LUMEN ET VIRTUS
ISSN: 2177-2789

THE CYTOKINES IL-1B AND TNF-A AND THE METABOLIC PROFILE IN COWS WITH HYPERKETONEMIA: A LITERATURE REVIEW

https://doi.org/10.56238/levv15n43-113

Submitted on: 24/11/2024 **Publication date:** 24/12/2024

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ABSTRACT

Hyperketonemia is the most prevalent metabolic disease in the transition period of productive cows. It is characterized by the increase of ketone bodies in body tissues and fluids, in addition to changes in metabolic profiles and cytokine expression, which can result in significant economic losses and damage to animal health. Objective: To conduct a literature review addressing the main aspects related to hyperketonemia, with emphasis on changes in the metabolic profile and expression of the cytokines IL-1β and TNF-α during the transition period. Methodology: The review was based on articles found in the databases, published between 1972 and 2024, using specific terms such as "hyperketonemia", "transition period" and "metabolic profile". Literature review: Hyperketonemia develops in response to negative energy balance, when energy demand exceeds dietary intake, leading to mobilization of body reserves and increased non-esterified fatty acids (NEFAs) in the blood. These fatty acids are metabolized in the liver, favoring ketogenesis and resulting in metabolic disorders. The metabolic profile is an essential tool to assess the metabolic health of the herd, allowing the identification of changes in biochemical parameters, such as glucose, NEFAs, proteins and hormones. This monitoring reveals the relationship between metabolism and inflammatory responses, and is crucial for the early detection of disorders. The cytokines IL-1β and TNF-α, released during inflammatory processes, play an essential role in the transition period of dairy cows, contributing to the amplification of the inflammatory response associated with hyperketonemia. Conclusion: Early detection through biomarkers, such as β-hydroxybutyrate and NEFAs, is essential to prevent hyperketonemia. Inflammatory alterations, such as the elevation of IL-1β and TNF-α, reinforce the importance of integrated strategies for nutritional, genetic and immunological management, aiming to minimize damage to animal health and reduce economic losses.

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Keywords: Ketosis, Interleukins, Transition period, Negative energy balance.

INTRODUCTION

The transition period of productive cows occurs in the space between three weeks before and postpartum and is linked to the high demand for nutrients resulting from rapid fetal development and early lactation. In addition, it is marked by adaptive changes with the objective of preparing the cow for the end of gestation and the beginning of the lactation process, as well as maintaining its homeostatic state and avoiding metabolic disorders, such as hyperketonemia (Wittwer, 2000a; Torres et al., 2024).

Hyperketonemia is the most prevalent metabolic disease among dairy cows. It occurs in response to the negative energy balance (NEB) that affects cows in the transition period and is linked to significant metabolic changes in these animals. In addition, the disease can also serve as a gateway to other pathologies, decrease reproductive performance and result in loss of milk production (Soares et al., 2018; Gulinski, 2021; Bergmann et al., 2022; Monteiro, 2023).

In situations of BEN, caused by the imbalance between nutrient intake and nutrient intake, peripheral tissues develop a state of insulin resistance and glucose is directed mainly to the fetoplacental unit and mammary gland. To meet the need for energy, the body initiates beta-oxidation of fatty acids, leading to the production of acetyl-CoA, which relies on oxalacetate to enter the Krebs cycle. In the absence of this, acetyl-CoA is transformed into ketone bodies, which can accumulate and generate metabolic disorders (Wittwer, 2000a; Regnault, 2004; Thrall et al., 2012; González; Silva, 2017; Law; Simões, 2021).

For the study of metabolic diseases, since the 70's, the analysis of metabolic profiles has been used, whether of a single individual or of the entire herd. The evaluation consists of the analysis of metabolic variables by means of blood tests, and the analysis of indicators of the energy, protein, enzymatic, hormonal and mineral profile can be performed (Wittwer, 2000b; Cozzi et al., 2011; Silva; Bondan; González, 2022). In addition, the development of hyperketonemia has been linked to genetic factors such as single nucleotide polymorphisms (SNPs) and dysregulation in the gene expression of pro-inflammatory cytokines (such as TNF-a) (Zhang et al., 2016; WU et al, 2020).

The cytokines IL-1β and TNF-a play a fundamental role in cows in the transition period because they stimulate the uterine cells, expanding and inducing the calving process. In addition, they also promote collagen remodeling, contributing to the weakening of the fetal membrane and the expulsion of the fetus. Despite this, the increase in pro-inflammatory cytokine levels is associated with some common occurrences of the transition period, such as reduced food intake and clinical and subclinical mastitis (El-Deeb; El-Bahr, 2017; Tizard, 2018; Kuhla, 2020; Vitenberga-Verza, 2022).

Thus, the study aimed to review the literature addressing the main aspects related to hyperketonemia in productive cows, with emphasis on changes in the metabolic profile and expression of the cytokines IL-1β and TNF-α during the transition period of these animals.

LITERATURE REVIEW

TRANSITION PERIOD OF PRODUCTIVE COWS

The transition period is known as the most critical interval for productive cows. It is characterized by a phase of metabolic and oxidative stress that results in a pro-inflammatory and immunosuppressive state in cattle (Torres et al., 2024). Occurring in the space between the three weeks before and after calving (Alvarenga et al., 2015), the transition period is a phase marked by adaptive changes with the aim of preparing the cow for the end of gestation and the beginning of the lactation process, as well as maintaining its homeostatic state and avoiding metabolic disorders (Wittwer, 2000a).

Homeorresis, a concept used by Bauman and Currie (1980) was defined as "orchestrated or coordinated changes in the metabolism of body tissues, necessary to support a physiological state". Years later, the author applied the concept to illustrate the physiological states of pregnancy and lactation, and later of growth (Bauman, 2000). In this sense, homeorrhesia occurs when physiological adaptations happen without causing damage to the animal's metabolism. On the other hand, when the cow's body is unable to adapt to the physiological changes of the transition period, there may be the emergence of multiple metabolic diseases (Ortolani, 2009).

The transition period is linked to the high demand for nutrients resulting from rapid fetal development and early lactation. However, this period is characterized by greater occupation of the uterus in the abdominal cavity and consequent decrease in rumen volume, which results in a decrease in dry matter intake (Sargison, 2007). In the last three weeks prior to delivery, consumption is approximately 1.5% to 1.7% of body weight, but in the week of delivery it can be less than 1.5%, making this drop even more visible on the day of conception (Hayirli et al., 2003).

The decrease in nutritional intake goes against the energy demand of cows in the last days of gestation, a stage where, according to Gomes et al (2009), colostrum formation occurs, as well as the mammary gland's energy demand for glucose, amino acids, fatty acids, minerals and vitamins. In the transition phase, especially in high-producing cows, the mammary gland needs high amounts of glucose, especially in the first days after calving, for milk synthesis. A few days after delivery, the mammary gland's demands for glucose, amino acids, and fatty acids are, respectively, 2.7, 2.0, and 4.5 times greater than that of the uterus in the final stage of gestation (Bell et al, 1995; Pereira, 2018).

The body's high demand for energy and insufficient intake of nutrients to match it result in negative energy balance (NEB). In this hypoglycemic situation, the cow mobilizes energy from its energy reserves, so there is greater movement of hepatic glycogen and adipose tissue. Fatty tissue, in particular, undergoes an increase in lipolysis, releasing non-esterified fatty acids (NEFAs) into the bloodstream, where in the blood they will be converted into energy and in the liver into ketone bodies. The latter will be used mainly by glucose-dependent tissues, which in states of caloric deficit, malnutrition or metabolic diseases, need to adapt to alternative energy sources (Drackley, 1999; Gomes et al, 2009; Santos et al, 2010; González; Silva, 2017).

In this sense, it is necessary to maintain an adequate nutritional balance, especially in periods of greater requirements, since energy imbalances can cause the so-called Metabolic Diseases or Production Diseases. These are caused by the imbalance between the nutrients ingested by the animal, its metabolism and those exited through feces, urine, milk and fetus, as is the case of hyperketonemia (Wittwer, 2000a; Vallenari et al., 2024).

HIPERCETONEMIA

In order to circumvent the negative energy balance (NEB) caused by the insufficiency in gluconeogenesis, the body starts to transform triglycerides into fatty acids (FA) to generate energy. In this reaction, there is an increase in the beta-oxidation of FAs in hepatocytes and consequent formation of acetylcoenzyme A (acetyl-CoA). On the other hand, the presence of a derivative of carbohydrate fermentation, oxaloacetate, is necessary for acetyl-CoA to be oxidized and generate energy in the Krebs cycle. In situations where there is an excess of acetyl-CoA and insufficient oxaloacetate, acetyl-CoA is used in the process of ketogenesis (Thrall et al., 2012; González; Silva, 2017; Law; Simões, 2021).

Ketogenesis occurs mainly in the liver, where the ketone bodies produced will be routed to the other tissues and serve as a source of energy. The main ketone bodies are acetoacetate, acetone, and β hydroxybutyrate (BHB), in which case acetoacetate is converted into acetone and BHB through chemical reactions. These soluble compounds will be found mainly in body fluids such as blood, urine and milk, with BHB being the ketone body with the greatest stability and predominance in circulation (Duffield et al., 2009; Thrall et al., 2012; Ospina et al., 2013; González; Silva, 2017).

When the body has large concentrations of ketone bodies in the body fluids and when the production of these compounds exceeds the amount used by the tissues for energy production, hyperketonemia is established. Due to their instability, acetone and acetoacetate are not used as markers to measure the levels of ketone bodies, and BHB is the most common indication for the diagnosis of hyperketonemic condition. The detection of BHB in the blood is the most common, and it can also be traced in milk, due to its abundance and preservation in fluids (Oetzel, 2004; Duffield et al., 2009; Schein, 2012; González; Silva, 2017; Benedet et al., 2019).

The current reference values for the diagnosis of hyperketonemia in cows are ≥1.2 mmol/L. In this sense, the blood concentration of BHB ≥ 1.2 mmol/L and < 3 mmol/L was established for the diagnosis of subclinical ketosis and ≥ 3 mmol/L for the diagnosis of clinical ketosis. However, more recent studies such as the one by Vallenari et al (2024) use the reference value ≥ 1.2 mmol/L as a marker of both forms of hyperketonemia, whether clinical or subclinical. Thus, despite the subdivision, the term hyperketonemia now encompasses both presentations of the disease (McArt; Nydam; Overton, 2015; Benedet et al., 2019; Djokovic et al., 2019).

Hyperketonemia in cows can be categorized into three types, according to the etiology and duration of the disease: type I, spontaneous ketosis or underfeeding where there is low insulin concentration due to hypoglycemia; type II, occurs mainly in early lactation and is characterized by fatty liver and high food intake; the latter type is known as "butyric acid silage ketosis", it occurs when cows consume silage fermented with a ketogenic precursor, butyric acid (Oetzel, 2007; Zhang; Ametaj, 2020).

Ketosis is the most prevalent metabolic disease among high-performance dairy cows during the transition period, with the main symptom being the increase in ketone bodies in body fluids. This period is marked by increased energy demand for fetal development and milk production, along with the difficulty of meeting this need through feeding, leading to negative energy balance (NEB). When this condition intensifies, it can trigger metabolic disorders, such as reduced glycemic index and hyperketonemia (Constable et al., 2017; Gulinski, 2021).

Hyperketonemia can lead to clinical and subclinical ketosis, the signs of the first form are usually the strong smell of acetone due to the volatile content of this compound, decreased activity and appetite, weakness, apparent blindness, and marked loss of body condition. Subclinical ketosis, on the other hand, is characterized by an increase in ketone bodies in the circulation, but without the clinical symptoms of the disease. In more severe cases, some animals may manifest the nervous form of the disease, exhibiting signs such as walking in circles, pressing the head against surfaces, loss of balance, motor incoordination, hyperesthesia, aggressiveness or abnormal behavior, loss of visual acuity, muscle tremor, among others. However, the clinical signs are nonspecific and difficult to detect, making it difficult to distinguish the forms of the disease in order to make an accurate diagnosis (Duffield, 2000; Berge; Vertenten, 2014; Constable et al., 2017; Soares et al., 2019).

In this sense, Gordon, LeBlanc and Duffield (2013) report that in their experiments, when examined, animals with high levels of ketone bodies may not show clinical signs, while animals with low levels may be weakened by the disease. Therefore, the disease can be better described as hyperketonemia, instead of distinguishing between clinical and subclinical, since the severity of the clinical signs of the disease seems to depend on the adaptation of each animal to process and tolerate ketone bodies in the body. In addition, it is understood that both presentations of the disease negatively impact milk production, reproductive performance, and the health of dairy cows (Herdt, 2000; Gordon; Leblanc; Duffield, 2013; McArt et al., 2013; Raboisson et al., 2014).

Studies such as those by Santschi et al. (2016) and Weigel et al. (2017) point to the prevalence and incidence of hyperketonemia in dairy cattle of 22.9% and 24%, respectively. Confirming the significant numbers of the disease, the study by Loiklung, Sukon, and Thamrongyoswittayakul (2022) shows a global prevalence of more than 22%. The highest prevalence of the disease is detected in the first two weeks of lactation, with a significant decrease after this period, as seen in the study by Drift et al. (2012) where there was a 60% decrease in the prevalence of hyperketonemia between the first and second months of lactation.

Among the risk factors for the occurrence, a higher frequency of the disease was observed in multiparous cows than in primiparous cows, suggesting that there is a direct relationship between hyperketonemia and the increase in parity, which can be justified by the simultaneous need for pregnancy and lactation. In addition, spring has been pointed out as the season of the year with the highest frequency of the disease, even though there are no biological elucidations for this. It is therefore believed that the higher frequency of the disease in this period occurs due to the use of silage with lower quality throughout the first half of the year (Berge; Vertenten, 2014; Vanholder et al., 2015; Santschi et al., 2016; Chandler et al., 2018).

Breed and nutritional management are also pointed out, where in the first case a higher prevalence of hyperketonemia is seen in Jersey cows (19%) than in Holstein cows (14%). A negative association was also observed between large herds and the prevalence of hyperketonemia, as well as a lower notification of the disease in herds that offer forage and concentrate at different times. In addition, the decrease in the disease extends to herds that use the total mixed ration (RMR) during pasture periods (Berge and Vertenten, 2014; Santschi et al., 2016; Chandler et al., 2018; Benedet et al., 2019).

This disease is linked to several problems, such as the risk of contracting other diseases of the transition period, poor reproductive performance and economic losses in milk production that are around $289 in each case. Disease prevention is an important factor to reduce costs, losses and ensure animal welfare, and an efficient diet, body score assessment and periodic fluid screening for the detection of ketone bodies can be applied in dairy cattle farms (Raboisson et al., 2014; McArt et al., 2015; Madreseh-Ghahfarokhi; Dehghani-Samani; Dehghani-Samani, 2018).

There is a widespread consensus on the use of quantification of β-hydroxybutyrate and non-esterified fatty acids (NEFAs) for the diagnosis of the disease. In addition to energy indicators, other biomarkers of metabolism have been used to monitor the disease, such as the concentration of proteins in the body and milk, changes in the hormonal picture, changes in the mineral profile, as well as in cardiac biomarkers. In addition, the nutritional status, milk production and health condition of cows in the transition period are also analyzed (Antunovic et al., 2011; McArt et al., 2013; Soares et al., 2018, 2019; Benedet et al., 2019).

In addition to the metabolic profile, the development of hyperketonemia in the transition period has been inherently associated with genetic factors (WU et al, 2020). In the study by Nayeri et al. (2019), the presence of several single nucleotide polymorphisms (SNPs) and hyperketonemia susceptibility genes was observed. Other studies reported that pre- and hyperketonemic cows had a higher amount of circulating pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor (TNF-a), compared to healthy cows. Thus showing that cows go through a process of chronic inflammation even before the establishment of the disease (Zhang et al., 2016). Therefore, along with the metabolic profile, genetic analysis is an important parameter for optimizing the diagnosis and prevention of the most severe cases of the disease.

METABOLIC PROFILE

In order to carry out the study of metabolic and nutritional diseases, the metabolic profile test, developed by Payne (1972), in Compton, England, has been used since the 70's. This evaluation consists of a combination of complementary tests for the investigation and diagnosis of metabolic and nutritional imbalances, and an unlimited number of variables can be analyzed, as long as there is prior and adequate knowledge of the biochemical and physiological specificities (González, 2000; Wittwer, 2000b).

From the analysis of the metabolic profile (PM) it is possible to identify the blood concentration of the indicators of the energy, protein, enzymatic, hormonal and mineral profile of a single specimen or an entire herd. The test can be used in conjunction with traditional assessment methods, such as body condition score, dietary assessment, productivity, milk composition, and others. However, it takes weeks or even months for these symptoms to be observed, thus making their application more useful in retrospective analyses, reserving the priority of the PM for obtaining more previous results (Wittwer, 2000b; Cozzi et al., 2011; Silva; Bondan; González, 2022).

In order to reach a result, the values of the biomarkers collected in the blood sample must be compared with the reference values established for that group. However, the interpretation of these data is more complex than it seems because it must take into account the aspects of each specimen such as age, breed, sex, physiological state, as well as environmental and management aspects. In the absence of any of these factors, there may be an inaccurate diagnosis, and the authors recommend the use of reference values of individuals similar to those analyzed and that these preferably come from similar management systems or climatic zones (Kida, 2002; Quiroz-Rocha, 2009; Cozzi et al., 2011; González; Silva, 2017; Silva; Bondan; González, 2022).

The application of PM has been widespread in the diagnosis, prognosis, and monitoring of metabolic and nutritional imbalances that affect ruminants in a transition period, it is possible to observe the application of the technique in sheep (Borowsky, 2021), goats (Soares et al., 2018), and cows (Alvarenga et al., 2015). Since metabolic or production diseases occur mainly in the transition period (Wittwer, 2000b) and hyperketonemia is the main disease at this time, PM analysis becomes an essential factor for adequate screening and monitoring of the disease.

Energy Profile

β-hidroxibutirato (BHB)

β-hydroxybutyrate (BHB) is the main, most stable and abundant ketone body present in the ruminant circulation. It is a product of lipid metabolism, with its production occurring mainly in the mitochondria of hepatocytes, from the metabolization of NEFAs, and can also be synthesized by butyric acid present in the rumen mucosa. In the tissue that is demanding energy, BHB can be used as an energy source, if oxalacetate is available for its oxidation, otherwise, this compound will accumulate in the blood causing acetonemia or metabolic ketoacidosis (Wittwer, 2000a; González; Corrêa; Silva, 2017)., Therefore, the increase of BHB in the bloodstream reveals a condition of negative energy balance (NEB) in the animal, since, in situations where the demand for glucose corresponds to its availability, the ketone bodies produced in the liver through oxidation will go to the tissues as an energy source, being mobilized by oxaloacetate. However, if glucose availability is low, oxaloacetate will be used to produce it. As a consequence, other energy production pathways will be triggered, and there will be a drop in the concentration of circulating oxaloacetate (Souto et al., 2013; Silva Filho, 2016).

Unlike other metabolites, whose concentration varies in response to feeding, BHB remains stable throughout the day, and is therefore the main biomarker for the diagnosis of hyperketonemia in ruminants. To diagnose hyperketonemic conditions in cows, the reference value ≥ 1.2 mmol/L is currently used (Leblanc, 2015; Benedet et al., 2019; Vallenari et al., 2024). BHB concentration can be determined in the bloodstream through commercial kits, despite the high cost, or through mobile electronic equipment (Panousis et al., 2012; Pichler et al., 2014).

Non-Esterified Fatty Acids (NEFA)

Although its levels vary throughout the day, together with BHB, NEFA is the most appropriate metabolite to indicate imbalances in the energy metabolism of cows. Higher levels of this metabolite are linked to lipomobilization of body reserves, and it is therefore used to indicate insufficient energy intake by these animals. This is the case of cows in the transition period, where a higher concentration of circulating NEFAs is observed a few days before calving, to meet the body's energy demands at that stage (Alvarenga et al., 2015; Leblanc, 2015; Benedet et al., 2019).

In dairy cows, NEFA concentrations are higher postpartum than prepartum, being approximately 0.2 mmol/L prepartum and 0.4 mmol/L on the day of conception, with the values extending up to 3 weeks postpartum. In the course of gestation, NEFAs, which are now at higher levels, will serve as a source of energy and develop a state of insulin resistance in cows by inhibiting the release of this hormone by pancreatic beta cells. This hinders the uptake of glucose by the peripheral tissues, directing it mainly to the feto-placenta unit (Regnault, 2004; Alvarenga et al., 2015; González; Corrêa; Silva, 2017).

Some studies, such as the one by Santos et al. (2012), reveal that nutritional management during the transition period is linked to serum concentrations of NEFAs. In this sense, the study observed that sheep that received propylene glycol (glucose precursor) supplementation in their diet during peripartum had lower mean NEFA values, while the control group had higher mean values.

Glucose

Glucose is an important variable in the metabolism of ruminants, on the other hand, these animals have homeostatic mechanisms that regulate blood glucose, which makes this carbohydrate a less appropriate marker to assess energy balance. Measuring this metabolite only once does not reveal the complete glycemic picture of the individual, as its concentrations undergo rapid and frequent changes throughout the day, due to diet and individual factors (Leblanc, 2010; Souto et al., 2013).

Despite this, in ruminants, the diet has little effect on blood glucose, because in addition to practically no glucose from the digestive tract entering the bloodstream, there is endocrine control of insulin and glucagon over glycogen, as well as glucocorticoids over gluconeogenesis. Thus, if energy intake is inadequate, these hormones stimulate the degradation of liver glycogen and the synthesis of new glucose in the liver. And in the cases of BEN, they stimulate the mobilization of triglycerides to provide fatty acids as an energy source, in addition to glycerol as a precursor of hepatic glucose (González; Silva, 2017).

In the final third of pregnancy, it is possible to observe a hypoglycemic condition, it is believed that due to the high demand of the fetus, with attenuation of this glycemic deficit in cases of twin pregnancy. However, in the peripartum period, it is possible to observe a condition of hyperglycemia, probably due to stress due to the increase in cortisol, as reported by Soares et al. (2018), in addition to insulin resistance in peripheral tissues. In the first weeks postpartum, the return of hypoglycemia is noted, especially in the first week and in cows with high milk production, as well as a greater presence of BHB during this period, which indicates the use of glucose mainly for lactation (Regnault, 2004; Raoofi et al., 2013; González; Silva, 2017).

Protein Profile

Proteína Total (PT)

Blood proteins are produced mainly by the liver, and the rate of production is intrinsically related to the nutritional status of the animal and liver functionality. The increase in total protein (TP) can occur in cases of dehydration due to hemoconcentration, as well as the decrease occurs when liver failure, intestinal and renal disorders, hemorrhage or caloric deficit occur (González; Silva, 2017; Kirovski; Sladojevic, 2017).

The concentration of total proteins tends to decrease in the days before calving and, after it, it is restored. This decrease is linked to the use of amino acids for the synthesis of fetal proteins, since, in the last weeks before delivery, the growth of the fetus is exponential. Another factor responsible for the reduction in total proteins is the mobilization of immunoglobulins for colostrum synthesis. Thus, dry cows may have higher levels of TP than cows in the transition period, and this drop in serum protein concentration also contributes to the reduction of milk production (Balikci; Yildiz; Gurdogan, 2007; Sadjadian et al., 2013; González; Silva, 2017).

In metabolic diseases such as hyperketonemia/toxemia of pregnancy, characterized by energy deficit, PT levels are usually lower. But, in addition to energy imbalance, PT levels are also influenced by inflammatory and infectious diseases, a period when it usually decreases. It is also possible to observe that cows with lower protein levels in the pre-calving period have a higher risk of presenting other conditions such as mastitis and placental retention at the beginning of lactation (Puppel; Kuczynska, 2016; Ruprechter, 2018; Soares et al., 2018).

Albumina

Accounting for about 50% of all plasma proteins, albumin is the most abundant protein in the bloodstream. Synthesized in the cytoplasm of liver cells, it is considered an acute negative phase protein, since its concentration tends to decrease in inflammatory and infectious diseases, in addition to having its synthesis reduced in cows with energy imbalances (Krause et al., 2014; Puppel; Kuczynska, 2016; González; Silva, 2017).

Its plasma concentration is affected by availability in the diet, protein loss in some diseases and especially by the functioning of the liver. Liver functionality can decrease with the fatty infiltration resulting from lipomobilization that occurs assiduously at the beginning of lactation, thus generating hypoalbuminemia in dairy cows. The decrease in albumin is caused by protein deficiency when, along with it, there is a reduction in urea levels. In cases of liver injury or failure, albumin levels are reduced, while urea levels remain normal or elevated and are accompanied by high levels of enzymes (González; Silva, 2017; Kirovski; Sladojevic, 2017).

It is possible to observe a decrease in albumin and total protein concentrations in the prepartum period of healthy ruminants, but this is due to the exponential growth of the fetus in the final third of gestation and the great mobilization of amino acids for this. However, animals with toxemia during pregnancy may also have albumin values within the normal range, thus indicating that there was no change in liver functioning (Sadjadian et al., 2013; Souto et al., 2019).

Globulina

The concentration of these proteins is obtained by the difference in concentration between the total proteins and albumin. Globulins can be divided into three types, α, β, and γ, identified from electrophoresis, and despite this, they are not considered good indicators of protein metabolism. Its importance is mainly focused on indicators of inflammatory processes. Thus, high levels of globulins are linked to infectious diseases or recent vaccinations (González; Silva, 2017).

Decreased globulin levels at the end of gestation and day of calving, as reported in Soares et al. (2018) are associated with the migration of gamma globulins for colostrum synthesis (Anwar; Ramadan; Taha, 2012). On the other hand, it is possible to observe that in animals affected by pregnancy toxemia/hyperketonemia, globulin levels can remain within normal limits (Souto et al., 2013). González and Silva (2017) explain that these variations are due to adaptation to stress. Thus, changes in globulin levels can be used to assess states of adaptation to stress and to identify adapted and non-adapted animals.

Urea

Urea is synthesized in the liver from ammonia, whether recycled from the rumen or from the catabolism of amino acids. Its levels are analyzed in relation to the level of protein ingested and kidney function, which makes it a sensitive and immediate indicator of protein intake. However, for kidney function, it is not the most appropriate indicator, since the degree of recycling of this metabolite between the blood and the rumen is very high, with creatinine being the most suitable metabolite for this (Souto et al., 2013; González; Silva 2017).

Blood urea values fall moderately before and after calving in healthy cows. This decrease in urea concentration is attributed to the reduction in food intake caused by stress during peripartum. In animals with toxemia during pregnancy, high urea values are observed, probably due to renal failures, such as reduced glomerular filtration and increased protein catabolism for milk synthesis (Santos et al., 2011; Sadjadian et al., 2013; Souto et al., 2019).

Creatinina

Creatinine is a compound that results from the metabolism of creatine and phosphocreatine, which are primarily stored in skeletal muscles. Unlike urea, blood creatinine levels are not significantly influenced by diet, but they can increase sporadically in situations of intense muscle metabolism, leading to higher blood concentrations. The blood elevation of this compound, as well as urea, reveals a picture of azotemia, which may be related to the decrease in the glomerular filtration rate and reduction in the urinary excretion of these compounds. In addition, its urinary excretion does not seem to vary according to the protein content in the body, as it does for urea (Souto et al., 2013; Thrall, 2015; Fischer et al., 2016).

The presence of this metabolite in blood is a highly sensitive marker to metabolic changes that occur during the reproductive cycle of ruminants, since studies show higher serum creatinine concentrations at the end of gestation and in the peripartum compared to the lactation period. This issue is attributed to the high mobilization of muscle protein to meet the energy demand generated by the accelerated development of the fetus. (Soares et al., 2014; Elzein; Osman; Omer, 2016; Soares et al., 2018).

For hyperketonemia, research suggests that kidney function can affect small ruminants, particularly in the later stages of the disease. This condition is usually associated with a specific prognosis for these animals, underlining the relevance of monitoring creatinine levels as an essential indicator of kidney health in these cases (Santos et al., 2011; Souto et al., 2013).

Hormonal Profile

Insulina

It is a hormone related to energy metabolism because it acts by increasing the uptake of glucose by cells, stimulates its storage in the form of glycogen and stimulates lipogenesis in the liver and adipocytes. Insulin sensitivity and response will depend on body tissue and the physiological stage in which the animal is. In this sense, the liver responds to insulin by decreasing gluconeogenesis, adipose tissue with fat synthesis, and skeletal muscles with glucose absorption (Bell, 1995; Araújo et al., 2014).

In cows in the transition period, a lower aptitude for the use of glucose in the peripheral tissues is noted, which is available to the tissues with greater demand at that time, as is the case of the feto-placental unit, as well as the mammary gland. In addition, it is also possible to detect the effects of insulin decrease through the high mobilization of NEFAs to meet the high demand for energy, a fact that ends up attenuating insulin resistance (Regnault, 2004; Raoofi et al., 2013; González; Corrêa; Silva, 2017; González; Silva, 2017).

In general, in ruminants affected by hyperketonemia, it is common to observe reduced insulin values. However, some ruminants, such as primiparous goats, do not show this reduction in insulin levels at the beginning of lactation, even in the face of the great demands of the mammary gland. It is believed that this limitation in the use of body reserves is a strategy to favor their own growth, considering that these animals are still in the maturation phase. (Souto et al., 2013; Araújo et al., 2014; Magistrelli; Rosi, 2014; Soares, 2017).

Cortisol

This hormone is used as an indicator of stress, although this position is questionable, as this glucocorticoid is influenced by several circumstances, which makes it difficult to interpret its results. However, its role in peripartum is widely recognized as a stimulator of gluconeogenesis to maintain blood glucose, reducing the ability of tissues to use glucose and acting in the opposite way to insulin. In addition, it works as a signal for the moment of calving because it is common to find levels above the reference values in females at the end of gestation (Beerda et al., 2004; Magistrelli; Rosi, 2014; González; Silva, 2017).

In several studies on hyperketonemia, there have been reports of elevated cortisol values in ruminants. According to the authors, this increase in the production of the hormone occurs, in fact, in response to the severe stress triggered by the pathological process and/or by the impaired ability of the fatty liver to metabolize and excrete it (Santos et al., 2011; Souto et al., 2013; Soares et al., 2018).

CYTOKINES

Cytokines are polypeptides secreted by a wide range of cells such as lymphocytes, macrophages, natural killer (NK) cells, mast cells, and others involved in pathological processes. These proteins play an essential role in the immune response, acting mainly in communication within this protection system. They also impact the activity, differentiation, and development of various immune cells, as they act as mediators of autoimmune and inflammatory diseases (Tizard, 2018; Shea; Gadina; Siegel, 2019).

Cytokines can be grouped into different categories, such as tumor necrosis factors (TNFs), interleukins (ILs), lymphokines, monokines, and interferons (IFNs). According to their function, cytokines are also classified as pro-inflammatory (such as L-1β, IL-6, and TNF-α) or anti-inflammatory (such as IL-10, IL-11, and IL-13). In addition, a single cytokine can be secreted by different cells and have both pro-inflammatory and anti-inflammatory activities (such as IL-6), which can generate multiple responses in an inflammatory condition (Boshtam et al., 2017; Monastero; Pentyala, 2017; Liu, Chao et al., 2021).

Variations in cytokine levels in biological fluids provide important information about the diagnosis, stage, and prognosis of various diseases. Abnormal or excessive cytokine production, also known as a cytokine storm, can result in organ failure and even lead to death. Thus, cytokine levels are considered an essential indicator for the evaluation of clinical disorders of cows in the transition period (Mehta et al., 2020; Liu, Chao et al., 2021; Poole et al., 2021).

Studies in the literature show that increased levels of pro-inflammatory cytokines in cows is associated with decreased fertility (Samir et al., 2017). In addition, in cows in the transition period, higher concentrations of pro-inflammatory cytokines are related to reduced feed intake, which results in a negative energy balance (Kuhla, 2020). High levels of these proteins are also observed in cases of clinical and subclinical mastitis (Vitenberga-Verza, 2022) and in situations of hyperketonemia (El-Deeb; El-Bahr, 2017).

Interleukine 1-beta (IL-1β)

When sentinel cells (such as macrophages) are exposed to infectious agents or their pathogen-associated molecular patterns (PAMPs), their signaling pathways activate genes that produce and secrete three major cytokines, including IL-1β. The synthesis of IL-1β and IL-1α occurs when sentinel cells are activated by CD14 and TLR4 proteins, and IL-1β production is 10 to 50 times higher than IL-1α; in addition, IL-1α remains associated with the macrophage, while IL-1β is secreted (Tizard, 2014; 2018).

IL-1β is initially synthesized as an inactive 31 kDa precursor, pro-IL-1β. The maturation and release of IL-1β are tightly controlled processes, which depend on the cleavage of pro-IL-1β to the active form of the cytokine through the action of caspase 1. In its active form, IL-1β acts as a small pro-inflammatory cytokine agonist (17.5 kDa), being produced mainly by CD4+ helper T lymphocytes, monocytes and macrophages. In addition, it is also synthesized by non-immune cells, such as fibroblasts, endothelial cells, microglia, and astrocytes in the central nervous system, in response to cell lesions, infections, and inflammatory processes (Lopez-Castejon; Brough, 2011; Tizard, 2018).

This cytokine is a very potent pro-inflammatory mediator that has its effects on several cell types, acting on several inflammatory diseases, such as stroke, diabetes, and genetic autoinflammatory disorder. In the immune system, it induces Th17 cells to produce other cytokines, making the body susceptible to the development of chronic inflammatory diseases. In its pro-inflammatory action, IL-1β promotes the attraction of granulocytes to the inflamed tissue. As well as, from the hypothalamus, it induces the expression of prostaglandins during the acute inflammatory response, which, at a systemic level, triggers fever (Sutton et al., 2009; Hong et al., 2019; Galozzi et al., 2021).

In humans, IL-1β is a fundamental cytokine for labor (PT), being released by leukocytes infiltrated in the uterus and myometrium, where it enables the metabolism of progesterone and induces cervical maturation. It is also believed that IL-1β acts at the beginning of labor by inducing the entry of calcium into the smooth muscle cells of the myometrium, stimulating uterine contractions. As in the human species, cows also have high concentrations of pro-inflammatory cytokines (such as IL-1β) in the placenta, amniotic membranes, and other tissues during gestation. During calving in cows, IL-1β, along with other cytokines, stimulates uterine stromal cells, amplifying the calving process. In addition, it acts on collagenase to remodel collagen, which weakens the fetal membrane, relaxes the cervix, and increases myometrial contractility, resulting in the expulsion of the fetus. In the postpartum period, the uterus involutes, since most of the cells that stimulate cytokines disperse or are destroyed (Tizard, 2018; Patel et al., 2018; Pierce et al., 2018; Menon; Richardson, Lappas, 2019).

Elevation in serum IL-1β levels has been reported in the stages involving the transition period of dairy cows. In this sense, most studies suggest that high concentrations of this cytokine are associated with behaviors and clinical conditions characteristic of the transition period, such as a higher number of mastitis cases, lower feed intake, and reduced milk production (Trevisi, 2015; Kuhla, 2020). In addition, Tizard (2018) reports that during severe infections, IL-1β acts in conjunction with tumor necrosis factor alpha (TNF-α), and the synergistic effect of this interaction induces unhealthy behavior in cows by acting on the brain, causing fever, lethargy, malaise, and loss of appetite.

Fator de necrose tumoral alfa (TNF-α)

As with IL-1β, TNF-α production results from sentinel cell signaling in response to infectious agents and their PAMPs. TNF-α is the first cytokine to be produced by activated sentinels, with its synthesis occurring early in inflammation, followed by a heavy flow of IL-1 and later IL-6. TNF-α can also be produced by endothelial cells, stimulated fibroblasts, T lymphocytes, and B lymphocytes. It can also be generated both in soluble form and bound to the membrane, the latter of which is cleaved from the cell surface by a protease called TNF-α convertase (Tizard, 2018; Holbrook et al., 2019).

TNF-α is synthesized as a 26 kDa type II transmembrane protein, forming a stable and active homotrimeric molecule, mTNF-α. This molecule can be processed by proteolytic cleavage by the TNF-α converting enzyme, resulting in a 17 kDa monomeric protein, also biologically active, called sTNF-α. TNF-α triggers multiple cellular responses through its interaction with two transmembrane receptors: the type I receptor (TNFR1) and the type II receptor (TNFR2). In physiological terms, TNFR1 is expressed constitutively in various cell and tissue types, while TNFR2 is predominantly expressed at low levels in immune and endothelial cells (Leung; Cahill, 2010; Urban; Socool; Azevedo, 2014).

Therefore, TNF-α is a primary mediator of inflammation because, together with IL-1, it triggers changes in the endothelial cells of small-caliber blood vessels. Thus, a local increase in TNF-α concentration generates the classic signs of inflammation, such as heat, swelling (tumor), redness, and pain. TNF-α amplifies and prolongs inflammation by stimulating macrophages to synthesize other inflammatory mediators such as NOX2 and COX-2. This cytokine also induces macrophages to increase their own production, along with that of IL-1. As its name suggests, TNF-α has the ability to destroy some tumor cells and virus-infected cells (Tizard, 2014; 2018; Holbrook et al., 2019).

TNF-α is among the most important cytokines in labor induction in human females. It can be found in large quantities in the placenta and embryonic appendages, being produced mainly by the membrane that protects the embryo in placental mammals, the chorionic decidua. TNF-α can intensify or trigger the labor process by inducing the expression and secretion of proinflammatory cytokines and chemokines, in addition to increasing the expression of the prostaglandin synthase 2 enzyme, thus promoting the production of COX-2 and PGE2, fundamental regulators of myometrial contraction (Lappas, 2016; Edey et al., 2018; Patel et al., 2018; Menon; Richardson, Lappas, 2019). According to Tizard (2018), for calving cows, TNF-α will act in a similar way to IL-1β, since, together with other cytokines, it stimulates uterine cells, expanding the calving process. In addition, it promotes collagen remodeling, contributing to the weakening of the fetal membrane and the expulsion of the fetus.

CONCLUSION

Hyperketonemia, prevalent in dairy cows in the transition period, is associated with negative energy balance, influenced by metabolic, nutritional and genetic factors, and negatively impacts the health, productivity and reproduction of the animals. Early detection through metabolic monitoring, especially of biomarkers such as β-hydroxybutyrate and non-esterified fatty acids, is essential for diagnosis and prevention. Inflammatory changes, including elevation of cytokines such as IL-1β and TNF-α, reinforce the need for strategies that integrate nutritional, genetic, and immunological management. In addition, practices such as balanced diets and the use of quality silage are essential to reduce the risks of the disease and promote the sustainability and profitability of cattle production.

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