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Clinical, Some Serum Biochemical and Hematological Alternation in Cows With Subclinical Ketosis

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Abstract: *Subclinical ketosis is usually unnoticed and typically associated with serious disease risk, lower milk production, poor reproductive performance, and decreased profitability of dairy farms. This study was conducted to achieve clinical, Some serum biochemical and hematological alternation in cows with subclinical ketosis. From the period between October 2020 and end of January 2021, 80 blood samples were obtained from recently calved cows through a cross-sectional survey in different areas of Mosul city. The findings of the current study indicated that the prevalence of subclinical ketosis in cross-breed cows was 22/80 (27.5%) depending on the concentration of BHB in the serum as determined by ELISA test at the cut-off point (≥1.2-≤ 1.4 mmol / L). No significant difference (p <0.05) was noted in pulse rate, respiration rate, and temperature, while, significant decrease (p<0.05) of the rumen contractions in animals with subclinical Ketosis (4.21 ± 0.30) was noted compared to the control group (9.4 ± 1.22). Significant increase (p <0.05) was manifested in the serum concentrations of BHB and NEFA (1.289 ± 0.014 mmol / L, 0.539 ± 0.11 mmol / L) respectively in the cows affected with Subclinical ketosis, compared with the control group. Coefficient Correlation analysis showed a positively significant (p <0.01) relationship (r = 0.610) between the concentration of NEFA and BHB in animals with subclinical ketosis. There was significant decrease in serum glucose 37.981 ± 0.987 mg / dL for the subclinical ketosis cows, in comparison with the control group. Therefore, this study indicates that subclinical ketosis is prevalent among dairy cows. Blood BHBA and NEFA concentrations are potentially useful tool for the routine monitoring of subclinical ketosis in early postpartum dairy cows. To minimize economic losses from SCK disease, it is highly recommended that there is regular monitoring of metabolic tests for the duration of the transition period.*

Key words*: Prevalence ,Subclinical ketosis, BHB, NEFA, Glucose*

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I. Introduction

Increased ketone bodies Beta-hydroxybutyrate (BHB), acetoacetate (Ac Ac), and acetone (Ac) in the blood, urine, and milk that may occur in dairy cows post-calving period usually refers to Ketosis. Exogenous (diet) or endogenous (adipose tissue) are the main sources of ketones. The acceptable hypothesis of ketosis is the initiation of a negative energy balance (NEB) state around the period of calving, switches on the mobilization of long-chain fatty acids (Triglyceride) from adipose tissue and formation of ketone bodies (1).

Subclinical ketosis SCK is known as an elevation of ketone bodies in the various body fluids (blood, urine, or milk) without obvious signs of disease. The ordinary cut-off points of BHB concentrations in the serum between 1.200 and 1.400 mmol/L are usually used for differentiation of SCK. On the other hand, clinical ketosis (CK) is characterized biochemically by hypoglycemia, hyperketonemia besides recognized symptoms being present such as body weight loss, lower milk production and appetite with higher levels of BHB in the blood ranging from 2.600 to 3.000 mmol/L (2,3).

The transition period (three weeks before and after calving) is the most critical time frame for dairy cows during which energy is required for growing fetus as well as for milk production. If energy requirement exceeds the energy available for the animal, a negative energy balance occurs. Several metabolic events take place to cope with the energy needs, such as the mobilization of adipose tissue. Cows with a disqualified adaptive response to the NEB may be addicted by metabolic diseases such as subclinical ketosis (4).

The liver picks up about 15–20% of the total non-esterified fatty acids NEFA from the blood and employs it in hepatocytes to produce energy through the tricarboxylic cycle. NEFA can be re-esterified in trilglycerols (TAG), and exported as very-low-density lipoproteins (VLDL) and be converted into ketone bodies. Pronounced plasma NEFA concentration could prompt hyperketonemia and promote gluconeogenesis which moves the oxaloacetate to the Krebs cycle, thus increasing the formation of ketone bodies (5 , 6 , 7).

Several studies in the literature have documented the negative effects of ketosis on dairy cattle, including lowering reproductive performance, decreasing milk production, and eventually, negatively affecting

the profitability of the dairy industry (8 , 9). Most researches that have been done usually focused on SCK as it is prevalent and the affected animals are more at risk for other disorders like abomasal displacement, metritis, mastitis, retained placenta, diminished fertility, decreased reproductive efficiency, and weakened immune functions (10 ,11).

Basically, measurement of the blood BHB concentration is the accepted standard test for diagnosis of ketosis. BHB is more stable in blood than the other ketone bodies acetone or acetoacetate. The most frequently applied cut-off points for subclinical ketosis are 1.2 mmol/L or 1.4 mmol/L for BHB in the blood. In general, clinical ketosis involves significantly elevated levels of BHB, approximately 3.0 mmol/L or more. SCK detection is typically done by testing ketone body concentrations in blood, urine and milk. A range of laboratory and cowside tests can also be performed to monitor ketosis in dairy herds. However, cow side test lack perfect sensitivity and specificity in comparison with blood BHB which is the gold standard test (12 , 13).

The objectives of the current study were to investigate the clinical, some serum biochemical and hematological alternation in cows affected with subclinical in Mosul, Iraq.

II. Materials And Methods

Ethical approval

The blood samples were obtained in accordance with the recommended "standard sample collection procedure" which ensured that animals were not subjected to any stress or harmed in any way.

Animals and study area

In a cross-sectional survey, 80 blood samples were collected from recently calved cows located in various areas of Mosul city, which included , Shalalat, Bazawayah, Al-Intisar, Julechwan, and Tehrawa, over a period from the beginning of October 2020 to the end of January 2021. Epidemiological information (age, management systems, Parity and origin) were recorded. Clinical signs, and clinical parameters (pulse, respiratory rate, rumen movements, and heart rate) were also recorded in a preformed clinical card.

Samples

A total of 10 ml of blood was drawn from the middle coccygeal vein of individual animals using a sterile syringe after cleaning the area and sterilizing it with 70% ethyl alcohol in the morning and before the animal consumed any type of feed. Blood samples were divided into two parts, the first (3 ml) was placed in a sterile test tube containing EDTA anticoagulant for the purpose of blood tests, and the second part of the blood sample (7 ml)) was placed in a sterile 10 ml glass without anticoagulant (gel and clot activator, from Biomed Bulgaria) to obtain blood serum for biochemical tests (BHB and non-esterified fatty acids NEFA and blood sugar Glucose).

Laboratory analysis

1- Enzyme-linked immunosorbent assay for BHB and NEFA concentration in serum

The concentration of ketone body Beta hydroxy butyrate BHB, and concentration of non-esterified fatty acids NEFA in serum samples were measured utilizing an ELISA kit (Bioassay Technology Laboratory, Shanghai, China), according to the manufacturer's instructions. The plate was read by an Elisa Reader at 450 nm wavelength.

2- Blood glucose

Blood glucose concentration in serum was obtained spectrophotometrically (Spectrophotometer, APEL, Japan) according to the manufacturer's instructions (BioLab, France). Glucose level extracted was determined using the formula:

$$= \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \text{ard concentration}$$

3- Blood picture analysis

Hemogram investigations which included RBCc , PCV , Hb , WBCc, MCV, MCH , and MCHC were analyzed on a CBC Spinreact (Spincell 3 Spinreact, Spain analyzer).

Statistical analysis

The prevalence was calculated applying descriptive statistics and using Microsoft Excel, version 2010 for Windows 10. The significant ($P < 0.05$) differences between infected groups and control group were represented by mean and standard error of mean was calculated using T-Test in SPSS program for Windows

(version 21; IBM SPSS, USA). All data with p>0.05 were considered significant. Pearson's correlation coefficient was used to analyze the correlation between BHB and NEFA in the SPSS program for Windows (version 21; IBM SPSS
, USA).

III. Results

In the current study the prevalence of subclinical ketosis in the crossbreed cows in Mosul was 27.5%, based on the concentration of BHB in the serum using ELISA test at the cut-off point (≥1.2-≤ 1.4 mmol / L), and the prevalence of clinical ketosis was 5% at threshold (2.9 mmol / L) (see Table 1).

Table 1: The prevalence of subclinical and clinical ketosis according to BHB concentration in the serum by ELISA test

BHBA mmol/L	Total No. tested	No. of Animals	Percentage (%)
≥1.2 - ≤ 1.4	80	22	27.5
≥2.9		4	5
≥0.7 - ≤1		26	32.5
<0.7		28	35

Results of this study show that animals with subclinical ketosis exhibited unmarked clinical signs represented by partial loss of appetite and fluctuated decrease in milk production. The outcomes also indicated no significant (p <0.05) difference in the clinical parameters of pulse rate, respiration rate, and temperature, while there was significant (p <0.05) decrease of the rumen contractions in animals with subclinical Ketosis (4.21 ± 0.30) compared to the control group (9.4 ± 1.22) (see Table 2).

Table 2: The clinical parameters in Subclinical, Clinical ketosis cows and control group

Parameters	SCK cow (n=22)	Control (n=28)
Body temperature, °C	37.4 ± 0.21	38.2 ± 0.32
Respiratory rate / min	29.0 ± 0.17	28.0 ± 0.64
Heart rate / min	57.9±0.03	58.3 ± 0.34
Rumen contraction / 5min	4.21 ± 0.30*	9.4±1.22

*P<0.05 between diseased cow and control group mean ± standard error of mean
SCK Subclinical ketosis

In this study, there was significant (p <0.05) increase in the concentrations of BHB ketone bodies for the serum ELISA test, as the concentrations was 1.289 ± 0.014 mmol / L in the cows affected with Subclinical ketosis, compared to the control group (0.539 ± 0.11 mmol / L) (see Table 3). The outcomes also indicated a significant (p <0.05) rise in the concentrations of NEFA in the serum (0.510 ± 0.028 mmol / L) for the cows affected with subclinical ketosis, compared to the control group (0.205 ± 0.011 mmol / L) (see Table 3). Coefficient Correlation analysis indicated a positively significant (p <0.01) relationship (r = 0.610) between the concentration of NEFA and BHB in animals with subclinical ketosis (Figure 1). The results of the current study showed a significant decrease in the concentration of glucose, as its serum concentrations were 37.981 ± 0.987 mg / dL for the subclinical ketosis cows, compared to the control's 71.221 ± 2.240 mg / dL (see Table 3)(Figure 1).

Table 3: Serum biochemical parameters in affected cows with subclinical and clinical ketosis compared to control group

	BHB mmol/L	NEFA mmol/L	Glucose mg/dL	P<0.05
Subclinical cow	1.289±0.014[a]	0.510±0.028[a]	37.981±0.987[a]	0.00
Control group	0.539±0.11[b]	0.205±0.011[b]	71..221±0.240[b]	0.00

Values significantly (P < 0.05) different between parameters are labeled with different letters (a, b or c)
mean ± standard error of mean

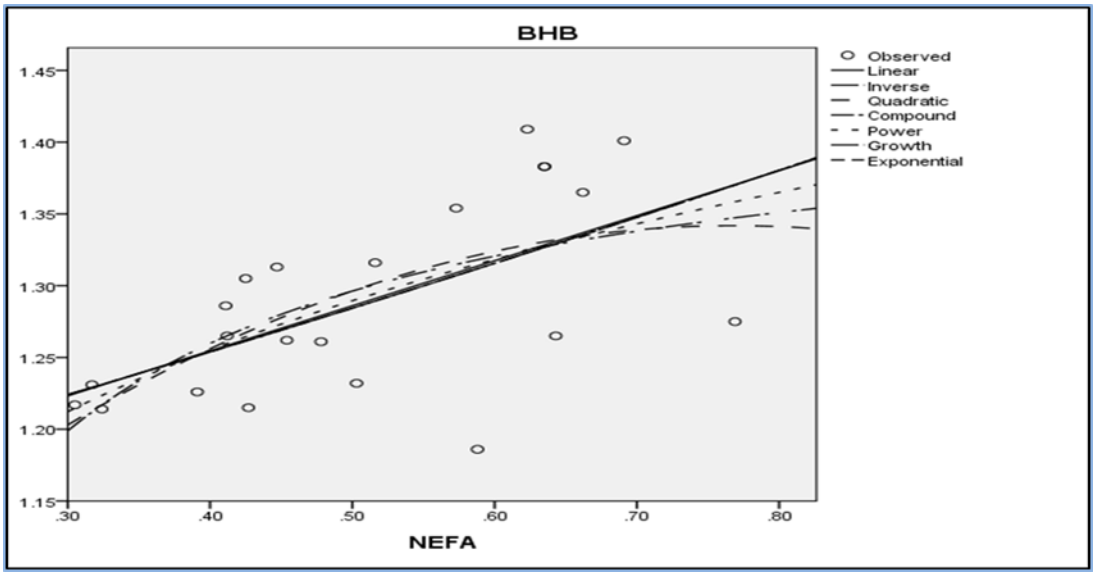

Figure 1: Linear regression showing the correlation between NEFA and BHB concentration in serum for SCK cows

The results of blood picture in the study revealed that animals with subclinical ketosis showed no significant ($p < 0.05$) difference in the values of the examined criteria, as their values reached 5.95 ± 0.98, 8.58 ± 8.17, 25.98 ± 2.55, 16.14 ± 1.04, 35.82 ± 7.57, 46.20 ± 2.95. , 2.84 ± 3.27, 4.61 ± 0,21, 0.31 ± 1.90 for TECc, Hb, PCV, MCH, MCHC, MCV, LS, and Eosinophil standards respectively, with a significant ($p < 0.05$) increase in the total number of white blood cells (11.01 ± 0,21), neutrophil cells (0.47 ± 0,113) and mononuclear cells (0.47 ± 0.13) compared to the control group (Table 4).

Table 4: Blood parameters in affected cows with subclinical ketosis compared to control group

Blood parameter	**Control group *n*=28**	**SCK Cow *n*=22**
TECc (10^6/mm^3)	6.71± 0.85	5.95±0.98
Hb (g/dL)	9.21±8.52	8.58±8.17
PCV (%)	26.72±3.00	25.98±2.55
MCH (pg.)	16.76±1.33	16.14±1.04
MCHC (%)	36.16±8.24	35.82±7.57
MCV (fl)	46.67±3.61	46.20±2.95
TLCc (10^3µl)	8.79±0,07	11.01±0,21*
Lymphocyte (10^3µl)	4.38±0,06	4.61±0,21
Neutrophil (10^3µl)	3.69±0,01	5.62±0,02*
Monocyte (10^3µl)	0.38±0,001	0.47±0,013*
Eosinophil (10^3µl)	0.32±1.10	0.31±1.90

*P<0.05 between diseased cow and control group, mean ± standard error of mean

IV. Discussion

The prevalence of subclinical ketosis in the cows was 22/80 or 27.5%, depending on the concentration of BHB in the serum using ELISA test at the cut-off point (≥1.2-≤ 1.4 mmol / L). Determination of concentration of Beta hydroxybutyrate in the serum and/or plasma is a gold standard test for diagnosis of Subclinical Ketosis. This result is in agreement with previous studies (14 ,15), which revealed that estimation of BHB was accepted as a gold standard for the diagnosis of SCK because if its stability in blood and the cutoff value of 1.2 to 1.4 mM of BHB in blood samples is utilized to differentiate between cows with and without SCK.

Animals affected with subclinical ketosis showed no prominent signs, but exhibited a partial loss of appetite and fluctuated decrease in milk production. These results were in agreement with (13,16). Subclinical ketosis is a metabolic condition in which ketone body concentrations are elevated in body fluids without obvious clinical signs. Several studies (17,18) among others, reported that subclinical ketosis was not

accompanied by clinical signs, and were predisposed to many metabolic and other diseases, such as abomasal displacement, retained placenta, mastitis, prolonged estrus interval, in addition to increased rate of culling. which often led to high economic losses. Results also showed no significant ($p < 0.05$) difference in the clinical parameters of pulse rate, respiration rate, and body temperature, while there was significant ($p < 0.05$) decrease of the rumen contractions in animals with subclinical ketosis (4.21 ± 0.30) compared to the control group (9.4 ± 1.22). Our results were in accordance with (19, 20, 21). The decrease in amplitude of rumen movements could be attributed to the effect of excessive ketone bodies production. Earlier studies (22 , 23) reported the decrease of rumen contractions to the effect of excessive ketone bodies that had an inhibitory effect on the rumen wall, keeping it partially empty and causing rumen depression. Youssef *et al*. (2010) (24) attributed rumen stasis to inhibition of rumen stimulants such as the hormone cholecystokinin, which stimulates rumen emptying.

This research confirmed increases of serum BHBA concentrations in cows with SCK (3.733 ± 0.728 mmol / L), compared with to the control group (0.539 ± 0.11 mmol / L). This result is similar to several those of previous researches (14 , 21, 25). The reason for the high concentration of BHB could be due to the occurrence of negative energy balance, that is associated with the high demand for energy, fats, and protein after calving for lactation and milk production, which trigger the mobilization of fat from the body store to use them as a source of energy, concomitant with incomplete oxidation of excessive NEFA in the liver and increase in circulating BHB ketone bodies. Preliminary researches indicated that the decreased production of glucose by the liver could reduce its concentration in the blood and lower the secretion of insulin, which in turn increases the mobilization of fats from the adipose tissue and increases the rate of absorption of hepatic fatty acids, and subsequent increase in the levels of NEFA and BHBA (26 , 27). It is acknowledged that fat mobilization usually is accompanied by the production of large amounts of acetyl-CoA, from incomplete metabolized fatty acids through the citric acid cycle and as a result, the acetyl-CoA is converted to acetoacetate which is reduced to BHB by an enzyme, α-dehydrogenase (28 , 29).

The findings illustrated a significant ($p < 0.05$) rise in serum concentrations of NEFA (0.510 ± 0.028 mmol / L) for the cows affected with subclinical ketosis compared with the control group (0.205 ± 0.011 mmol / L). CCA indicated a positive significant ($p < 0.01$) relationship ($r = 0.610$) between the concentration of NEFA and BHB in animals with subclinical ketosis. The findings of the current study correspond with (9 , 30 , 31 , 32). The reason for this change in the level of NEFA could be the negative energy balance after birth to meet the need for milk production, so the animals resort to the body's fat stores for energy production, and for this reason, NEFA concentrations in the blood can be used as an indicator of energy consumption. Gross *et al.* (2013) (33) indicated that the serum NEFA concentration reflects the level of reserve fat mobilization as compensation for the imbalance between the nutrients consumed by the cow and the nutrients excreted in the milk. Hence, elevated NEFA levels have an association with an increased rate of lipolysis, which is positively stimulated by the hormone glucagon, and in turn stimulates a cycle by hypoglycemia, a direct effect of negative energy. Results also indicate the possibility of relying on the concentration of fatty acids NEFAs in the serum as diagnostic indicators of the risk of metabolic diseases in dairy cows as the correlation between NEFA and BHB $r=0.610$. Initial studies reported that increased serum NEFA and BHBA concentrations had a detrimental effect on reproductive performance and milk production (34). In contrast to these results, (35) revealed that BHB levels were more sensitive to the detection of subclinical status than NEFA in high-yield dairy cows with a high degree of fat mobilization. Moreover, mobilization of high fat negatively affects liver function but it does not mean that ketosis may be present.

This study reported a significant reduction in the concentration of glucose, as serum concentrations were 37.981 ± 0.987 mg / dL for the subclinical, compared to the control group (71.221 ± 2.240 mg / dL). The result in this current study is in agreement with other previous studies (35 , 36 , 37). The reason here may be the fact that most dairy cows experience negative energy balance at the time of early lactation, when energy requirements for lactation and milk production are higher than their consumption, which concomitantly can lead to metabolic stress and fat mobilization from body fat stores. Initial studies revealed that the regulation of lipolysis and lipogenesis balance is done through glucose and insulin concentration. Moreover, in early lactation, production of lactose increases dramatically, and increases the demand for glucose. This, in turn, speeds up the process of gluconeogenesis and often reduces glucose consumption. Despite these compensatory efforts, the blood glucose concentration decreases, especially in older dairy cows due to higher milk production and the consequent higher demand for lactose (38 ,39).

The results from the blood picture showed no statistically significant difference in TECc, Hb, PCV, MCH, MCHC, MCV, LS, and Eosinophil. This result suggests that animals with subclinical ketosis in this study are not accompanied by changes in these blood parameters and is in agreement with (40). In contrast, (37) revealed significant decrease in hemoglobin (Hb) in subclinical affected ketotic cows. Result also showed significant ($p < 0.05$) rise in the total number of white blood cells ($11.01 \pm 0,21$), neutrophil cells ($0.47 \pm 0,113$) and mononuclear cells (0.47 ± 0.13) in comparison with the control group. The outcomes of the current study agree with the findings of (39). These changes in the haemogram findings may be due to an increased BHB

concentration and hypoglycemia due to negative energy balance. On the other hand, high neutrophil and lymphocyte counts can result from stress and elevated cortisol levels. Previously, a researcher had demonstrated that an increase in neutrophils and lymphocytes in subclinical cows with ketosis occurred as a result of the mobilization of nonsteroidal fat and the formation of ketone bodies (41 , 42).

V. Conclusion

In conclusion, successful control and subsequent prevention of economic loss depend highly on early detection of subclinical ketosis in dairy farms, when it can be efficaciously obtained by estimation of blood BHB with a cut-off point between ≥1.2-≤ 1.4 mmol / L, and NEFA >0.4 mmol / L in postpartum period. Changes in hemogram have little value for the diagnosis of subclinical ketosis. Significant decrease in serum glucose, was observed in subclinical kenotic cows compared to normal healthy cows.

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Conflict Of Interest

Authors declare no conflict of interests of the manuscript.

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