Brien, 2008; Aldous, 2012).

In this study, troponin I in both high and low yielding Holstein cows was found to be 0.55±0.02 and 0.67±0.04 ng/mL, respectively. Therefore, the serum concentration of troponin I in low yielding Holstein cows was higher than that of high yielding Holstein cows significantly (p<0.05). Therefore, according to the normal range of troponin I, less than 1.3 ng/ml, it can be concluded that the increase of cardiac dysrhythmias in low yielding Holstein cows may be resulted from metabolic and electrolyte disorders. Troponin is released from injured myocardiocytes in to the circulation within hours, peaks within 2 days, and remains elevated for as long as the injury continues (Willis et al., 2007; Varga et al., 2009). Cardiac troponin I and T parameters are usually investigated for cardiac muscle injuries. Although CTnT was claimed to be specific for cardiac muscle, CTnI was reported to have high sensitivity in the diagnosis of cardiac disease (Antman et al., 1996; Apple et al., 1998; Willis et al., 2007). This protein is the gold- standard biomarker of myocardial injury in humans and animals because of its high tissue specificity and persistence in the blood. (Wells and Sleeper, 2008; Varga et al., 2009) To date, the protein has been studied in rats, mice, rabbits,
dogs, pigs, horses, cats, cattle, non-human primates, hamsters, and sheep (Gunes et al., 2010). The peak sensitivity of troponin I was at 12 to 24 h after onset of cardiac damage (Adams et al., 1993).

Gunes et al. (2008) used the cardiac troponin kits for the qualitative determination of myocardial cell damage due to traumatic reticuloperitonitis in twenty cattle. They observed that the mean serum concentrations of total protein, globulin, glucose, and calcium and the mean activities of creatine kinase Mb, aspartate aminotransferase, lactate dehydrogenase, and gamma-glutamyl transferase were higher in the cattle with TRP than in the control group (Gunes et al., 2008).

Tunca et al. (2008) determined the changes of the cardiac troponin I (cTnI) expression in blood and tissue during the myocardial degeneration in calves with foot-and-mouth disease (FMD). They observed that the mean serum concentrations of total protein, globulin, glucose, and calcium and the mean activities of creatine kinase Mb, aspartate aminotransferase, lactate dehydrogenase, and gamma-glutamyl transferase were higher in the cattle with TRP than in the control group (Gunes et al., 2008).

In this study, in both high and low yielding Holstein cows, the serum concentration of CK-MB was 27.85±3.68 U/L and 39.89±5.12 ng/mL. The peak sensitivity of CK-MB occurred at 8 to 12 h after onset of cardiac damage (Adams et al., 1993). Also, the serum concentration of CK was 57.23±11.96 U/L and 73.53±13.62 U/L in high and low yielding Holstein cows, respectively. This finding is in accordance with previous studies which have shown that CK lacks specificity for myocardial cell injury and that the measurement of CK is not sensitive enough to detect micropathology of the heart in cattle. In contrast, CK-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High yielding cows</th>
<th>Low yielding cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/L</td>
<td>67.25±9.21</td>
<td>127.89±13.16(*)</td>
</tr>
<tr>
<td>LDH U/L</td>
<td>117.16±1005.9</td>
<td>1402.96±153.14(*)</td>
</tr>
<tr>
<td>CK U/L</td>
<td>57.23±11.96</td>
<td>73.53±13.62</td>
</tr>
<tr>
<td>Ck-MB U/L</td>
<td>27.85±3.68</td>
<td>39.89±5.12(*)</td>
</tr>
<tr>
<td>Troponin I ng/mL</td>
<td>0.55±0.02</td>
<td>0.67±0.04(*)</td>
</tr>
</tbody>
</table>

Table 1. Mean±SEM of serum concentration of cardiac biomarkers in high and low yielding Holstein cows. (*)Values are significant at p<0.05.

Table 2. Type of dysrhythmias in high and low yielding Holstein cows.

<table>
<thead>
<tr>
<th>Type of dysrhythmias</th>
<th>High yielding cows</th>
<th>Low yielding cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus Arrhythmia</td>
<td>86.95</td>
<td>34.7</td>
</tr>
<tr>
<td>Wandering Pacemaker</td>
<td>13.05</td>
<td>22.45</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>0</td>
<td>18.37</td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>0</td>
<td>10.20</td>
</tr>
<tr>
<td>Atrial Premature Complex</td>
<td>0</td>
<td>2.04</td>
</tr>
<tr>
<td>Atrial Fibrilation</td>
<td>0</td>
<td>8.16</td>
</tr>
<tr>
<td>SA Block</td>
<td>0</td>
<td>2.04</td>
</tr>
<tr>
<td>Atrial tachycardia</td>
<td>0</td>
<td>2.04</td>
</tr>
</tbody>
</table>

Varga et al. (2009) investigated the correlation of serum cardiac troponin I and myocardial damage in cattle with monensin toxicosis. Their study confirmed that cTnI is a specific and sensitive biomarker for the detection of myocardial cell damage in cattle. A serum concentration of cTnI 1.04 ng/mL is an indicator of histopathologically detectable myocardial necrosis in cattle after monensin administration (Varga et al., 2009).

Karapinar et al. (2010) reported the high cardiac troponin I plasma concentration in a calf with myocarditis. They concluded that the cTnI assay may be useful in diagnosis of myocarditis in cattle (Karapinar et al., 2010).

Schober et al. (2002) indicated that serum cardiac troponin concentrations are associated with severity of ECG abnormalities and outcome (Schober et al., 2002).

Jesty et al. (2005) analyzed cardiac troponin I concentration in a cow with idiopathic pericarditis. They reported its value as 0.89 ng/mL (Jesty et al., 2005).

Buczinski and Belanger (2010) reported an increase of Serum cardiac troponin I at 3.52 ng/mL in bovine tricuspid endocarditis (Buczinski et al., 2010).
MB, an isoenzyme of CK, which is more specific to the cardiac muscle, has a higher specificity for myocardial cell injury (Varga et al., 2009).

In this study, the serum concentration of AST in high and low yielding Holstein cows was 67.25 U/L and 127.89 U/L, respectively. As a result, the serum concentration of AST in the low yielding Holstein cows was significantly higher than that of the high yielding Holstein cows (p<0.05). Therefore, according to the normal range of AST, 78-132 U/L, it can be concluded that the increase of cardiac dysrhythmias in the low yielding Holstein cows may be resulted from metabolic and electrolyte disorders.

AST enzyme is found in almost all cells including red blood cells; however, it is considered a diagnostic enzyme for liver and muscle disease because of its high activity in these tissues. Plasma half life of AST is above. AST is not an organ specific enzyme (Smith, 2009). Al-Habsi et al. reported that the reference levels of AST are 48 - 132 U/L in cattle (Al-Habsi et al., 2007).

In this study, the serum concentration of LDH in high and low yielding Holstein cows was 1005.9±117.167 U/L and 1402.96±153.14 U/L, respectively. Hence, the serum concentration of LDH in the low yielding Holstein cows was significantly higher than that of the high yielding Holstein cows (p<0.05). Therefore, according to the normal range of LDH, less than 692-1449 U/L, it can be concluded that the increase of cardiac dysrhythmias in the low yielding Holstein cows may be resulted from metabolic and electrolyte disorders. LDH enzyme is found in most tissue such as heart, liver, erythrocyte, leukocytes, and kidney. LDH enzyme has 5 isoenzymes, namely LDH1 (H4), LDH2 (H3M1), LDH3 (H2M2), LDH4 (H1M3), and LDH5 (M4). LDH1 is the principal isoenzyme in cardiac muscle and kidney. In cattle and sheep, LDH1 also is found in the liver. LDH5 is the principal isoenzyme in skeletal muscle and erythrocyte (Smith, 2009).

Consequently, it can be concluded that despite significant differences in cardiac biomarkers and based on the normal range of cardiac biomarkers in the both groups, the increase in cardiac dysrhythmias in low- yielding Holstein dairy cows may be metabolic and electrolyte disorders.

Acknowledgments

This research was founded by Shahrekord University, Shahrekord, Iran.

References

10. Gunes, V., Ozcan, K., Citil, M., Onmaz, A.C., Erdogan, H.M. (2010) Detection of myocardial...
ارزیابی دیس ریتمی‌ها و پیومارکرهای قلبي در گاوهای پر تولید و کم تولید

افشین جعفری دهکردی ۱

(۱) بیمارستان دامپزشکی ایران

چکیده

زمینه مطالعه: قلب و عروق یکی از مهم‌ترین ارگان‌های بدن می‌باشد که نقش حیاتی در عملکرد بالغ‌ها دارد. رشد و تولید شیر در دام‌های مزرعه به سلیم‌سازی قلبی و روانی است. هدف: سلامت قلب و عروق در گاوهای پر تولید و کم تولید ارزیابی شد. روی کار آمد: در این مطالعه از ۳۰ راس گاو هشترانگیژ کم تولید و ۵۰ راس گاو هشترانگیژ پر تولید استفاده گردید. ادراک‌های طبیعی یا تولید نمونه (CK, CK- MB, LDH, AST and troponin I) خون از ورید و وادی جهت اندام گیری پیومارکرهای قلبي (کار ۱) گیری گردید. نتایج: دیس ریتمی‌های قلبي در گاوهای هشترانگیژی پر تولید (۶۲٪) بیشتر از گاوهای هشترانگیژی کم تولید (۳۸٪) محسوس گردید. دیس ریتمی‌های قلبي که در گاوهای هشترانگیژی کم تولید مشاهده گردیده‌اند در نسبت سینوسی/۷۳/۷٪ پیش‌گزاری رشده‌اند که در این گروه پر تولید مشاهده گردیده‌اند. نکته کاربردی: در دام‌های پر تولید ریتمی‌های قلبي نسبت به دام‌های کم تولید بهتر است. نتایج: دیس ریتمی‌های قلبي در گاوهای هشترانگیژی پر تولید (۶۲٪) بیشتر از گاوهای هشترانگیژی کم تولید (۳۸٪) محسوس گردید. دیس ریتمی‌های قلبي که در گاوهای هشترانگیژی کم تولید مشاهده گردیده‌اند در نسبت سینوسی/۷۳/۷٪ پیش‌گزاری رشده‌اند که در این گروه پر تولید مشاهده گردیده‌اند. نکته کاربردی: در دام‌های پر تولید ریتمی‌های قلبي نسبت به دام‌های کم تولید بهتر است.

Email: jafari-a@vet.sku.ac.ir

 embargo: ۱۵۱