Levels of some biochemical markers in sera of pregnant and non-pregnant lactating dairy cows in Baghdad, Iraq

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Abstract

This study aimed to measure the biochemical markers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), total proteins, albumin, globulin, albumin / globulin (A/G) ratio, glucose, blood urea nitrogen (BUN), and total bilirubin in sera of dairy cows from the period of February-2016 to March-2018. For this purpose, 78 dairy cows of >3 years old reared at some areas of Baghdad province were subjected to blood sampling from the jugular vein. Study values (mean ± standard error) of ALT, AST, GGT, A/G ratio and total bilirubin markers were found outside the normal ranges of international reference values. Conversely, ALP, CK, total protein, albumin, globulin, glucose and BUN were found within the normal ranges of international reference values. To investigate changes that occur during late stage of pregnancy, study animals were divided into two groups (non-pregnant lactating and pregnant dairy cows). Statistically, significant decrease (P<0.05) was encountered in values (M ± SE) of ALT, AST, and GGT enzymes; and significant increase (P<0.05) in ALP and CK values of pregnant compared to non-pregnant dairy cows. Data concerning the serum proteins, results also indicated a significant increase (P<0.05) in values of total protein, whereas there no significant differences (P>0.05) were showed in values of albumin, globulin and A/G ratio. Pregnant dairy cows were showed a significant increase (P<0.05) in values of BUN and total bilirubin; however, glucose appeared without significant differences (P>0.05). It has been concluded that the lactation and gestation periods have a great impact on activity of biochemical indices in blood of dairy cows; therefore, there is a need for constant periodic monitoring for the described indices in this study to maintenance on animal’s health status.

Keywords: Biochemical markers, Dairy cows, Pregnant, Lactating, Serum, Iraq

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مستوى بعض المؤشرات الكيميائية في مصول أبقار الحليب الحوامل وغير الحوامل - المدرة للحليب

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الخلاصة

هدفت الدراسة الحالية إلى قياس بعض المؤشرات الكيميائية التي تشمل (الأمينات، أمينات القولون، البروتينات، الكالسيوم، الكالسيومات، النسيج، الدم، البوليمرات) في مصروف أبقار الحليب خلال الفترة من شباط ٢٠١٦ إلى آذار ٢٠١٨. لهذا الغرض، أخذت ٧٨ قررة حليب بعمر ٣ سنوات في بعض مناطق محافظة بغداد إلى جمع عينات الدم من الوريد الودائي. تواجهت قيم الدراسة (المعدل ± الخطأ القياسي) لمؤشرات الأمينات، أمينات القولون، البروتينات، الكالسيومات، الكالسيومات، النسيج، الدم، البوليمرات. وأخذت قيم الدراسة (المعدل ± الخطأ القياسي) لمؤشرات الأمينات، أمينات القولون، البروتينات، الكالسيومات، الكالسيومات، النسيج، الدم، البوليمرات. ويرجى المعدل الطبيعي لقيم المصدر العالمي وعلى النقيض، تواجهت مؤشرات الكالسيومات، كرياتين، كلوبيولين، كليوبيولين، كلوبيولين، كليوبيولين، كليوبيولين.
Introduction

In medicine of farm animals, the health and metabolism are assessed partly by measuring the serum biochemical parameters as practical tools for evaluating the pathological conditions or for monitoring the health status (1). During gestation, the dairy cows can expose to additional changes that cause alteration in biochemical and hematological blood parameters, especially, at late stage of gestation and early lactation (2). In order to correlate of liver functions with the possible tissue damage, several serum parameters can be measured to evaluate these functions and management of liver diseases during gestation, which mostly include alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), and creatine kinase (CK) (3,4). ALT and AST enzymes are abundant in cytoplasm (cytosol) of hepatocytes. These enzymes participate in gluconeogenesis, and any injury to hepatocytes causes leakage of these enzymes into the extracellular compartment (5). GGT is a membrane-bound enzyme having emphasized functions in secretion and absorption with high relative activity in liver of cows, sheep, goats, and horses (3). In plasma, GGT is significant for hepatobiliary system diseases that related with cholestasis, and being used extensively for diagnosis of liver diseases (6). ALP localized mainly in cellular membrane of hepatocytes, is increased physiologically during growth and pregnancy periods or after administration of some drugs (7). Tumor metastases in liver, liver poison intoxication, hepatitis, cholestasis, biliary disorders and steroid-hepatosis can increase the levels of ALP, pathologically (8). Creatine kinase (CK) is a muscleconcerning enzyme act as a necessary part in energy transferring through converting ATP to ADP (9). This enzyme can be detected in serum of pregnant animals as a result of higher metabolic demands in late gestation, or due to mechanical and metabolic stress (10). Serum proteins composed mainly several types of proteins. Albumin (A) is one of the most important and efficient proteins that contribute in maintaining of osmotic plasma pressure (11). Globulins (G) are group of proteins such as the inflammatory molecules and antibodies, fibrinolytic and hemostatic proteins, hormones and vitamins, and act as carriers of lipids (12). In addition, numerous physiologic or pathologic conditions can cause shift in albumin (A) and globulin (G) concentrations especially in late gestation (13).

Glucose is one of the most important elements of milk in dairy cattle, level of blood glucose is vital as dairy animals undergoing an elevated need for glucose to encounter the demands for milk yield and to regulate concentrations of other blood metabolites (14). In dairy practice, measurement of blood urea nitrogen (BUN) refers to that sufficient amounts of diet and efficiency in utilization of nitrogen (15). Concentrations of both BUN and blood glucose are recommended at most for detection of abnormalities that occur during gestation such as acetonemia in cattle and pregnancy toxemia in ewes (16). Bilirubin is a waste-product derived from hemoglobin (Hb) of erythrocytes that breakdown either normally due to removal of aged and affected cells, or abnormally due to intravascular and extravascular hemolysis (17). The aim of this study is to measure the levels of some serum biochemical markers in pregnant dairy cows, at late stage of gestation, and in non-pregnant lactating dairy cows in some areas of Baghdad province, Iraq.

Materials and methods

Samples collection

At some areas in Baghdad province during about two years extended from February-2016 to March-2018, 78 cross-breed dairy cows aged >3 years were submitted for this study. Study cows composed 39 cows at late stage of gestation and 39 non-pregnant lactating cows. From each animal, 10 ml of venous blood were drained using heparinized anticoagulant vacutainer tubes (AFMA, Jordan), which transported to the laboratory within specialized darkbox to be centrifuged at 4000rpm for 15 minutes. Sera samples were kept frozen until beinganalyzed using specific kits.
Biochemical analysis

Glucose, BUN, CK, total bilirubin, ALT, AST, GGT, ALP, and CK were measured by commercially available kits (Roche, Germany) using the chemistry analyzer (Cobas c 111, Roche, Germany). Concentrations of total serum protein and albumin were measured by the spectrophotometric method using commercial kits provided by Roche-Germany. Concentration of globulins (G) was calculated by the difference between the total protein and albumin (A). (A/G) ratio is also detected (18).

Statistical assessment

Using two computerized programs, Microsoft Office Excel (2013) and IBM/SPSS (V23), the obtained data were arranged, tabled, and illustrated in graphics. Significant variations in values [mean ± standard error (M ± SE)] were showed between pregnant and non-pregnant dairy cows at (P < 0.05) level.

Results

In this study, overall results of biochemical markers were compared with the international reference values of (19) as presented in Table (1). Values (mean ± standard error) of study’s ALT, AST, GGT, A/G ratio and total bilirubin were significantly outside the ranges of normal reference values (P < 0.05). However, values (mean ± standard error) of study’s ALP, CK, total protein, albumin, globulin, glucose and BUN were significantly included within or nearby the normal reference values (P > 0.05).

Table 1: Overall study results in comparison with normal values of international reference

<table>
<thead>
<tr>
<th>Markers</th>
<th>Unit</th>
<th>Total study values (M ± SE), (n= 78)</th>
<th>International values (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>IU/L</td>
<td>67.57 ± 2.19 **</td>
<td>11 - 40</td>
</tr>
<tr>
<td>AST</td>
<td>IU/L</td>
<td>146.15 ± 3.12 **</td>
<td>78 - 132</td>
</tr>
<tr>
<td>GGT</td>
<td>IU/L</td>
<td>21.7 ± 1.89 **</td>
<td>6.1 - 17.4</td>
</tr>
<tr>
<td>ALP</td>
<td>IU/L</td>
<td>197.79 ± 3.51</td>
<td>0 - 200</td>
</tr>
<tr>
<td>CK</td>
<td>IU/L</td>
<td>195.23 ± 4.5</td>
<td>35 - 280</td>
</tr>
<tr>
<td>Total Protein</td>
<td>g/dl</td>
<td>8.23 ± 0.42</td>
<td>5.7 - 8.1</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dl</td>
<td>3.71 ± 0.13</td>
<td>2.1 - 3.6</td>
</tr>
<tr>
<td>Globulins</td>
<td>g/dl</td>
<td>4.52 ± 0.06</td>
<td>3.6 - 4.5</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>-</td>
<td>0.82 ± 0.31 **</td>
<td>0.70</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dl</td>
<td>54.6 ± 2.69</td>
<td>45 - 75</td>
</tr>
<tr>
<td>BUN</td>
<td>mg/dl</td>
<td>21.49 ± 1.29</td>
<td>6 - 27</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>mg/dl</td>
<td>0.81 ± 0.04 **</td>
<td>0.01 - 0.5</td>
</tr>
</tbody>
</table>

Mean ± Standard errors (M±SE), Range (R), Significance ** (P<0.05).

Among 39 pregnant dairy cows at late stage of gestation, and 39 non-pregnant lactating dairy cows, the results of serum biochemical enzymes are showed in (Table 2). Statistically, significant decreases (P<0.05) were encountered in values (M ± SE) of ALT, AST, and GGT. In addition, values (M ± SE) of ALP and CK are increased significantly (P<0.05) among pregnant dairy cows.

Table 2: Serum enzymes of pregnant and non-pregnant dairy cows

<table>
<thead>
<tr>
<th>Markers</th>
<th>Unit</th>
<th>Non-Pregnant values Mean ± SE (R)</th>
<th>Pregnant values Mean ± SE (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>IU/L</td>
<td>73.16 ± 2.82</td>
<td>61.98 ± 1.57 **</td>
</tr>
<tr>
<td>AST</td>
<td>IU/L</td>
<td>154.09 ± 2.57</td>
<td>138.21 ± 3.69 **</td>
</tr>
<tr>
<td>GGT</td>
<td>IU/L</td>
<td>22.04 ± 2.31</td>
<td>21.37 ± 1.48 **</td>
</tr>
<tr>
<td>ALP</td>
<td>IU/L</td>
<td>183.42 ± 2.99</td>
<td>212.15 ± 4.02 **</td>
</tr>
<tr>
<td>CK</td>
<td>IU/L</td>
<td>181.3 ± 3.57</td>
<td>209.15 ± 5.46 **</td>
</tr>
</tbody>
</table>

Mean ± Standard errors (M±SE), Range (R), Significance ** (P<0.05).

Data concerning the serum biochemical proteins of pregnant dairy cows, significant increase (P<0.05) were detected only in values (M ± SE) of total protein, whereas there no significant variations (P>0.05) were indicated in values of albumin, globulin and A/G ratio, (Table 3).

Table 3: Serum proteins of pregnant and non-pregnant dairy cows

<table>
<thead>
<tr>
<th>Markers</th>
<th>Unit</th>
<th>Non-Pregnant values Mean ± SE (R)</th>
<th>Pregnant values Mean ± SE (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>g/dl</td>
<td>7.92 ± 0.68</td>
<td>8.54 ± 0.17 **</td>
</tr>
<tr>
<td>Protein</td>
<td>g/dl</td>
<td>3.61 ± 0.12</td>
<td>3.81 ± 0.15</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dl</td>
<td>4.31 ± 0.01</td>
<td>4.73 ± 0.11</td>
</tr>
<tr>
<td>Globulins</td>
<td>g/dl</td>
<td>0.84 ± 0.06</td>
<td>0.81 ± 0.54</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>-</td>
<td>0.68 ± 0.9</td>
<td>0.75 - 0.92</td>
</tr>
</tbody>
</table>

Mean ± Standard errors (M±SE), Range (R), Significance ** (P<0.05).

In other serum biochemical constituents, BUN and total protein of pregnant dairy cows were encountered a significant elevation (P<0.05) in their values (Table 4). However, glucose values were appeared without significant differences (P>0.05).
Discussion

Worldwide, the maintenance and optimization the activity of reproductive system in dairy herds is still to be challenged for dairy farmers especially when there are programs adopted for increasing of milk production (20). Modern milk industry often puts the production capabilities of cows at risk due to the metabolic disorders. In order to predict such disorders in addition to subclinical diseases, it is necessary to determine the physiological and biochemical parameters that reflected the metabolic demands (21). Hence, the metabolic profile tests can display on a pathological feature of illness and help in identifying the biomarkers that may affect on reproductive or productive performance (22). In this study, the total results of some serum biochemical markers (ALT, AST, GGT, ALP, CK, total protein, albumin, globulin, A/G ratio, glucose, BUN, and total bilirubin) revealed on variable levels on that provided by the international reference values. In fact, cross-breed dairy cows have different blood profile from local (indigenous) ones is attributed to the differences in rumen physiology, genetic mapping, productivity, management and feeding practice (23,24).

Also, the examined dairy cows involved in this study were classified into two groups; pregnant at late stage of gestation and non-pregnant lactating dairies. In serum enzymes, though the highest levels of ALT and AST activity were recorded during lactation; ALP and CK were increased during late stage of gestation, while GGT was significantly unchanged. As reported by (6, 25), the activity of ALT was decreased during the last three months of gestation until birth, to be increased during 2nd and 3rd periods of lactation. Related to AST, the highest levels of this enzyme during lactation may reflect an existence of ketosis, lower dry matter intake, or fatty liver syndrome (26). In addition, highest serum AST may indicative for trauma, necrosis or neoplasia in muscle, which can occur during animal restraint procedures that lead to mild muscle trauma in non-clinical cases (27). In dairy cows, AST activity can be changed during early lactation and gestation as a reflection of metabolic events (6).

The finding of present study showed no significant variation in levels of GGT activity in both dairy cow groups; however, it was slightly higher during lactation. Although the level of GGT may change irregularly during pregnancy and lactation periods, it positively correlates with milk yield and the mammary gland activity (28,29). In fact, the excessive activity of ALP detected in pregnant cows of present study could occur due to presence of corpus luteum that responsible on production of progesterone required establishment and maintenance of pregnancy (30). In addition, the high metabolic rate or increasing bone metabolism due to absence of mineral feed additives in feeds of dairy herds may result in an elevation in ALP activity (31).

The increased levels of CK were observed in pregnant cows involved in this study. During pregnancy, CK elevates could occur as a result of alterations in metabolic demand of uterus. Uterus is considered as the third highest tissue performs high energy transfer, after skeletal and cardiac muscle (10). Cows having elevated levels of AST and CK over the physiological ranges might show additional abnormalities in abomasal wall that appear with considerable activities for CK (32).

The significant elevation in values of total protein in pregnant cows, but not in albumin, globulin and A/G ratio are reported in this study. In dairy cows, the periods of late gestation and early lactation are recognized to be associated with important alterations in metabolic changes, physical stress, and mobilization of body fat, proteins and mineral stores to meet newborn requirements (33). Although, pregnancy is accompanied by a substantial and progressive increase in plasma volume that decreases after parturition, the normal albumin with A/G ratio usually increase the effect of decreased plasma volume because albumin and globulin are affected to the same extent (34). In general, the lactating period when compared to pregnancy phase is characterized by a decline in total protein due to the decrease of γ-globulin and to consequent increase of A/G ratio (35).

Although, the glucose observed without significant differences, BUN and total bilirubin were show higher levels in pregnant cows than in lactating dairy ones. In dairy cows, concentration of glucose is low since is produced mostly in process of gluconeogenesis, and great quantities are secreted in milk as a form of lactose (36). Urea is a good and sensitive indicator for energy or protein imbalance and protein utilization efficiency. Correlation between levels of dietary proteins and reproduction can be evaluated using the sera of blood or milk (20). It was detected that the relationship between high BUN and diminished of conception or pregnancy seem stronger when the urea levels over threshold (37). Several studies
suggested that the variation in BUN concentration between cows at the same breed might be attributed to the genetic differences in nitrogen efficiency (38). Other possible causes include age of animal, time of sample collection, hydration status at time of sampling, different grazing habits, dry matter intake, energy levels, differences in rate of urea excretion, and applied method of analysis (39). In dairy cows, normal BUN and glucose levels might be varied significantly at the same geographical location or breed.

In this study, high levels of plasma total bilirubin were in agreement with the fact that the values of blood bilirubin elevated during gestation as a consequence of additional bilirubin derived from degradation of fetal hemoglobin or due to inadequate glucuronic acid synthesis (40, 41).

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