

A STUDY OF SUBCLINICAL KETOSIS IN COWS IN BASRA PROVINCE

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ABSTRACT

Subclinical ketosis (SCK) is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs. Measurement of blood β -hydroxy butyric acid (BHBA) concentration is considered as the gold standard method to detect SCK. The objective of this study was to determine the presence of SCK in cows in different physiologic status in Basra, Iraq. The cows were classified into 5 groups according to the physiologic group; G1: 2nd trimester pregnant cows ($n =9$), G2: 3rd trimester pregnant cows ($n =8$), G3 Fresh cows (1-30 days postpartum) ($n=41$), G4: Lactating cows (2 and 3 months postpartum) ($n =21$), and G5: 4 months – more postpartum cows ($n =14$) . Cholesterol, ALT activity and BHBA concentrations were determined. The cutoff point $1000 \mu\text{mol L}^{-1}$ - $2600 \mu\text{mol L}^{-1}$ was used. The mean concentrations of the parameters of the cows of the five groups were; cholesterol: 3.7 ± 0.34 , 3.21 ± 0.38 , 2.59 ± 0.16 , 3.15 ± 0.24 and 2.5 ± 0.28 mmol/L, ALT: 45.11 ± 6.16 , 37.125 ± 3.98 , 44.83 ± 3.36 , 45.19 ± 4.57 and 41.29 ± 4.37 IU/L and BHBA: 2.014 ± 0.15 , 2.086 ± 0.085 , 1.94 ± 0.07 , 1.89 ± 0.18 and 1.92 ± 0.075 mmol/L. The concentration of cholesterol was within the normal limits in the all groups but it showed descending trend from early pregnancy to parturition then elevated with advancement of lactation, while the activity of ALT showed highness in each group with different percent, whereas the concentration of BHBA was significantly high in the all groups. The present findings reveals that prevalence of subclinical ketosis in Basra is extremely high. To prevent the consequences of subclinical ketosis, feeding a good feed stuff with propylene glycol and implementing a good management with regular test for detecting SCK to reduce the economic losses caused by SCK.

INRODUCTION

Dramatic increases in energy requirements during late gestation and early lactation superimposed on an animal with a profound drop in Dry Matter Intake (DMI) before calving and make the cow highly susceptible to the metabolic diseases e.g., ketosis. Ketosis is caused by a negative energy balance, and typically occurs within 2 months after calving. Sub-Clinical Ketosis (SCK) is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs of ketosis (1). Total energy intake in early lactation is usually less than what is required for maintenance and milk production (2). The animal attempts to supply the needs for milk production by drawing on body fat reserves. This release of free fatty acids results in the production of the major ketone bodies, acetone, acetoacetate and beta-hydroxybutyrate (BHB) (3). These compounds are important source of energy when carbohydrate levels are reduced (1). The gold standard diagnostic test for SCK is the measurement of BHB in serum or plasma because of its stability (1), (4). The objective of the present study to:

- Detection of subclinical ketosis in cows pre and post calving.
- Figuring out the affection of subclinical ketosis on liver.
- Prediction of subclinical ketosis by using of some metabolic parameters.
- Measuring the some blood metabolites and serum enzyme.

MATERIALS AND METHODS

The present study were carried out in Basra province, south of Iraq, in winter 2013. Almost all the cows were owned by a private sector and some of them were belonged to the farms of the College of Veterinary Medicine and the College of Agriculture. The cows were classified according to the physiologic status which contained five groups: [1] 2nd trimester pregnant cows (G1) ($n = 9$), [2] 3rd trimester pregnant cows (G2) ($n = 8$), [3] Fresh cows (1-30 days postpartum) (G3) ($n = 41$), [4] Lactating cows (2 and 3 months postpartum) (G4) ($n = 21$), and [5] four months – more postpartum cows (G5) ($n = 14$). The blood samples were collected from the jugular vein by use of 10 ml sized syringes. Blood was collected in a vacuum tubes without anticoagulant agent and was allowed to clot to be centrifuged for 15 minutes (3000 rpm) to obtain serum. Serum had been collected in eppendorf tubes and kept frozen in (-20°C) to be used for measuring the concentration cholesterol, the activity of ALT and the level of BHBA in serum.

RESULTS

1. Cholesterol:

The concentration of cholesterol was within the normal limits but it showed descending trend from the early pregnancy to the parturition then began to elevate with lactation period. The concentration of cholesterol of G1 was significantly higher than that of G3 and G5 ($p < 0.05$). Whereas the concentration of cholesterol of G2 and G3 showed no significant difference with the cholesterol of any group at level ($p > 0.05$). The concentrations of cholesterol of G3 and G5 were significantly the lowest among the groups. The mean values of cholesterol concentration of G1, G2, G3, G4 and G5 were 3.7 ± 0.34 , 3.21 ± 0.38 , 2.59 ± 0.16 , 3.15 ± 0.24 and 2.5 ± 0.28 respectively. The mean values of cholesterol concentration are shown in Table (1).

2. ALT:

No significant difference in the mean values of ALT activity has been reported between any group and other at level ($p > 0.05$). The mean values of ALT activity of G1, G2, G3, G4 and G5 were 45.11 ± 6.16 , 37.125 ± 3.98 , 44.83 ± 3.36 , 45.19 ± 4.57 and 41.29 ± 4.37 respectively. The results of the mean values of ALT activity are shown in Table (1).

3. BHBA:

The mean values of BHBA concentration was significantly higher than normal in almost each cow in each group, but there was no significant difference between any group and other at level ($p > 0.05$). The mean values of BHBA concentration of G1, G2, G3, G4 and G5 were 2.014 ± 0.15 , 2.086 ± 0.085 , 1.94 ± 0.07 , 1.89 ± 0.18 and 1.92 ± 0.075 respectively. The mean values of BHBA concentration are shown in Table (1).

Table (1): Shows the mean values of serum biochemical parameters in cows during different physiologic periods.

| Parameter | G1 | G2 | G3 | G4 | G5 |
|----------------------|--------------------|---------------------|--------------------|--------------------|--------------------|
| Cholesterol (mmol/L) | 3.7 ± 0.34 a | 3.21 ± 0.38 ab | 2.59 ± 0.16 a | 3.15 ± 0.24 ab | 2.5 ± 0.28 b |
| ALT (GPT) (U/L) | 45.11 ± 6.16 a | 37.125 ± 3.98 a | 44.83 ± 3.36 a | 45.19 ± 4.57 a | 41.29 ± 4.37 a |
| BHBA | $2.014 \pm$ | $2.086 \pm$ | 1.94 ± 0.07 | 1.89 ± 0.18 | $1.92 \pm$ |

| | | | | | |
|----------|--------|---------|---|---|---------|
| (mmol/L) | 0.15 a | 0.085 a | a | a | 0.075 a |
|----------|--------|---------|---|---|---------|

Values denote the mean \pm standard error of measurements.

The small letters in same row mean no significant differences.

DISCUSSION

1. Cholesterol:

The reduction of serum cholesterol of G3 compared to the other groups (G1, G2 and G4) is related to the switch in synthesis of lipoproteins from the hepatocytes. In the postpartum period there is an increase in circulating high density lipoproteins and a drastic decrease in low density lipoproteins (LDL), as well as reductions in very low density lipoproteins. Low density lipoproteins contain the largest proportion of cholesterol amongst the lipoproteins (5). Low cholesterol content in serum has been noted in G3 (2.59 ± 0.16) compared to that of G4 (3.15 ± 0.24). Similar results were observed by (6). They found that cholesterol content was the lowest in early stage of lactation and then increased gradually during lactation. (7) found the same results. Cholesterol concentrations are low at calving and increase slowly over the first weeks after calving (8), (9). The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements. The descending trend in the serum cholesterol concentration in dry pregnant cows compared to lactating cows was observed. That in coincidence with (10). The hormonal level of estrogen along with thyroxin played a vital role in reducing the cholesterol levels during pregnancy. The decrease observed in G3 compared to G4 could be ascribed to the increased cholesterol uptake by tissues involved in milk synthesis, because of the normal insulin responsiveness compared to late pregnancy (11). The significant decreased cholesterol concentrations have been associated with metabolic disorders (12), hepatic lipidosis (13), and feed intake (14) that was not found in the present study.

2. ALT:

Alanine Transaminase (ALT) is a cytoplasmic enzyme that catalyzes the transamination of L-alanine and 2-oxoglutarate to pyruvate and glutamate (15). High ALT activities detected and also reported in ketotic cows by (3), (16), that partially agrees with the present findings, as we found there was increase in ALT activity in

almost half of the cows of each group and decrease ALT activity in the other half. The explanation of that might be because the livers of the cows with no ALT activity were not affected because our study tested subclinically ketotic cows. Increase in the activities of liver enzymes may have been associated with cholestasis and thus, with the disruption of normal hepatobiliary circulation. (17) in their study presented information that ALT activity decreased in the seventh and eighth months of pregnancy and that it remained stable until the end of pregnancy, and in the first month of lactation. These findings partially agrees with our findings, ALT increased in 37% of G2 and 51% in G3.

3. BHBA:

SCK may start at serum BHB concentrations above $1000 \mu\text{mol L}^{-1}$ and clinical ketosis at about $2600 \mu\text{mol L}^{-1}$. In the Therefore, all the cases is considered as subclinical ketosis. Recent work has suggested that elevated BHBA in the first week postcalving has more impact on both cow health and milk yield reduction than elevations later in lactation (18). Energy source requirements of insulin-sensitive tissues are more flexible and can shift from glucose to other fuels, including metabolites like (Non-Esterified Fatty Acids) NEFA and BHBA (19). Subclinical ketosis is considered present in cows with an elevated concentration of ketone bodies in blood but without clinical symptoms of ketosis (20). Similar findings were observed in the present study. The present study agrees with the study that carried out by (21) who presented that elevated BHBA is common to the transition period. Elevated BHBA concentration in cattle occurs during the first two months of lactation when appetite is limited and the mammary gland has priority for glucose (22-25). This finding is in line with the results of our study. We found that the cows of G3 and G4 showed significant elevation of BHBA. In addition to that, the concentrations of BHB increase postpartum and peak 2–4 weeks postpartum (8), (26). The present results agree with this observation. (1) (27) reported that nearly 30% of dairy cows have subclinical ketosis in the first two weeks after parturition. Whereas, in the present study, 97% of the cows in the early lactation (G3) were having subclinical ketosis. That could be attributed to feeding a poor feed stuff. It is possible to induce ketosis via overfeeding of highly ketogenic feeds (1), (25), (28), (29). That could interpret the elevated BHBA concentration in many cows who were not in the transition period, as we found some owners were feeding dates to their cows. In the present study, the

cows with the elevated concentration of BHBA showed decreased in the concentration of glucose. These findings are in coincidence with that of (30) who reported that the cows with primary, or type I, ketosis that occurs occurs 3–6 weeks postpartum affected cows led to hyperketonemia and low concentration of both glucose and insulin in plasma.

دراسة الكيتوسز تحت السريري في الأبقار في محافظة البصرة

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الخلاصة

يعرف الكيتوسز تحت السريري على انه ارتفاع تركيز الأجسام الكيتونية في الدم مع عدم ظهور العلامات السريرية للكيتوسز. اعتبر قياس تركيز حامض بيتا هيدروكسي بيوتريك (BHBA) كطريقة قياسية مثلى لكشف الكيتوسز تحت السريري. هدفت الدراسة إلى كشف وجود الكيتوسز تحت السريري في الأبقار في حالات فسلجية مختلفة في محافظة البصرة, جنوب العراق. صنفت الأبقار الى خمسة مجاميع حسب الحالة الفسلجية: G1: أبقار حوامل في الثلث الثاني (9 أبقار), G2: أبقار حوامل في الثلث الثالث (8 أبقار), G3: أبقار والدة حديثاً (1-30 يوم) (41 بقرة), G4: أبقار حلوب (2 و 3 شهور بعد الولادة) (21 بقرة) و G5: 4 شهور- أكثر بعد الولادة (14 بقرة). تم قياس تركيز الكولستيرول, فعالية ALT, و تركيز BHBA. اعتمدت نقطة الفصل بين 1000 $\mu\text{mol L}^{-1}$ و $2600\text{-}\mu\text{mol L}^{-1}$. كانت متوسطات تراكيز البارامترات لأبقار المجاميع الخمسة كالتالي: الكولستيرول: 0.34 ± 3.7 , 0.38 ± 3.21 , 0.16 ± 2.59 , 0.15 ± 0.15 و $2.5 \pm$ 0.28 mmol/L ALT, 6.16 ± 45.11 , 3.98 ± 37.125 , 3.36 ± 44.83 , 4.57 ± 45.19 و 41.29 ± 4.37 IU/L و BHBA: 0.15 ± 2.014 , 0.085 ± 2.086 , 0.07 ± 1.94 , 0.18 ± 1.89 و $1.92 \pm$ 0.075 mmol/L. كان تركيز الكولستيرول ضمن الحدود الطبيعية في كل المجاميع لكن كان هنالك انحدار من فترات الحمل المتأخرة لحين الولادة ليشهد صعوداً مع تقدم فترة إدرار الحليب, بينما كان هنالك ارتفاع في فعالية ALT في كل مجموعة مع اختلاف في النسبة المئوية, فيحين اظهر تركيز BHBA ارتفاعاً "معنوياً" في كل المجاميع. من النتائج الحالية, يتبين أن الكيتوسز تحت السريري في محافظة البصرة موجود بشكل واسع. ولأجل منع مضاعفات الكيتوسز تحت السريري, يتوجب إعطاء علائق ذات نوعية جيدة وتقديم البروبيلين كلايكل و تطبيق نظام إدارة جيد و القيام بفحوصات منتظمة لكشف الكيتوسز تحت السريري لتقليل الخسائر الناجمة عن هذا المرض.

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