



Impact of Retained Fetal Membranes on Concentration of Some Biochemical Parameters and Liver Enzymes in Cattle



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CURRENT study was aimed to investigate effect of retained fetal membrane on the concentration of some biochemical parameters and liver enzymes in cows. Total forty five multipara local Iraqi Holstein (n=35) cows suffered from retained fetal membrane were considered as treatment group and 10 normal cows (n=10) as control group, in Salah El-Din province, were used in the current study at the period September 2020 up to April 2021. Blood samples were collected and serum extracted for biochemical parameters and liver enzymes analysis from all animals. The concentration of calcium, phosphorous, iron, magnesium, copper, total serum proteins, glucose, cholesterol and triglyceride were measured by spectrophotometer. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also estimated. The results of present study revealed that the triglyceride showed significant ($P \leq 0.05$) increases in cows suffered from retained fetal membranes (20.863 ± 1.222 mg/dl) compared with healthy cows (16.3 ± 2.172 mg/dl), while the total protein, Glucose and cholesterol didn't show significant differences between groups. Both Calcium and phosphorous showed significant ($P \leq 0.05$) decreases in cows suffered from retained fetal membranes (1.598 ± 0.166 mg/dl and 1.105 ± 0.118 mg/dl respectively) compared with healthy animals (2.058 ± 0.191 mg/dl and 1.52 ± 0.0393 mg/dl respectively). The liver enzymes didn't show any significant changes between the groups. In conclusion the present study elucidated that calcium and phosphorous are the main minerals which have important role in occurrence of retained fetal membranes in cows.

Keywords: Retained placenta, Cow, biochemical, Calcium, phosphorus,

Introduction

Retained fetal membrane (RFM) is one of important disorders occurring post-partum in cattle which causing decreased reproductive performance [1], it consider as one of main causes of metritis and post-partum endometritis [2] and resulting in increased the period between calving to first estrus,

days open, services per conception and calving interval [3]. Retained fetal membranes occur when the fetal membrane failed to expulse from the uterus within 24 hrs after parturition [4]. There were many predisposing factors lead to RFM in cattle such as shorten or prolonged gestation, abortion, dystocia, uterine inertia, and hormonal disturbance [5]. Nutrition deficiency may play

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important role as a predisposing factors for occurrence of RFM in cattle [6]. Blood biochemical parameter analysis is important in that it complements clinical findings and often used for assessing the health status of individual animals and homeostatic impairments [7]. Khanthusaeng *et al.*, [8] reported that minerals supplementation before parturition have a positive influence on the birth process and lead to decreased the period of fetal membrane expulsion in cattle. Regular uterine contraction is the most requirements for occurrence of normal expulsion of fetal membranes after parturition [5]. Serum Ca^{++} is the main element which must be provide to maintain the sufficient uterine contraction [9], and decrease blood concentration of Ca^{++} may interact with normal expulsion of fetal membranes in cattle through interfere with normal myometrium contraction [10]. Akar and Yildiz, [11] reported that the deficiency of Zink and copper increased the risk of incidence of retained fetal membranes in cattle. Mordak *et al.*, [12] demonstrated that the decreased phosphorus and zinc concentration accompanied with neutropaenia which it seems to be an important co-factor connected with the pathogenesis of RP in dairy cows. Immune suppression is the main factor lead to occurrence retained fetal membrane, and the metabolic disturbances are the important causes result in this suppression [13]. Metabolic disorder associated with dystocia lead to an additional stressors result in elevated of cortisol secretion and increased suppression of immunity in cattle [6]. The development of fatty liver in cows may adversely affect immune defense mechanisms and increase the susceptibility to retained fetal membranes [14]. Mordak *et al.*, 2009 [12] presented the most important metabolic, mineral and enzymes disturbances as conditions for occurring retained fetal membranes in cows.

The current study was designed to investigate the correlation between the levels of some biochemical and liver enzymes with the occurrences of retained fetal membranes in local cross breed cattle in Salah El-Din province. Iraq.

Material and Methods

Experimental Animals:

The present study was conducted on total 45 multiparous Holstein cross breed cows, aged 4 to 8 years, (n=35) cow suffering from retained fetal membrane (RFM) and 10 healthy cows (n=10) which were brought to the Veterinary Hospital in Salah El-Din province from September 2020 to April 2021. All animals in treated group were come to normal calving without any hormonal treatment and case history and a clinical examination was performed for all the studied animals to identify the

cows that suffering from another diseases and these cows were failed to expelled fetal membrane after 24 hrs of parturition were considered as retained fetal membrane case.

Blood samples

Blood samples were collected from jugular vein of all animals (10 ml) in free anti-coagulant collecting tubes and directly transported to the laboratory of clinical Pathology/ Collage veterinary medicine/ Tikrit University and left for 10 minutes at room temperature until clotting, then the serum was separated in a centrifuge at 3000 rpm for 10 minutes. The serum samples were kept at -20 until the biochemical tests were performed [15].

Biochemical and minerals estimation

The biochemical parameters and minerals Values were estimated in the laboratory of clinical Pathology/Collage Veterinary Medicine. Tikrit city. Iraq. The concentration of calcium, phosphorous, iron, magnesium and copper in the blood serum of the samples were measured by the colorimetric method According to way described by [16] and using measuring kits LINEAR (Spanish), then the color absorption of the resulting complex light was read by using a spectrophotometer. Total serum proteins, glucose, cholesterol and triglyceride were measured by spectrophotometer with special kits (BIOLABO/ France) as according to [17]. The Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also measured by spectrophotometer with special kit as described by [18]. All the protocols were run according to manufactural notes.

Statistics analysis

The data presented as mean \pm SE and were analyzed by using independent *t*-test by using of SPSS program (Version 19) and the differences were set as significant at $p < 0.05$ [19].

Results

Levels of total protein, glucose, triglycerides and cholesterol

The results of current study as shown in the table 1 revealed non-significant decrease in the levels of total protein, glucose and cholesterol in cows suffered from retained fetal membranes (6.22 ± 0.26 g/dL, 50.481 ± 5.424 mg/dl and 104.708 ± 7.363 mg/dl respectively) compared with healthy cows (6.4 ± 0.13 g/dL, 53.067 ± 2.543 mg/dl and 110.8 ± 11.655 mg/dl respectively). The concentration of triglycerides exhibited significant increases ($p \leq 0.05$) in cows that had suffered from retained fetal membranes (20.863 ± 1.222 mg/dl) compared to healthy cows.

TABLE 1. Levels of total protein, glucose, triglycerides and cholesterol in the blood of retained fetal membrane and healthy cows (mean ± standard error).

Parameters	Animals group	Retained fetal membranes cows N =35	Healthy cows N =10
Total protein (g/dL)		6.22±0.26	6.4 ±0.13
Glucose (mg/dl)		50.481±5.424	53.067±2.543
Triglycerides (mg/dl)		20.863±1.222 ^a	16.3±2.172 ^b
Cholesterol (mg/dl)		104.708±7.363	110.8±11.655

Different letters between columns referred to significant differences at (P≤0.05).

Levels of minerals in the blood

The result of current study (Table 2) revealed significant decreases (P≤0.05) in the concentration of calcium in the cows suffered from RFM (1.598±0.166 mg/dc) compared with healthy group (2.058±0.191 mg/ dc). Also, significant decreases (P≤0.05) in the concentration of phosphorus in the current study were observed in cows suffered from RFM (1.105±0.118 mg/ dc) compared with healthy group (1.52±0.0393 mg/ dc). While the results didn't revealed significant differences in the

concentration of Mg, copper and zinc between the cows suffered from RFM (0.669±0.0385 mg/ml, 60.473±4.017 mg/ml and 61.817±5.019 mg/ml respectively) and healthy (0.818±0.0428 mg/ml, 59.02±2.711 mg/ml and 58.58±3.101 mg/ml respectively).

Concentration of ALT and AST in the animals of study

The result of present study didn't recorded differences in the concentration of ALT and AST in the groups of animals study (figure 1).

TABLE 2. levels of minerals in the blood of retained fetal membrane and healthy cows (mean ± standard error).

Minerals	Animals group	Retained fetal membranes cows N =35	Healthy cows N =10
Ca (mg/ dc)		1.598±0.166 ^b	2.058±0.191 ^a
Mg (mg/ml)		0.669±0.0385	0.818±0.0428
Phosphorus (mg/ dc)		1.105±0.118 ^b	1.52±0.0393 ^a
Copper (mg/ml)		60.473±4.017	59.02±2.711
Zinc (mg/ml)		61.817±5.019	58.58±3.101

Different letters between columns referred to significant differences at (P≤0.05).

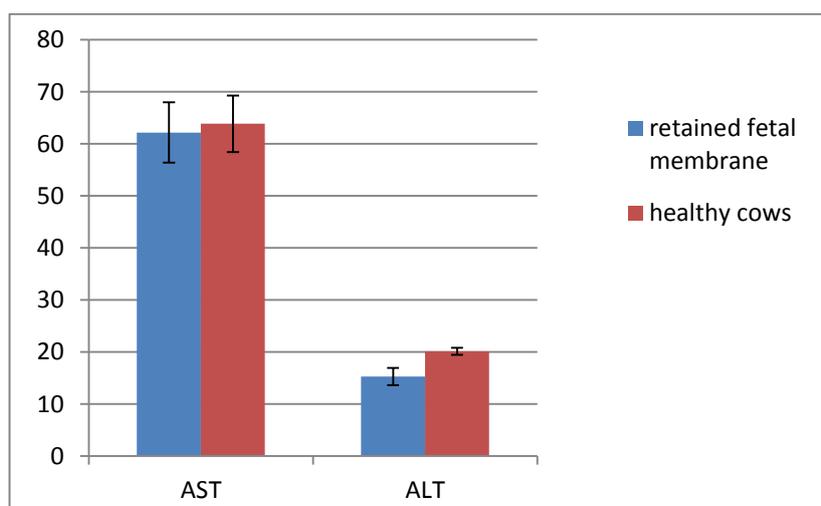


Fig. 1. Concentration of AST and ALT (Mean± SE) in serum blood of cows suffered from retained fetal membranes and healthy cows.

Discussion

Data of present study referred to non-significant decrease in the levels of total protein, glucose and cholesterol in cows suffered from retained fetal membranes, these data were in agreement with the finding of some authors in cattle [20] and [21-22] in buffalo. The significant increases ($P \leq 0.05$) in triglyceride concentration in cows suffered from retained fetal membranes is agree with some investigators [23 - 24] and this higher concentrations of triglycerides in cattle suffered from retained fetal membranes may occurs due to the increased energy requirement.

The result of current study (Table 2) revealed significant decreases ($P \leq 0.05$) in the concentration of calcium in the cows suffered from retained fetal membranes (1.598 ± 0.166 mg/dc) compared with healthy group (2.058 ± 0.191 mg/ dc). These results was agreed with previous reports [5], [11], [23-24] who found significant decreases in calcium level in cattle with retained placenta than control group. These decreases may lead to poor uterine contractions, which might be the cause of retained fetal membranes in cattle [5]. Goff, 2008 [25] referred to hypocalcemia has been proposed as a risk factor for RP in early lactation by explain action of intra- cellular calcium who have a main function in cellular signaling [26]. Also the low concentration of calcium might be affecting the mechanism of detachment of cotyledon from uterine caruncles, as the collagenase activity of cotyledon villi is involved in the process of releasing of fetal membranes [27]. Collagenase (a calcium-dependent enzyme) [28] activity is increased during delivery in healthy cows and decreased in cows with RFM [27] Perhaps marginal calcium concentrations are sufficient to reduce collagenase activity and alter the immune function, thereby causing a higher incidence of RFM [20].

The Significant decreases ($P \leq 0.05$) in the concentration of phosphorus in cows suffered from retained fetal membranes in current study was agreed with some reports [29-30]. Tillard *et al.*, [31] reported that the lowering levels of Phosphorus during the post-calving period may consider a predisposing factor for RFM in cattle. As Phosphorus is integral to many physiological functions (e.g., ATP, phospholipids, nucleic acids) the decrease of Phosphorus lead to decreased uterine muscle contraction after parturition.

The non-significant differences in the concentration of Mg, copper and zinc between the cows suffered from RFM and healthy was agree with some studies [32,11].

The non-significant differences in the concentration of ALT and AST between the groups in current study were in agreement with [21] and

[29], who found no differences in these levels between cows suffered with retained fetal membranes and normal cases. In contrast, the result was disagree with [33] who found high significant increases in liver enzymes in retained placental cows, these differences may be due to the liver dysfunction rather than retained placenta. the activity of liver enzymes are a very sensitive indicator for liver disorders [34] and the severity of fatty livers in cattle is related to the loss of body condition score after calving [35] and these problem seems to be more severe in older high yielding cows [36], which lead to accumulation of lipids in the liver tissues.

Conclusion

The present study elucidated that calcium and phosphorous are the main minerals which have important role in occurrence of retained fetal membranes in cows. The triglyceride showed significant ($P \leq 0.05$) increases in cows suffered from retained fetal membranes while the total protein, Glucose and cholesterol didn't showed significant differences between groups.

Conflict of Interest

There are no conflicts of interest to be declared.

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Authors Contribution: The authors each contributed equally.

Ethical approve:

All Ethical consideration had been taken during dealing and sampling with study animals

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تأثير الأغشية الجنينية المحتبسة على تركيز بعض المعايير الكيموحيوية وأنزيمات الكبد في الأبقار

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هدفت الدراسة الحالية لمعرفة تأثير احتباس الاغشية الجنينية على مستوى بعض المعايير الكيموحيوية وانزيمات الكبد في الابقار. استخدمت في الدراسة 45 بقرة ذات ولادات متعددة محلية مضرية بالهولشتاين (35 بقرة تعاني من احتباس الاغشية الجنينية وعشرة ابقار سليمة كمجموعة سيطرة) تراوحت اعمارها من 4 الى 8 سنة في محافظة صلاح الدين في الفترة من ايلول 2020 الى نيسان 2021. جمعت عينات الدم واستخلص المصل لغرض تحليل المعايير الكيموحيوية وأنزيمات الكبد من جميع الحيوانات. تم قياس تركيز الكالسيوم والفوسفور والحديد والمغنيسيوم والنحاس وبروتينات المصل الكلية والجلوكوز والكوليسترول والدهون الثلاثية بواسطة جهاز المطياف الضوئي. كما تم تقدير ناقلة أمين الأستاتات (AST) وناقلة أمين الألانين (ALT). اظهرت نتائج الدراسة ارتفاعا معنويا (P<0.05) في مستوى الدهون الثلاثية الابقار التي كانت تعاني من احتباس الاغشية الجنينية (20.863±1.222 mg/dl) مقارنة مع السليمة (16.3±2.172 mg/dl) بينما انخفض (P<0.05) تركيز كل من الكالسيوم والفوسفور في الابقار التي كانت تعاني من احتباس الاغشية الجنينية (1.598±0.166 ملغم/ديسلتر و 1.105±0.118 ملغم/ديسلتر على التوالي) مقارنة مع السليمة (2.058±0.191 ملغم/ديسلتر و 1.52±0.0393 ملغم/ديسلتر على التوالي). لم تظهر انزيمات الكبد اي تغيرا ملحوظا بين المجموعتين. نستنتج من الدراسة الحالية ان كلا من الكالسيوم والفوسفور من اهم المعادن التي لها دور مهم في حدوث احتباس الاغشية الجنينية في الابقار.

الكلمات المفتاحية: احتباس الاغشية الجنينية ، بقرة ، معايير حيوية ، الكالسيوم ، الفوسفور.