Diagnosis of Ketosis in Postpartum Dairy Cows by Rapid On-Farm Tests in the Peri-Urban Area of Dakar, Senegal

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT
The objective of this study is to contribute to the improvement of the productivity of dairy farms through the rapid detection of metabolic diseases of dairy cows on the farm. The study involved 42 dairy cows from a dairy farm located in the village of Niacoulrab. Four blood and urine samples were taken from each cow every two weeks for the first eight weeks after calving. Blood and urine ketone and glucose determinations and urine pH measurements were performed by FREESTYLE OPTIUM device, CUMBUR Test and pH paper during the first 8 weeks postpartum. The following results were obtained: the prevalence and incidence of subclinical ketosis were very high 45% and 32% respectively. At the 4th week of lactation, the blood concentration of Beta-Hydroxy-Butyrate was very high respectively 1.9 ± 0.31 mmol/l and 4.2 ± 0.85 mmol/l in subclinical and clinical ketosis cows. At the 4th week of lactation, urinary acetoacetate concentration was very high in subclinical and clinical cows with values of 8.8 ± 2.3 mmol/l and 15 ± 3.8 mmol/l respectively. Blood glucose concentration was very low in subclinical and clinical ketosis cows with values of 0.37 ± 0.03 mmol/l and 0.2 ± 0.03 mmol/l respectively around the 4th week of lactation. There was a strong correlation between the concentrations of glucose (r²= -0.88) and Beta-HydroxyButyrate (-0.86) in blood and also with those of acetoacetate in urine.

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1. INTRODUCTION

Under the effect of high population growth (3%), the demand for milk and dairy products in Senegal continues to grow and reached a record level of 546.6 million liters in 2018 [1]. To meet this high demand, milk production has developed and is estimated in 2004 at 114.2 million liters, including 95.6 million for cow's milk (84%) and 18.3 million for small ruminant milk (16%) [2].

Dairy production is carried out by extensive or pastoral/agropastoral farms located in rural areas and by semi-intensive and intensive farms in peri-urban areas. Extensive farms are based on the use of local breeds with low milk production potential (an average of 2 l/d for cattle) [3] and natural pastures.

In order to express its high genetic potential, the dairy cow requires a maximum energy metabolism to valorize a very rich ration. Energy metabolism disorders are frequent in dairy farming, especially those related to the energy deficit at the beginning of lactation, when low ingestion capacity and the lactation peak coincide [4]. To make up for this deficit, the animal mobilizes its fat reserves, which leads to an accumulation of ketone bodies (breakdown product of triglycerides) in the blood. This accumulation can lead to clinical disorders (depletion, anorexia, even neurological signs: compulsive licking, salivation...) when it is excessive [5]. However, most of the time, it does not cause any clinical signs, even though it has major negative repercussions on the animal's production and health status (drop in milk production, deterioration of milk quality rates, delay and lower success in reproduction, increased prevalence of certain pathologies: metritis, displacement of abomasum...). The management of this subclinical ketosis is a major issue in dairy farming.

This metabolic disease is even more marked during the postpartum period. Indeed, the peripartum is an important period in the physiological cycle of a dairy cow, starting at dry-off and ending at confirmed pregnancy. Drying off is a key period that will strongly influence the success of the postpartum period, i.e. lactation, but also the reproductive career of the cow. The beginning of lactation is a period during which the needs of the dairy cow increase, but which she struggles to cover because of the decrease in her ingestion capacity after calving. Thus, a good management of the dry period and the beginning of lactation is necessary to avoid the development of metabolic diseases in farms, especially in high milk producing cows [6,7,8].

The detection of these ketoses is a key point for the implementation of corrective measures essential to the good economic health of the farm. Several means of detection are proposed, including rapid diagnostic tests that are increasingly used on the farm. They allow farmers to know more quickly the health status of the cow in order to implement a treatment or to prevent the disease [9].

The general objective of this study is to assess the prevalence of ketosis in postpartum cows (using rapid tests), identify the causes of metabolic diseases, and evaluate the impact of metabolic diseases on milk production.

2. MATERIALS AND METHODS

2.1 Study Site and Period

Our study took place on a farm located in the peri-urban area of Dakar, in particular in the Niayes area and more precisely in the village of Niacoulrab. This farm was created in 2003 by a Senegalese businessman. It is a limited company with a dairy vocation (production and transformation of milk) installed on an area of 3.5 ha. It exploits Holsteins and Normandes dairy cows. The farm also has a dairy for the transformation of curdled milk and yogurt.

The present study was conducted over two periods, the first one from February 13 to April 30, 2015 and the second from September 27 to December 20, 2015. The choice of these periods is related to the calving schedule of the farm.

2.2 Materials

2.2.1 Animal material

The study involved forty-two (42) dairy cows including 36 Holsteins and 6 Normandes (Figs. 1 and 2).

2.2.2 Technical equipment

To carry out our study, we used the following equipment:
Portable reader "FREESTYLE OPTIUM" was used for the determination of ketone bodies (BHB) and glucose in blood in cows.

COMBUR Test: COMBUR Test® strips were used to measure ketones (Ac-Ac) and glucose in the urine of cows.

pH paper: for measuring the pH of the cows' urine.

Barymetric tape: was used to estimate the weight of dairy cows.

Bromatological analysis equipment: several laboratory materials were used to analyze the nutritional values of the feed components of the ration (corn silage, peanut meal, ground corn, dairy cow feed) distributed to the dairy cows. The following equipment was used: a 0.1 mg precision digital balance, a grinder, an oven, a muffle furnace, a desiccator, porcelain glass crucibles, a magnetic stirrer, and a digester (mineralization tube).

A data collection form: to collect data such as: cow identification number, breed, lactation rank, calving date, Body Condition Score (BCS), weight and feed intake.

Sampling sheets: to collect data on glucose and ketone concentrations (BHB, Ac-Ac) in blood and urine, and urine pH of cows.
2.3 Methods

2.3.1 Determination of glucose, Beta-HydroxyButyrate (BHB), and Aceto-Acetate (Ac-Ac)

The portable meter named "FREESTYLE OPTIUM" was used for the determination of ketone bodies and glucose in blood. This device, designed for human medicine, is used daily by diabetics to measure the concentration of ketone bodies and glucose in the blood in order to adapt, if necessary, the treatment. Its use is recent in veterinary medicine in the diagnosis of subclinical ketonemia, where the measurement of blood BHB is recognized as the "gold standard", due to its stability and predominance in the blood stream [10,11]. According to its mode of use, the reader must be calibrated each time using a calibrator electrode. It is advisable to wash hands thoroughly and dry them before opening the individual strip package. Also, after inserting the strip into the device, the reader automatically starts up, and displays the lot number of the individual strip to be used. A drop of blood of about 0.6 to 1.5 μl is then placed on the white area at the end of the strip, the blood will migrate by capillary action to the test area. The result is displayed after 10 seconds for ketone bodies and 20 seconds for glucose.

The COMBUR test is used for the determination of ketones and glucose in urine. The test strips are dipped into the bottles containing the cows' urine. After 5 to 10 seconds, the strip is removed and, depending on its coloring, a concentration is read on the COMBUR test label. The principle of determination of ketones is based on the Legal reaction and for the determination of glucose on the glucose-oxidase/peroxidase reaction.

2.3.2 Measurement of urine pH

We found it useful to use urine pH and not ruminal pH to study ruminal acidosis in early lactation dairy cows for several reasons. Collecting urine by spontaneous micturition is easy and straightforward for farmers and technicians, but collecting rumen juice is a tedious process for them. Furthermore, according to ENEMARK [12], there is a positive linear correlation between urine pH and rumen pH. The normal urine pH of a lactating cow is between 7.8 and 8.4 below 7.8 urine can be considered acidic and one must ask if the ration is the cause [13].

The pH of the urine is measured using a pH paper. The pH paper is immersed in the urine for a few seconds, its coloring will correspond to a pH read on the label of the bottle.

2.3.3 Statistical analysis of the data

Excel software was used for data entry. The calculation of means, standard deviations and analysis of variance of means was done with STATISTICA software version 10.0. The significance level was set at 5%.

3. RESULTS

3.1 Blood (BHB) and Urine (Ac-Ac) Ketone Concentrations in Dairy Cows

The average concentration of BHB in the blood of dairy cows in normal physiological condition (NPS) did not vary between the 2nd and 4th week of lactation (0.7 ± 0.2 mmol/l), but it increased in the 6th and 8th week of lactation (0.8 ± 0.2 mmol/l) (Fig. 3).

The mean blood BHB concentration in subclinical cows was 1.7 ± 0.27 mmol/l at the 2nd week of lactation. This concentration increased to 1.9 ± 0.31 mmol/l at the 4th week and then dropped to 1.5 ± 0.22 mmol/l at the 6th and 8th week of lactation.

In clinically ketotic dairy cows, blood BHB concentration was 2.9 ± 0.28 mmol/l at week 2, increased to 4.2 ± 0.85 mmol/l at week 4. Then, it dropped progressively from the 6th to the 8th week of lactation, i.e. 2.7 ± 0.14 mmol/l and 1.9 ± 0.07 mmol/l respectively.

The mean concentration of acetate (Ac-Ac) in urine of normal physiological dairy cows did not vary during the 8 weeks of lactation, ranging from 0 to 1 mmol/l.

In contrast, in subclinical ketosis dairy cows, the mean concentration was 6.3 ± 1.88 mmol/l at week 2, peaked significantly at the 4th week of lactation (15 ± 3.8 mmol/l), and then decreased from the 6th to the 8th week of lactation to 10 ± 2.3 mmol/l and 5 ± 1.2 mmol/l, respectively (Fig. 4).
3.2 Blood and Urine Glucose Concentration in Dairy Cows

The normal blood glucose level in ruminants is between 0.45 and 0.7 g/l. Thus, a cow is hypoglycemic when her blood glucose concentration is below 0.45 g/l and hyperglycemic when it is above 0.7 g/l.

Our results showed that in cows in Normal Physiological State (NPS), blood glucose was normal during the first 8 weeks postpartum. Indeed, the average glucose concentration (0.53 ± 0.05 g/l) obtained at the 2nd postpartum week, which decreased slightly from the 4th to the 6th week (0.51± 0.07 g/l; 0.5 ± 0.42 g/l respectively), followed by a slight increase at the 8th week (0.51 ± 0.05 g/l).

Our results also showed that in subclinical ketosis (CSb) and clinical ketosis (CC) dairy cows, blood glucose was low during the first 8 weeks postpartum. The mean blood glucose concentration in subclinical ketosis (CSb) dairy cows was 0.39 ± 0.05 g/l at week 2, followed by a decrease at week 4 (0.37± 0.03 g/l) and an increase from week 6 to week 8 of lactation (0.41± 0.05 g/l and 0.44 ± 0.05 g/l respectively). Dairy cows in clinical ketosis (CC) had a blood glucose concentration of 0.35 ± 0.04 g/l at week...
2, followed by a decrease at week 4 (0.2 ± 0.03 g/l), and an increase at weeks 6 and 8 of lactation (0.26 ± 0.03 g/l and 0.44 ± 0.05 g/l, respectively) (Fig. 5).

We did not detect any cases of glycosuria in the cows during the entire period of our experiment.

3.3 Correlation between the Concentrations of Glucose, Beta-Hydroxy Butyrate (BHB), and Acetoacetate (Ac-Ac) in Blood and Urine

We therefore sought to study the correlations that exist between blood and urine concentrations of glucose, Ac-Ac, and BHB.

The correlation coefficients between the concentration of blood glucose and blood BHB and that of Ac-Ac in urine were -0.88 and -0.86 respectively. These strongly negative correlation coefficients show that there is a strong relationship between the concentrations of glucose and BHB in blood and between glucose in blood and Ac-Ac in urine.

The correlation coefficient between blood BHB concentration and urine Ac-Ac concentration is 0.95. This correlation coefficient is strongly positive, which justifies that there is also a strong link between these concentrations (Table 1).

3.4 Urine pH in Dairy Cows

The normal urine pH of a lactating cow is between 7.8 and 8.4. In our study, we found that the urine pH of normal physiological dairy cows was below 7.8 during the first 6 weeks postpartum, resulting in a slightly acidic pH. This pH was 7.2 ± 1.47; 7.6 ± 1.26; and 7.4 ± 1.5 in the 2nd, 4th, and 6th weeks postpartum, respectively.

In subclinical and clinical ketosis cows, urine pH changes were similar during the 2nd, 4th, and 6th postpartum weeks (6.3 ± 1.17 and 6.1 ± 0.14; 5.75 ± 1.13 and 5.5 ± 0.71; 7 ± 1.51 and 6.8 ± 1.13, respectively), thus acidic pH. They are marked by a decrease at the 4th week followed by an increase at the 6th week of lactation. This increase was noted from the 4th to the 8th week of lactation (Fig. 6).

Fig. 5. Evolution of blood glucose concentration during the first 8 weeks of lactation

Table 1. Correlation coefficients between glucose, BHB, and Ac-Ac concentrations in blood and urine (significant correlations marked at p<0.05)

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<th>GLU-S</th>
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<td>GLU-S</td>
<td>1</td>
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<tr>
<td>BHB-S</td>
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<tr>
<td>Ac-Ac-U</td>
<td>-0.86</td>
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GLU-S: Blood Glucose, BHB-S: Blood BetaHydroxy-Butyrate, Ac-Ac-U: Urinary Acetate
Table 2. Correlation coefficients between urine pH and ketone body concentration (blood BHB and urine Ac-Ac) (significant correlations marked at p<0.05)

<table>
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<th>BHB-S</th>
<th>Ac-Ac-U</th>
<th>pH-U</th>
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<td>BHB-S</td>
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<td>Ac-Ac-U</td>
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<td>pH-U</td>
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3.5 Correlation between Urinary pH and Concentrations of Beta-HydroxyButurate (BHB) in Blood, and Aceto-Acetate (Ac-Ac) in Urine

The correlation coefficients between urine pH and the concentration of BHB in blood and Ac-Ac in urine were -0.95 and -0.87 respectively. These correlation coefficients were strongly negative, showing a strong relationship between urine pH and ketone body concentrations (Table 2).

3.6 Prevalence and Incidence of Subclinical and Clinical Ketosis

In total, of the 42 cows, the blood ketone body assay showed a prevalence of subclinical ketosis of 45% (19/42) and clinical ketosis of 5% (2/42) in the cows monitored.

The incidence of subclinical ketosis (CSb) was 21% between the 2nd and 4th week of lactation. This incidence increased from the 4th to the 6th week of lactation (32%), then decreased from the 6th to the 8th week postpartum, with a rate of 26% (Fig. 6).

The incidence of Clinical Ketosis (CK) was 4% from the 2nd to 4th week of lactation, then gradually decreased from the 4th to the 8th week postpartum (from 3% to 0%).

The proportion of cows in Normal Physiological State (NPS) was 75% between the 2nd and 4th week of lactation, then decreased to 65% from the 4th to the 6th week of lactation. This proportion increased from the 6th to the 8th week postpartum, with a rate of 71%.

4. DISCUSSION

4.1 Blood (BHB) and Urine (Ac-Ac) Ketone Concentrations in Dairy Cows

The distribution of the cows followed in three groups: Cows in Normal Physiological State (NPS), in Subclinical Ketosis (SC), and in Clinical Ketosis (CK) takes into account the classification made by some authors according to blood BHB concentrations. Indeed, according to Enjalbert et al [14], cows are in subclinical ketosis when their blood BHB concentration is between 1.2 and 2.6 mmol/l and in clinical ketosis when this concentration is higher than 2.6 mmol/l. Similarly,
according to Oetzel [15], cows with a blood BHB concentration between 1.4 and 3 mmol/l are considered to be in subclinical ketosis, and in clinical ketosis when the concentration is higher than 3 mmol/l. Therefore, we chose 1.2 mmol/l as the lower threshold for subclinical ketosis, because in our study we found that most cows with a blood BHB concentration greater than or equal to 1.2 mmol/l had hypoglycemia. The lower threshold value for clinical ketosis was 2.6 mmol/l.

Thus, the analyses carried out showed high average blood BHB concentrations at the 4th week of lactation of 1.9 ± 0.31 mmol/l and 4.2 ± 0.85 mmol/l respectively for cows in subclinical ketosis and those in clinical ketosis, followed by a decrease during the following weeks.

At the beginning of lactation, cows mobilize their own fat reserves to compensate for the energy deficit. This mobilization of fat reserves is at the origin of the production of ketone bodies (BHB, Ac-Ac, Acetone) in the blood. We also observed during the whole follow-up period that the blood concentration of BHB was higher in cows in clinical ketosis than in cows in subclinical ketosis, this means that cows in clinical ketosis mobilize more fat reserves than cows in subclinical ketosis. Indeed, lipomobilization leads to a release of non-esterified fatty acids (NEFA) into the bloodstream, which are then taken up by the hepatocytes and undergo incomplete oxidation in the mitochondria leading to the synthesis of ketone bodies [10].

Urinary Ac-Ac concentration was very high in subclinical and clinical ketosis cows, with higher values in clinical ketosis cows. A threshold value of 4.0 mmol/l is set by CARRIER et al, (2004) to distinguish cows in ketosis from normal cows. Thus, in our study, a minimum average concentration of Ac-Ac of 4.6 ± 1.1 mmol/l and a maximum of 8.8 ± 2.3 mmol/l were noted in cows in subclinical ketosis while in cows in clinical ketosis, minimum average concentrations of Ac-Ac of 5 ± 1.6 mmol/l and maximum of 15 ± 3.8 mmol/l were obtained. We considered a cow to be in subclinical ketosis when her urine Ac-Ac concentration was between 4 and 10 mmol/l, and in clinical ketosis when this concentration was higher than 10 mmol/l.

It is recommended to use urine tests for individual diagnosis and not for herd diagnosis of ketosis, because urine tests have a low specificity and therefore a high risk of false negatives [16].

4.2 Blood and Urine Glucose Concentration of Dairy Cows

Our results showed a decrease in blood glucose concentration in cows in subclinical and clinical ketosis, with a more marked decrease in cows in clinical ketosis. Hypoglycemia is evident in early lactation dairy cows, as during this period there is a rapid increase in milk production and a low intake capacity. Most of the glucose precursors are of dietary origin (propionate/C3, amino acids, lactate, glycerol). It is therefore not surprising that in conditions of under-nutrition, or during periods when glucose requirements are high (lactation) the dairy cow can become hypoglycemic. The mean blood glucose concentrations obtained in subclinical (0.37 ± 0.03 g/l) and clinical (0.2 ± 0.03 g/l) cows are below the physiological concentration (0.45 to 0.7 g/l). These results support those of Tehrani-Sharif et al; Sakha et al who stated that blood glucose levels are on average lower in ketotic cows [17,18].

Blood glucose alone is not sufficient to diagnosticate ketosis. Indeed, a glucose level below 2.2 mmol/L only predicts ketosis (BHB>1.2 mmol/L) with 44% sensitivity and 78% specificity [19]. Thus, blood glucose is not usable as a ketosis marker. Moreover, it is subject to strong daily variations because it is homeostatically regulated unlike NEFAs.

It is nevertheless important to specify that the determination of blood glucose remains of prognostic interest because a low blood glucose level associated with high BHB indicates a more severe ketosis [20]. On the contrary, hyperglycemia points to type 2 ketosis (MAIR et al., 2016) which allows for the best adjustment of treatment.

4.3 Urine pH in Dairy Cows

Urine pH measurements performed showed values below 7.8; whether in cows in normal physiological condition, subclinical ketosis, or clinical ketosis during the first six weeks of lactation, thus an acidic urine pH. The latter could be due to metabolic acidosis, because according to Peyraud et al [21], there is a linear correlation between urine pH and
blood pH. This metabolic acidosis can be of food origin (ration rich in concentrate) or due to respiratory disorders leading to hypovo2entilation. In our study, all cows had a more or less normal respiration, we did not observe any respiratory disorder that could lead to hypovo2entilation, so the metabolic acidosis observed would be related to the diet. This conclusion could be true because on the farm each dairy cow receives 8 kg of dairy cow feed and 2 kg of ground corn, which makes a total of 10 kg of concentrate per day, which is too high for a cow in early lactation. Thus, this concentrate-rich ration leads to ruminal acidosis leading to metabolic acidosis as a result of increased elimination of acid ions in the urine. Also, we noticed during our study that cows in clinical and subclinical ketosis have a very high concentration of ketone bodies (Ac-Ac) in the urine. These ketone bodies are strong organic ions and will contribute to the acidification of the urine. This makes it difficult, in the case of our study, to evaluate ruminal acidosis by measuring the urine pH, because we do not know concretely, if the acidic urine pH observed is due to the effect of ketone bodies (Ac-Ac) and or to the feed.

4.4 Prevalence and Incidence of Subclinical and Clinical Ketosis

The prevalence of subclinical ketosis was 45%. This prevalence in the cows followed is similar to the 44% prevalence obtained in France by MICHAUX, in 2008 on Holsteins cows. It is, on the other hand, higher than the 19.6% obtained also in France by ENJALBERT et al. in 2001 on Holsteins cows. This difference would be related to the many risk factors, namely the breeding conditions, the level of milk production and the number of cows followed.

The 5% prevalence of clinical ketosis is within the range of 2 to 15% predicted by Duffield and Herdt [10]. In general, the average inter-herd prevalence of clinical ketosis is often low as noted by Vanholder et al(11.6%), Itle et al (8.1%), Saillard and Quemere (5%); Berge and vertenten (1.5%)); Suthar et al (3.7%) and Seifi et al (3.6%) [22,23,24,25,26,27]. Indeed, the low prevalence of clinical ketosis obtained during our survey would be related to the good management of drying off. Indeed, a good feeding management at the time of drying off allows to obtain low fat cows at the beginning of lactation, which reduces the high risk of clinical ketosis (fat cow syndrome) during the first week of lactation (Bobe et al).

The very high incidence of subclinical ketosis (32%) obtained between the 4th and 6th week of lactation in the monitored dairy cows, shows that most of the monitored dairy cows reach their peak lactation in this period. This high incidence was also found by Alves De Oliveira et al., Saillard Et Quemere, Philippe Et RABOISSON, Berge Et Vertenten, Commun et al., Enjalbert et al., Grelet et al., 27.6% in France, by Vanholder et al. Iwersen et al., Van Der Drift et al., Van KNESGEL et al., Iwersen et al., 12% in the Netherlands and Germany, Berge and Vertenten, Suthar et al., Gantner et al., Rutherford et al.,Süss et al, 21.8% in the rest of Europe, Rathburn et al., Mcart et al., Mcart et al., Chapinal et al., Seifi et al., Ospina et al., Duffield et al., Leblanc et al, Macmillan et al, Denis-Robichaud et al, Wilson and Goodell, Denis-Robichaud et al, Carrier et al, OETZEL, Mccart et al,Rollin et al, 19.7% in USA and CANADA, Tehrani-Sharif et al, ASL et al, Voyvoda and Erdogan, Sakha et al, 25.8% in Turkey and Iran [7, 8, 14, 17, 19, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51] .

Indeed, at the peak of lactation, when the milk production is maximum, the ingestion capacity of dairy cows is low, and they are therefore unable to meet their production needs. According to LE BARS, (1991)[46], to produce one liter of milk it takes about 60g of glucose, so the udder takes about 60 to 85% of available glucose. This very high production need at the peak of lactation will accentuate the energy deficit and consequently increase the risk of subclinical ketosis. The incidence of clinical ketosis is low and decreases from the 2nd to the 8th week of lactation, because if the drying off is well done, the risk of ketosis is lower and moreover the appetite increases gradually.

4. CONCLUSION

The peripartum is a key period in the life of a dairy cow. It starts at dry-off and ends at confirmed pregnancy. It is characterized by unavoidable metabolic changes, and the challenge is to manage it well. If not, it can lead to numerous metabolic diseases such as ketosis, ruminal acidosis, hypocalcemia, herbage tetany, etc. These metabolic diseases have enormous economic consequences in dairy farms and often go unnoticed in subclinical form. Ketosis and ruminal acidosis are a real threat in dairy farms.
Their detection remains a concern for dairy farmers. Thus, knowledge of the risk factors, their occurrence, methods of early diagnosis and control measures is essential for a veterinarian in charge of cattle breeding.

In sum, this study shows that the determination of BHB and glucose in blood using the portable reader "FREESTYLE OPTIUM", and the determination of Ac-Ac and glucose in urine using COMBUR Test strips, allowed us to quickly evaluate the risk of ketosis in dairy cows on the farm during the 8 weeks postpartum. We would like to see the dissemination of these screening protocols in order to increase the awareness of the veterinary world and farmers on the usefulness of early diagnosis of metabolic diseases on the farm, for a better and faster management.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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