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Hemato-biochemical and Ultrasonographic Studies on Subclinical Ketosis in Dairy Cows

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Abstract: Seven hundred-eighty dairy cows in Kafr-Elshikh and El-Beheira provinces were tested for subclinical ketosis (SCK) using qualitative determination of β-hydroxybutyric acid (BHBA) in urine from January to December 2022. All 280 positive cows were gathered in a questionnaire. The clinical examination, serum biochemical analysis, complete blood picture, and liver ultrasound of 89 subclinical ketotic cows were all performed. Our findings showed that both ruminal motility and milk production had significantly decreased while serum levels of BHBA, non-esterified fatty acid (NEFA), TAG, GGT, AST, ALT significantly elevated, blood glucose, total protein, albumin, and calcium levels decreased. Hb, RBCs, PCV, MCV, MCH, and MCHC, whereas WBCs, neutrophils, eosinophils, basophils, monocytes, and neutrophil-lymphocyte (N/L) ratio significantly increased. These cow's ultrasonography examinations revealed the typical signs of fatty liver. The prevalence of subclinical ketosis was 35.9 %. The development of SCK in dairy cows is influenced by various risk factors such as cows at the first 60 days of lactation, body condition score (3.5-5), age greater than 6 years, high milk production, and parity greater than three times. Early detection of SCK in early postpartum dairy cows is crucial to prevent financial loss to the farmers. Routine estimation of SCK in early postpartum dairy cows can be achieved using BHBA and NEFA estimation, along with hepatic ultrasonography.

Keywords: Cows; Subclinical ketosis; β-hydroxybutyrate; Non-esterified fatty acid; Ultrasonography

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1. Introduction

In dairy herds, SCK with rising BHBA concentrations reduces milk output and increases the risk of peripartum illnesses, resulting in economic losses **(Duffield et al., 2009; McArt et al., 2013; Suthar et al., 2013; Gohary et al., 2016).** Low dry matter intake and higher energy reduction for milk production cause dairy cows to have a negative energy balance (NEB) **(Straczek et al., 2021).** Acute NEB causes fat to be mobilized **(Tessari et al., 2020)**, which then raises the levels of fatty acids and β-hydroxybutyrate (BHB) in the blood, leading to hyperketonemia **(Yepes et al., 2019).** Due to its effect on farm profitability, particularly in Holstein dairy farming, hyperketonemia develops into an economically significant postpartum metabolic condition **(Deniz et al., 2020).** Ketosis reduces the dairy industry's profit by raising the costs of diagnosis, treatment, milk losses, the likelihood of mortality, and early culling **(Martens, 2020).** The incidence of SCK in dairy cows is extremely high reaching 40-60% in the first two lactations **(McArt et al., 2012).** The average prevalence of SCK is around 24.1%, which is greater than that of clinical ketosis **(Brunner et al., 2019).** The presence of SCK is linked to economic losses because of the possibility of abomasum displacement, reproductive issues, infectious illnesses, decreased milk output, and increased chance of culling cows **(Kenez et al., 2016).** Increases in ketone bodies over the normal range in cow's blood, plasma, serum, urine, and milk are indicative of SCK **(Benedet et al., 2019).** When diagnosing SKC or hyperketonemia in high-yielding dairy cows without clinical symptoms, the blood BHB concentration is measured using a threshold of 1.0-1.4 mmol/l **(Fiore, 2020).** This study was conducted to determine the prevalence, risk factors, and hemato-biochemical, and ultrasonographic changes of subclinical ketotic cows in Kafr-Elshikh and El-Beheira provinces.

2. Materials and Methods

2.1. Ethical statement

The research protocol was authorized by Damanhur University Faculty of Veterinary Medicine's ethical committee (DMU/VetMed-2023/035). The nation's standards for animal care and welfare were followed in all animal handling and procedures.

2.2. Animal

Seven hundred- -eighty dairy cows from 8 small-scale dairy farms in Kafr-Elshikh and El-Beheira provinces, Egypt during the 5th - 60th day postpartum from January to December 2022 were investigated for subclinical ketosis. These cows ranged from 6 to 11 years old, their body weight ranged from 350 to 450 kg, the average body condition score was 2 to 4 according to **Wildman et al. (1982),** the average milk yield was 8 to 20 kg/day and parity ranged from 3-7. All Dairy cows were given diets that were appropriate for their physiological state (lactation). Total mixed rations (TMR) were managed as feed in the 8 farms. All cows receive routine therapy for internal and external parasites. All cows were tested for subclinical ketosis using qualitative determination of BHBA in urine. Out of 280 who tested positive for subclinical ketosis, 89 cows had full clinical examinations that included measuring their ruminal movements, rectal temperatures, respiration, and heart rates (**Constable et al., 2017).** A control group of ten dairy cows that were in good clinical condition were selected from the same farms.

2.3. Sampling, hematological, and biochemical analyses

Urine samples were collected from all examined cows in clean plastic containers during natural urination after perineal massage **(Rosenberger, 1979).** Urine was examined for qualitative determination of ketone bodies by using BHBA ketone test strips (Porta Check, Inc, Moorestown, USA) **(Bonini et al., 1988).** Ten millimeters of blood were drawn from the jugular vein of control and sub-clinically ketotic cows. The initial blood sample was obtained in sterile, dry test tubes that were angled downward, clean non-hemolyzed serum was then gathered, transferred, and maintained frozen at -20 ºC in clean, dry vials. Serum samples were used to measure BHBA **(Peden, 1964),** non-esterified fatty acid (NEFA) **(Soloni and Sardina, 1973)**, triacylglycerol in feed state (TAG) (**Dryer, 1970)**, Gamma-glutamyltransferase (GGT) (**Heersink et al., 1980),** aspartate aminotransferase (AST) and alanine aminotransferase (ALT) **(Reitman and Frankel, 1957),** total protein **(Henery, 1968),** albumin**(Dumas and Biggs, 1972)** and glucose **(Young, 2001)** using spectrophotometric methods and specific kits. Commercial test kits are used for the determination of ionized calcium (Ca) **(Clarck et al., 1975)**. To perform complete blood pictures, the second blood sample was drawn into a test heparinized tube. hemoglobin (Hb), erythrocytes (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood count (WBC) and indicators of leucocyte formula (neutrophils, lymphocytes, eosinophils, and basophils, monocytes) were measured using an Auto analyzer/Cell counter by Mindray (BC-2800vet) (**Jain, 2000)**.

2.4. Ultrasonographic assessment

Ultrasonography examination according to **Braun (1996)** was carried out using a 3.5MHz micro-convex transducer for liver examination of all subclinical ketotic cows. Before applying coupling gel, the entire abdomen, including the right 6^{th} and 12^{th} intercostal spaces (ICS) were trimmed, shaved, and alcohol swabbed to eliminate excess oil.

2.5. Statistical analyses

Data were collected and subjected to statistical analysis using a T-Independence test between healthy and subclinical ketotic cows using SPSS software (Version 21). The alpha level for determination of significance was $p < 0.05$. Two stages of Logistic regression analysis were performed to analyze risk factors that could be related to the development of SCK. Every possible risk factor (independent variables) was first subjected to a univariate analysis and the variables with $p \leq 0.25$ were selected for a subsequent multivariable logistic regression analysis. Each result was presented as a P value and odds ratio (OR) with a 95% confidence interval (CI 95%).

3. Results

Selected cows that tested positive for ketone bodies in urine but didn't exhibit any obvious clinical symptoms didn't exhibit any appreciable changes in their body temperature, pulse, and respiratory rates, but showed a significant decrease in the ruminal motility (2.36 ± 0.05 per 2 minutes) compared to the control cows (3.70 ± 0.21 per 2 minutes).

In comparison to the control group, sub-clinical ketotic cows had higher serum levels of BHBA and NEFA ($p < 0.05$). Additionally, there was a substantial increase in GGT, AST, and ALT serum activities ($p < 0.05$). On the other hand, serum levels of TAG, blood glucose, total protein, albumin, and ionized calcium were substantially decreased ($p < 0.05$) (**Table 1)**. There was a substantial decline in Hb, RBCs, PCV, MCV, MCH, and MCHC levels ($p < 0.05$). Conversely, subclinical ketotic cows had noticeably greater levels of WBCs, neutrophils, eosinophils, basophils, monocytes, and N/L ratio ($p < 0.05$) than control cows **(Table 2)**.

Unlike the ultra-sonogram of a control cow's normal liver, which displayed a uniform granular echo texture of parenchyma and sharp, visible blood vessel edges (**Figure 1**), Subclinical ketotic cow's liver ultrasonography revealed the existence of fatty liver characteristics, beam attenuation, backscattering, and fine echogenicity and improved blurring of the vessels. Several focal hyperechoic lesions were different in size, position, and shape. Additionally, larger livers, rounded liver borders, coarser echoes, and the liver parenchyma echogenicity near the abdominal wall were seen (**Figure 2**).

In this study, the prevalence of SCK was 35.9 % in Kafr-Elshikh and El-Beheira provinces. The main risk factors that increase the chance of development of subclinical ketosis in dairy cows are cows at first 60 days of lactation, good body condition score (3.5-5), age greater than 6 years, high milk production and parity greater than three times (**Table 3,4**).

Table 1. Biochemical findings of subclinical ketotic and healthy (control) cows

Parameters	SCK	Healthy cows
BHBA (mmol/l)	1.76 ± 0.03 ^{a}	0.83 ± 0.07 ^{b}
NEFA (mmol/l)	0.79 ± 0.01 ^{a}	0.23 ± 0.03 ^{b}
TG (mmol/l)	0.12 ± 0.01 ^{b}	0.37 ± 0.02 ^{a}
GGT (U/l)	30.67 ± 0.79 ^{a}	19.80 ± 1.74 ^{b}
AST (U/l)	62.31 ± 1.95 ^{a}	50.00 ± 4.38 ^{b}
ALT (U/l)	58.74 ±1.07 ^{a}	39.70 ± 2.41 ^{b}
Glucose (mg/dl)	40.96 ± 0.45 ^{b}	55.90 ± 2.16 ^{a}
Total protein (g/dl)	4.92 ± 0.07 ^{b}	7.03 ± 0.14 ^{a}
Albumin (g/dl)	1.95 ± 0.06 ^{b}	2.65 ± 0.12 ^{a}
Ca (mmol/l)	8.65 ± 0.06 ^{b}	10.60 ± 0.22 ^{a}

Values are means ±SE; Means with different superscripts in the same raw are significantly different ($p < 0.05$).

Table 2. Hematological values of subclinical ketotic and healthy (control) cows

Parameters	SCK	Healthy cows
Hgb (g/dl)	10.04 ± 0.21 ^{b}	11.99 ± 0.11 ^{a}
RBCs (10^6/µl)	5.98 ± 0.21 ^{b}	6.50 ± 0.11 ^{a}
PCV (%)	33.70 ± 0.79 ^{b}	36.47 ± 0.66 ^{a}
MCV (fl/cell)	56.3 ± 2.07 ^{b}	56.52 ± 0.96 ^{a}
MCH (Pg)	17.99 ± 0.68 ^{b}	18.49 ± 0.31 ^{a}
MCHC (g/dl)	29.80 ± 0.95 ^{b}	32.24 ± 0.70^{a}
WBCs (10^3/µl)	8.79 ± 0.50 ^{a}	7.18 ± 0.31^{b}
Neutrophils (10^3 µl)	4.44 ± 0.09 ^{a}	3.47 ± 0.15 ^{b}
Lymphocytes (10^3 µl)	4.98 ± 0.08 ^{a}	4.52 ± 0.14 ^{a}
Eosinophils (10^3 µl)	0.84 ± 0.01 ^{a}	0.33 ± 0.01 ^{b}
Basophils (10^3 µl)	0.20 ± 0.01 ^{a}	0.13 ± 0.01 ^{b}
Monocytes (10^3 µl)	0.67 ± 0.02 ^{a}	0.36 ± 0.01 ^{b}
N/L ratio	0.90 ± 0.03 ^{a}	0.76 ± 0.04 ^{b}

Values are means ±SE; Means with different superscripts in the same raw are significantly different ($p < 0.05$).

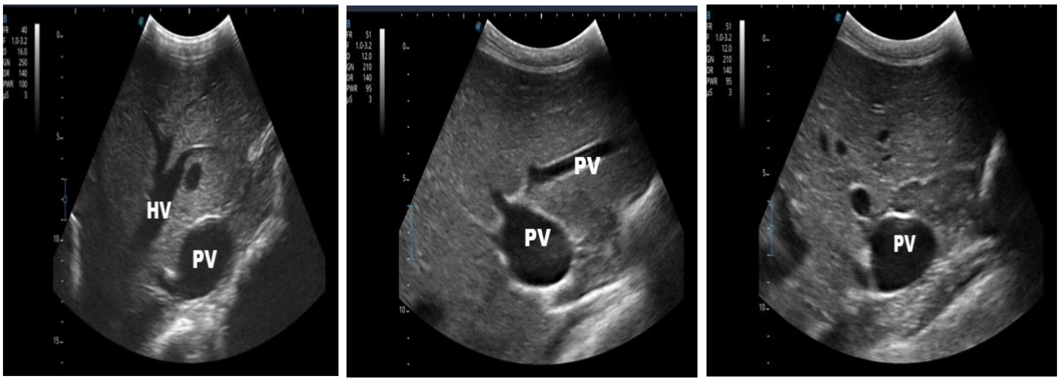

Figure 1. An ultrasonogram of normal liver in the control group was taken from 7-12 ICS. Note sharp blood vessel margins and the liver parenchyma has a uniform granular echo texture. Portal vein (PV), Hepatic vein (HV).

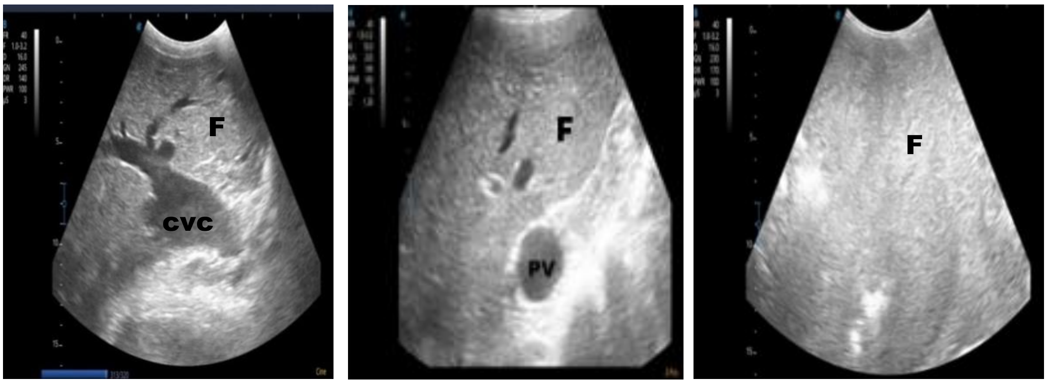

Figure 2. An ultrasonogram of fatty liver (F) in the diseased group was taken from 7-12 ICS. Note the brilliant pattern of the hepatic parenchyma, vessels blurring, and presence of focal hyperechoic lesions of varied shapes, sizes, and positions. Caudal vena cave (CVC); Portal vein (PV).

Table 3. Univariable analysis of risk factors associated with occurrence of subclinical ketosis in dairy cows

Variables	β	Odds ratio (OR)	Sig.
Days in milk			
Dry (reference)	0.00	1.00	
Less than 60 days	0.478	2.613	0.042
More than 60 days	-1.8	1.17	0.200
Body condition score			
(reference)	0.00	3.00	
(3.5-5)	-0.54	3.39	0.01
(1.5-2.25)	-0.112	4.894	0.535
Age			
Less than 6 years (reference)	0.00	1.00	0.06
More than 6 years	1.086	3.963	
Daily milk amount			
Less than 20 kg (reference)	0.00	1.00	
More than 20 kg	-0.818	1.441	0.022
Parity			
Less than 3 time (reference)	0.00	1.00	
More than 3time	-0.316	1.729	0.01
Housing system			
Indoor (reference)	0.00	1.00	
Outdoor	-0.299	2.742	0.20
Season			
Summer (reference)	0.00	1.00	
Autumn	1.30	3.69	0.21
Winter	-0.76	1.47	0.22
Spring	-0.21	2.81	0.3
Proper hygienic measures			
Applied (reference)	0.00	1.00	
Not applied	4. 1	4.05	0.11

β, regression coefficient; OR, odds ratio, $p \leq 0.25$.

Table 4. Final multivariable logistic regression analysis of positive risk factors associated with occurrence of subclinical ketosis in dairy cows

Variables	β	Odds ratio (OR)	95% CI	Sig.
Days in milk				
Dry (reference)	0.00	1.00		
Less than 60 days	0.478	4.5	0.958-2.714	0.01
More than 60 days	-1.8	1.17	0.09-0.55	0.001
Body condition score				
Medium (reference)	0.00	1.00	1.1-1.7	
Good (3.5-5)	-0.54	5.39	0.627-1.274	0.01
Poor (1.5-2.25)	-0.112	1.82		0.535
Age				
Less than 6 years (reference)	0.00	1.00	1.36-6.454	0.01
More than 6 years	1.086	6.93		
Daily milk amount		1.00	0.219-0.889	
Less than 20 kg (reference)	0.00	2.41		0.02
More than 20 kg	-0.818			
Parity				
Less than 3 time (reference)	0.00	1.00	0.601-0.884	
More than 3time	-0.316	5.73		0.001

Model statistics: $\chi 2$ =2.92; $P \leq 0.05$; β, regression coefficient; OR, odds ratio, $p \leq 0.05$.

4. Discussion

Body temperatures, respiration, and pulse rates of subclinical ketotic cows in our study showed no significant change, these results correlate with those of **Issi et al. (2016)**. Increased ketone body synthesis may have a negative impact on rumen movement amplitude **(Dar et al., 2018). Lean et al. (1991)** reported that a rise in ketone bodies inhibited the rumen wall, causing rumen depression and leaving it half empty. Rumen contractions decrease as a consequence of this.

Subclinical ketotic cows may have a markedly higher BHBA because of their high energy needs in late pregnancy and early lactation this is extremely prone dairy cows to negative energy balance. The majority of cows must mobilize their body fat to meet their energy needs for milk production. When significant amounts of body fat are required as an energy source to support production, the liver may not always be able to properly digest fat. In this case, the cow produces more ketone bodies than it can use, which leads to ketosis because the amount of glucogenic precursors is limited. When carbohydrates are scarce, ketone bodies give peripheral tissue energy. Acetoacetate, BHBA, and acetone are the three circulating ketone bodies. Acetoacetate is the parent ketone body and can undergo an enzymatic reduction to BHBA or a spontaneous non-enzymatic decarboxylation to acetone. In subclinical ketotic cows, BHBA is the primary circulating ketone body and is relatively stable in plasma, serum, and whole-body fluid (**Rodriguez-Jimenez et al., 2018**; **Djokovic et al., 2019).**

Additionally, our findings showed that subclinical ketotic cows had significantly higher serum NEFA levels. These results concur with those obtained by **Ospina et al. (2010)**, **Asl et al. (2011)**, and **Štolcová et al. (2020).** They attributed this development to the animal's necessity to use their body's fat reserves for energy after giving birth to meet the demand for milk production. Thus, blood levels of NEFA can be utilized as an indicator of energy consumption. According to **Gross et al. (2013)**, serum NEFA concentration indicates how much reserve fat is mobilized to balance off the nutrients that cows consume and the nutrients they excrete in their milk.

In the current investigation, all subclinically ketotic cows experienced a considerable reduction in TAG. This supports earlier research **(Djoković et al., 2013**; **Đjoković et al., 2016). Reichel and Sokoi (1987)** and **Veenhuizen et al. (1991)** suggested that the significant increase in blood FFA concentrations, which raises the lipid content in hepatocytes, is what caused the decline in blood TAG.

All subclinical ketotic cows showed a considerable rise in their serum levels of GGT, AST, and ALT. These concur with those mentioned by **Padmaja and Rao (2013)** and **Har (2015)**. This result was linked to the production of fat globules in the hepatocyte and enzyme leakage in the blood circulation. Regarding subclinical ketotic cows, serum ALT activity increased significantly when compared to control cows; this result is in line with the findings published by **Bali et al. (2016)**, **Li et al. (2016)**, and **Youssef et al. (2010)**.

All subclinical ketotic cows showed a considerable reduction in blood glucose. This is in line with the conclusions drawn by **González et al. (2011)**, **Mohebbi et al. (2019)**, and **Paramesh et al. (2020)**. They observed that the greatest negative energy balance occurs in early lactation in dairy cows when their intake of energy is less than what is needed for lactation and milk production. This may result in metabolic strain and the release of stored fat from the body.

A notable reduction in serum total protein levels in all subclinically ketotic cows was obtained, these results were in agreement with **Sainath (2015)**, who noted that protein catabolism could be the cause of the drop in serum total protein as the cows are experiencing an energy deficit due to a higher rate of gluconeogenesis, which provides a crucial energy source for the synthesis of milk lactose and milk protein.

All of the subclinically ketotic in the current study had a considerable drop in serum calcium levels. This study's conclusions concurred with those of **Padmaja and Rao (2013)** and **Akgul et al. (2017)**. A large drop in serum calcium may indicate an acidosis-related loss of base in the urine or a problem with the gut's ability to absorb minerals **(Padmaja and Rao, 2013)**. The same authors also noted the use of blood calcium content as a reference index for diagnosis of SCK and the early lactation period is when dairy cows with SCK experience impaired calcium uptake and utilization due to the high concentration of BHBA. **Singh et al. (2017)** state that the enhanced transfer of calcium required for the development of the fetal skeleton and for the milk synthesis during the early lactation phase is what causes the drop in serum calcium levels during late gestation and early lactation phase. An increase in the rate of bone mobilization or gastrointestinal absorption does not counterbalance this enhanced transfer of calcium and lower intake of dry matter.

Hemoglobin levels significantly decreased in subclinical ketotic cows. **Sahoo et al. (2009) and Har (2015)** reported similar outcomes. Additionally, a notable increase in the overall count of white blood cells, neutrophils, N/L ratio, and mononuclear cells excluding lymphocytes was seen. The hemogram findings may have changed as a result of an elevated BHB concentration and hypoglycemia brought on by negative energy balance **(Marutsova et al., 2018)**. Stressful situations, like ketosis, dystocia, and abomasal displacement in cows are associated with higher neutrophil counts **(Tornquist and Rigas, 2010; Roland et al., 2014).** In experimental animals, it has been demonstrated that the N/L ratio is a good indicator of both acute and chronic stress **(Swan and Hickman, 2014; Hickman, 2017)** as well as pigs may experience long-term stress **(Sanchez et al.,**

2019) and numerous other forms of stress in cattle **(Lynch et al., 2010)**. Neutrophils were elevated in subclinical cows due to the mobilization of non-steroidal fat and the synthesis of ketone bodies, as demonstrated by **Burton et al. (2005)** and **Belić et al. (2011)**. A link between NEFA levels and neutrophil activity was also demonstrated by **Hammon et al. (2006)**. Both NEFA and BHB prevent lymphocyte growth and function **(Qi et al., 2022)**. According to **Zhang et al. (2018)**, a rise in proinflammatory cytokine and polymorphic cells during the postpartum phase in cows with NEB causes an overabundance of neutrophil counts in subclinical ketotic cows and may encourage systemic inflammation.

Fatty liver disease often coexists with other metabolic conditions (**Rukkwamsuk et al., 1999; Heuer et al., 2000)**. When fatty liver disease is suspected, liver biopsy may be replaced by ultrasonography, a trustworthy and non-invasive diagnostic technique. It provides a high level of certainty and is a reasonable substitute for liver biopsy in some situations, particularly during the transition period (**Komelian et al., 2011)**. Subclinical ketotic cows were investigated using liver ultrasonography, which revealed beam attenuation, backscattering, elevated hepatic TAG, fine echogenicity, enhanced vascular blurring, and distinct hyperechoic lesions of varied sizes and shapes. Additionally, we noticed an enlargement of the liver and rounded edges of it. These results are in line with those of **Braun (1996)** who reported that, in comparison to normal liver tissue, fat has a lower acoustic impedance which contributes to its higher echogenicity.

A recent study found that 35.9 % of dairy farms in the provinces of Kafr-Elshikh and El-Beheira had SCK. In the first two lactation months, 8.9-43% of dairy cows have SCK according to **Dohoo et al. (1983)**, **Duffield et al. (1998)**, and **McArt et al. (2012)**. Furthermore, the prevalence of SCK in cows ranges from 7% to 41%, according to studies by **Geishauser et al. (2000)** and **Engalbert et al. (2001)**.

Cows were at greater risk for developing subclinical ketosis during their first 60 days of lactation. According to reports, the third and fourth weeks saw the highest incidence of **(Dohoo and Martin 1984; Anderson and Emanuelson, 1985).** Recent reports **(Suthar et al., 2013; Tatone et al., 2016)** state that in the first two weeks postpartum, SCK prevalence peaked. Most research revealed that during the first two weeks of lactation, there is a greater prevalence of ketosis which is followed by a significant decline in prevalence **(Santschi et al., 2016)**.

The development of SCK was significantly influenced by body condition score, cows with body condition score (3.5-5) were at high risk to develop SCK. In the early lactation phase, cows with over-conditioning (fatty), BCS of 3.5 or above are prone to suffer from ketosis **(Gillund et al., 2001; Seifi et al., 2011; McArt et al., 2015; Vanholder et al., 2015).** Recent studies have shown that cows with BCS of 3.0 or higher at calving are prone to suffer from ketosis and experience increased lipolysis, which raises BHBA and NEFA plasma concentrations **(Weber et al. 2015; Garzon-Audor** and **Oliver-Espinosa, 2019)**. There is an increased risk of ketosis whose BCS at calving is ≥ 3.75 out of 5.0 **(Herdt, 2000)**. After calving, cows with a BCS of at least 3.5 are 2.5 times more likely to be in ketosis than cows with scores as low as 3.25 **(Gillund et al., 2001)**. According to **Heuer et al. (1999)**, changes in BCS are a direct reflection of dairy cows' energy condition.

Garro et al. (2014) and **Vanholder et al. (2015)** stated that older cows over the age of were considerably more likely to experience subclinical ketosis**.** According to **Mohammed et al. (2019)**, cows 8 to 9 years of age have a higher prevalence of SCK, while cows older than 9 years have a lower frequency. The multiparty and increased milk production may be the reason. On the other hand, it might be because young cows may intensify their metabolic processes in response to stressor conditions like NEB during the early lactation stage. With advancing age, this adaption and activity gradually decline.

There was a correlation between subclinical ketosis and milk production as stated by **Gröhn et al. (1989)**, **Uribe et al. (1995)**, and **Garzon-Audor and Oliver- Espinosa (2019)**. High-producing cows have a higher frequency of ketosis **(Naher et al., 2020)**. The average daily milk output for cows in clinical ketosis, SCK, and healthy cows was found to be 28, 35, and 45 kg, respectively, according to **Samiei et al. (2013)**. The increased energy requirements of lactation and insufficient DMI to meet those requirements result in hyperketonemia and increased NEB, which may be more noticeable in dairy cows with high yields (**Gröhn et al., 1989; Herdt, 2000)**.

Subclinical ketosis prevalence was higher in multiparous cows. The majority of research showed that cows with higher parity (≥ 3) had a higher likelihood of developing SCK as stated by **McArt et al. (2012)**, **Suthar et al. (2013)**, **Berge and Vertenten (2014)**, and **Garzon-Audor and Oliver-Espinosa (2019).** Many authors have proposed a correlation between increased parity and the prevalence of ketosis **(Biswal et al., 2016; Chandler et al., 2018)**. According to major Australian studies **(Golder et al., 2021)** and a study carried out in the United States **(Golder et al., 2019)**, Cows with parity ≥2 were at 2 times higher risk for several diseases than parity 1 cows**.** According to **Berge and Vertenten (2014)**, lactation and gestation are two concurrent physiological processes that are linked to energy depletion, which are characterized by more lipid mobilization and NEB in multiparous cows compared to primiparous cows.

5. Conclusion

In conclusion, it is critical to identify SCK in dairy cows as soon as possible to protect the farmers from financial loss. BHBA and NEFA estimation as well as hepatic ultrasonography may prove to be effective tools for regular assessment of SCK in early postpartum dairy cows. Additionally, the main risk variables for the development of SCK in dairy cows include days in milk, body condition score, age, milk production, and parity.

Conflict of interest: There are no conflicts of interest stated by the authors.

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