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Biochemical Insights into Bovine Ketosis and its Mechanisms

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Abstract:

Dairy cows' early postpartum metabolic alterations can, in fact, cause an imbalance in energy use, which in turn causes an excess of ketone bodies to be produced. The health of the cow and the amount of milk it produces may suffer as a result, costing dairy farmers money. A major contributing element to the development of ketosis is the increased lipolysis that releases non-esterified fatty acids into the blood. A major contributing element to these results in modern dairy cows is abdominal obesity. Acetyl-CoA is converted into ketone bodies rather than entering the Krebs cycle as a result of energy and glucose being diverted towards lactose synthesis and milk production, which leaves a shortage of gluconeogenic precursors. Acetone, acetoacetate, and β-hydroxybutyrate are among the ketone molecules that build up in the blood and can be found in a variety of body fluids, including milk, urine, and blood, enabling diagnostic testing. In fact, the key to controlling ketosis in dairy cattle is prevention. Ketosis can be avoided by using monensin, either as a slow-release bolus or as a dietary supplement, or by include propylene glycol in the diet. To prevent ketosis and associated conditions, it is essential to prevent high body condition (subcutaneous fat), excessive abdominal adiposity during the dry period and parturition, and to ensure that cows are comfortable. By offering extra energy sources or improving the cow's capacity for energy efficiency, these interventions seek to lessen the need for excessive lipolysis and the formation of ketone bodies.

Introduction

A metabolic condition known as ketosis mainly affects dairy cows with high milk production. Acetone (Ac), acetoacetate (AcAc), and β-hydroxybutyrate (BHB) are among the ketone molecules that are elevated in the circulation. Both clinical and subclinical manifestations of this illness are possible. Neurological symptoms such as aberrant roaming, unstable movement, bellowing, and sporadic hostility are frequently present in the clinical presentation. Additionally, it may result in decreased milk production, decreased feed intake, and disturbances to other physiological processes (Chirivi *et al.,* 2023; Melendez, 2017).

Conversely, subclinical ketosis (SCK) happens when the blood contains high levels of ketone bodies, especially BHB. Even while the symptoms might not be as noticeable in this situation, they can nevertheless have serious consequences, including decreased fertility and heightened vulnerability to secondary illnesses such fatty liver, metritis, mastitis, and abomasal displacement (DA). It is important to note that, depending on a number of herd-related parameters, milk output may be normal, decreased, or even enhanced (Melendez, 2017; Melendez & Risco, 2021).

Furthermore, cows with SCK are more prone to disturbances in triglyceride (TG) and cholesterol metabolism. This is due to the downregulation of acetyl CoA acetyltransferase 2 in the liver, which inhibits cholesterol synthesis, promotes TG synthesis, and reduces the levels of very-low-density lipoproteins (VLDL) and low-density lipoprotein-C. Consequently, there is an increased risk of developing fatty liver (Zhou *et al.,* 2023).

Both clinical and subclinical cases of ketosis result in significant economic losses for the dairy industry, making prevention strategies crucial (Liang *et al.,* 2017).

Physiologiocal and pathophysiological aspects

Since the production of ketone bodies, or ketogenesis, is a normal physiological process in mammals, including dairy cows, it is difficult to define ketosis, a complex metabolic condition. Acetyl Co-A builds up in the mitochondria of cells, especially hepatocytes, as a result of this metabolic process, which involves the convergence of proteins, lipids, and carbs (Nelson & Cow, 2004).

Lack or depletion of typical precursors needed for the Krebs cycle, especially oxaloacetate, can contribute to the development of ketosis. This happens as a result of a higher rate of gluconeogenesis and the inhibition of many Krebs cycle enzymes brought on by the significant amount of NADH+ produced by the β-oxidation of acetylated fatty acids that reach the mitochondria. As a result, the accumulated acetyl Co-A is diverted toward the production of ketone bodies rather than undergoing additional oxidation within the Krebs cycle. Following their release into the bloodstream, these ketone bodies provide energy to certain bodily tissues, including the brain and heart (Nelson & Cox, 2004; Ruppert & Kersten, 2024; Zhou *et al.,* 2023).

More specifically, the liver is the primary site of ketone body formation, which is brought on by a number of conditions that lower blood glucose levels. One such element is the rise in milk production, which necessitates glucose as a precursor for the subsequent synthesis of lactose. As a result, the liver's glucagon activation suppresses lipogenesis, lowers the rates of glycogen synthesis and glycolysis, increases fatty acid oxidation, boosts gluconeogenesis and glycogenolysis, and encourages ketogenesis. On the other hand, insulin, which is decreased during the postpartum catabolic condition that cows go through, has the opposite impact (Melendez, 2017; Nelson & Cox, 2004; Ruppert & Kersten, 2024).

The onset of ketogenesis coincides with a decline in blood glucose levels due to the heightened synthesis of lactose in the mammary gland. However, increased ketone body synthesis can also arise from the development of insulin resistance, particularly in the early post-partum period (2–14 days after calving) (Drackley *et al.,* 2001; De Koster & Opsomer, 2013). Additionally, during proteolysis, certain amino acids with ketogenic properties, such as leucine, lysine, tryptophan, alanine and tyrosine, undergo deamination and contribute to the formation of AcAc (Nelson & Cox, 2004).

Indeed, the term 'bodies' is a historical designation that has been used in reference to ketone compounds. However, it is important to note that these compounds are highly soluble in blood and body fluids, despite occasional references to them as insoluble particles. Among the ketone bodies, Ac is produced in smaller quantities compared to the others. It is exhaled and gives the characteristic odour associated with cows experiencing ketosis. AcAc and BHB are transported through the bloodstream to extrahepatic tissues. Once there, they undergo conversion into acetyl-CoA and are oxidized in the Krebs cycle, providing essential energy for muscle, cardiac and renal cortex tissues. The brain, which primarily relies on glucose as its preferred energy source, can adapt to utilize AcAc and BHB during periods of severe negative energy balance when glucose availability is limited (Nelson & Cox, 2004; Ruppert & Kersten, 2024).

The enzyme thiolase facilitates the enzymatic condensation of two acetyl-CoA molecules, which is the first step in the synthesis of AcAc. In essence, this procedure is the opposite of the last stage of β-oxidation. After combining with acetyl-CoA, aceto-acetyl-CoA forms β-hydroxy-β-methylglutaryl-CoA, which is cleaved to release AcAc and more acetyl-CoA. BHB dehydrogenase has a role in the reversible reduction of AcAc to BHB. AcAc can be decarboxylated spontaneously or with the aid of aceto-acetate decarboxylase to produce Ac in trace amounts (Nelson & Cox, 2004).

In extrahepatic tissues, BHB is oxidized back to AcAc with the involvement of BHB dehydrogenase. AcAc is activated by the transfer of a CoA group from succinyl-CoA, a reaction mediated by β-ketoacyl-CoA transferase. The resulting aceto-acetyl-CoA is then cleaved by thiolase, resulting in the production of two acetyl-CoA molecules that enter the Krebs cycle. Through this process, ketone bodies serve as an energy source, enabling the continuous oxidation of fatty acids while minimizing the oxidation of acetyl-CoA (Ruppert & Kersten, 2024).

Table No 1: Hormonal effects in bovine ketosis

Compound	**Effect**	**Enzyme/receptor**
Insulin	↑ Glucose entry into the cell (muscle and adipocytes)	↑ GLUT4
Decreases ketogenesis	↑ Glucose entry into the cell (liver)	↑ Glucokinase
	↑ Glycogen synthesis in liver and muscle	↑ Glycogen synthetase
	↓ Glycogenolysis (liver and muscle)	↓ Glycogen phosphorylase
	↑ Glycolysis and acetyl-CoA (liver and muscle)	↑ PFK-1, ↑ pyruvate dehydrogenase
	↑ Fatty acid synthesis (liver)	↑ Acetyl-CoA carboxylase
	↑ Triglyceride synthesis (adipose tissue)	↑ Lipoprotein lipase
Glucagon	↑ Hepatic glycogenolysis	↑ Glycogen phosphorylase
Increases Ketogenesis	↓ Hepatic glycogen synthesis	↓ Glycogen synthetase

	↓ Hepatic glycolysis	↓ PFK-1
	↑ Hepatic gluconeogenesis en hígado	↑ FBPase-2, ↓ pyruvate kinase, ↑ PEP carboxykinase
	↑ Lipolysis	↑ Triacylglycerol lipase, phosphorylation perilipin
Epinephrine	↑ Heart rate	↑ Perilipin phosphorylation
Increases ketogenesis	↑ Blood pressure	
	↑ Respiratory tract dilation	
	↑ Glycogenolysis in liver and muscle	
	↓ Glycogen synthesis in liver and muscle	
	↑ Glycolysis in muscle	
Adiponectin	↑ Fatty acid entry in muscle	↑AMPK
Increases ketogenesis	↑ Beta-oxidation	
	↑ Glucose entry in muscle	
	↑ Glycolysis in liver	
Cortisol	↑ gluconeogenesis	
	↑ lipolisis	

More specifically, glucagon and epinephrine are released when blood glucose levels are low, such as during high lactose synthesis in the mammary gland of high-producing dairy cows. Adenylyl cyclase is stimulated by glucagon binding to its receptor on the adipocyte membrane via a G protein, which results in the synthesis of cAMP. The hormone-sensitive lipase and perilipin molecules on the surface of lipid droplets are phosphorylated as a result of phosphokinase A being activated. Hormone-sensitive lipase can reach the lipid droplet surface through phosphorylation of perilipin, where it hydrolyzes TG into free fatty acids.

After exiting the adipocyte, these fatty acids attach to blood serum albumin and travel throughout the body. Through certain fatty acid transporters, they are subsequently liberated from albumin and absorbed by other cells, including hepatocytes. Fatty acids undergo oxidation in these cells to produce CO2, and the energy from this process is stored in ATP, meeting the energy needs for milk production (Melendez & Risco, 2021; Nelson & Cox, 2004).

The majority of fatty acids produced from adipose tissue are fatty acids with 16 or more carbon atoms, which are unable to directly cross mitochondrial membranes. Rather, they proceed through three enzymatic processes linked to the carnitine shuttle. A family of isozymes known as acyl-CoA synthetases is involved in the first reaction, which takes place in the outer mitochondrial membrane. These enzymes catalyse the formation of a thioester linkage between the carboxyl group of the fatty acid and the thiol group of coenzyme A, resulting in the production of fatty acyl CoA and the cleavage of ATP to AMP and phosphate. Similar to acetyl-CoA, fatty acyl CoA is a high-energy compound that can be transported into the mitochondrion from the cytosolic side of the outer mitochondrial membrane, where it can be oxidized to generate ATP or used for the synthesis of membrane lipids within the cytosol (Nelson & Cox, 2004).

The second phase in the carnitine shuttle is transesterification, which involves temporarily attaching fatty acids that are intended for mitochondrial oxidation to the hydroxyl group of

carnitine. Carnitine acyltransferase I, which is found in the mitochondrial outer membrane, catalyzes this transesterification. The acyl-carnitine/carnitine transporter found in the inner mitochondrial membrane facilitates the entry of the resultant fatty acyl carnitine ester into the mitochondrial matrix by facilitated diffusion. Carnitine acyltransferase II enzymatically transfers the fatty acyl group from carnitine to intramitochondrial coenzyme A in the third and last stage of the carnitine shuttle. This particular isozyme, which is located on the inner face of the inner mitochondrial membrane, releases free carnitine and regenerates fatty acyl CoA into the mitochondrial matrix. The acyl-carnitine/carnitine transporter then allows carnitine to re-enter the intermembrane gap. Two separate pools of coenzyme A found in the cytosol and mitochondria are connected by this three-step process of fatty acid transfer into the mitochondrion, which includes esterification to CoA, transesterification to carnitine, and subsequent transport and reconversion to CoA. Each of these pools has a distinct function.

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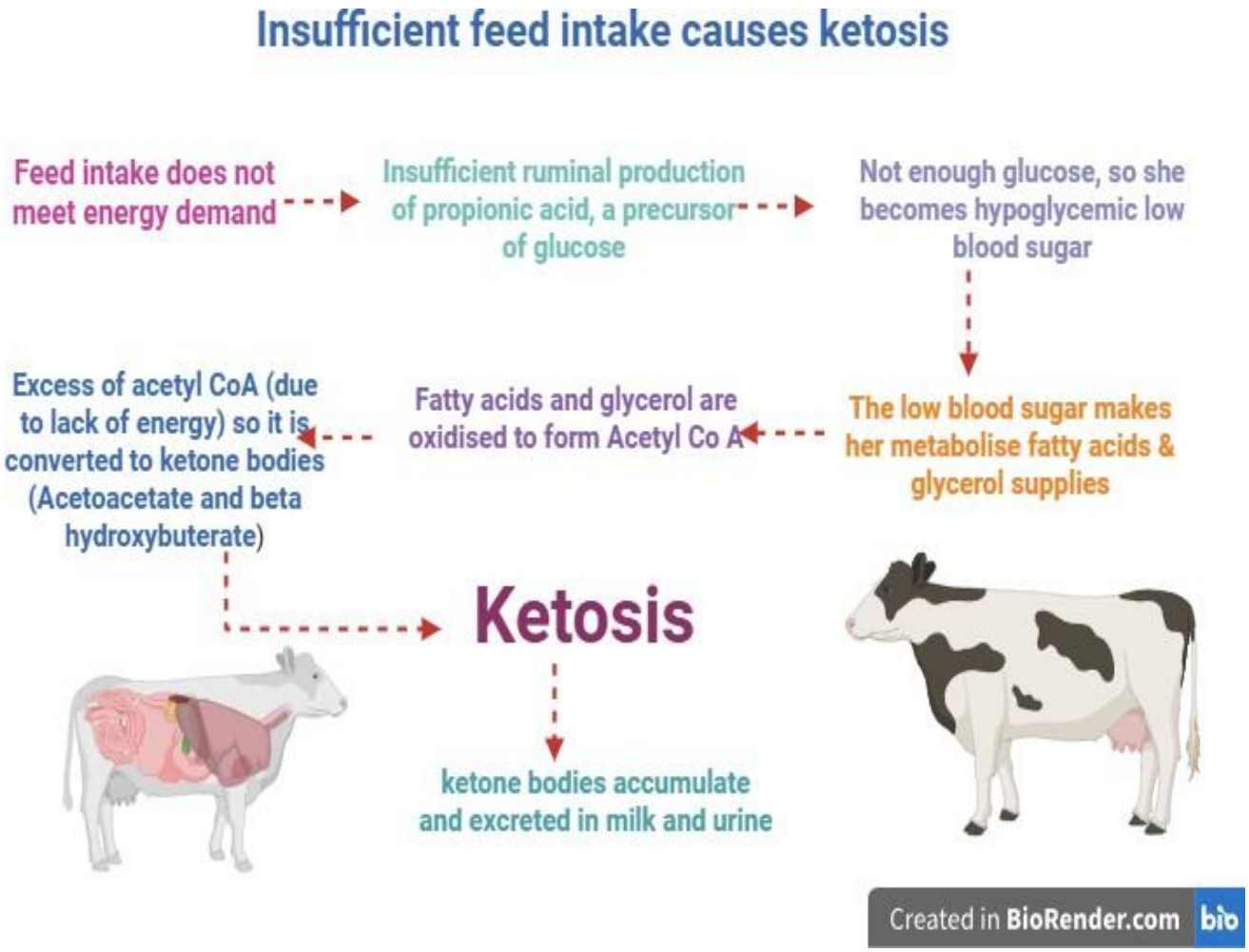

Figure No 1: Insufficient feed intake causes bovine ketosis

Diagnosis

Measuring the concentrations of ketone bodies, specifically AcAc and BHB, in different bodily fluids such urine, milk, serum, plasma, or blood is necessary to diagnose ketosis in dairy calves (Iwersen *et al.,* 2009; Melendez, 2017; Ospina *et al.,* 2013). There are various approaches for every fluid, and each has unique properties for sensitivity, specificity, positive predictive value, and negative predictive value.

Treatment and prevention

Due to the accompanying expenses and adverse effects, it is imperative that ketosis in dairy cows be prevented and treated efficiently. It is now crucial to control and monitor the risk variables associated with this metabolic disease (Melendez & Risco, 2021).

Numerous approaches of treating ketosis have been investigated in recent research. These include daily injections of vitamin B12 in comparison to a control group, the use of dexamethasone as an adjuvant therapy to propylene glycol, a product that combines but ophosphane and cyanocobalamin, or insulin in combination with propylene glycol (Gordonetal., 2013; Tatone, Gordonetal., 2016; Tatone, Duffieldetal., 2016; Weerathi Lake *et al.,* 2019). Studies assessing supplementary treatments to propylene glycol, however, have not consistently shown a substantial increase in postpartum health or reproductive performance when compared to treatment with propylene glycol alone (Jeong *et al.,* 2018). Propylene glycol, either in liquid form for oral treatment or as a powder incorporated with the entire diet, is therefore still the main treatment for ketosis (Ospina *et al.,* 2013; Mc Art et al., 2011). The effectiveness of propylene glycol as the only treatment without the need for other therapies such intravenous dextrose is supported by recent research.

This study discovered that adding intravenous dextrose did not improve the SC solution. Kor increased average daily milk production in comparison to propylene glycolone therapy. Additionally, the extravascular delivery of dextrose may result in jugular thrombosis and be labor - intensive. According to earlier research showing its benefits in correcting the metabolic state, preventing clinical ketosis, and enhancing milk production during early lactation, propylene glycolone is still the recommended treatment for clinical and SCK (Mc Art *et al.,* 2011). In conclusion, the use of propylene glycol as the primary treatment and the efficient management and prevention of ketosis in dairy herds are crucial in reducing the negative effects this metabolic disease has on the economy and human health.

A recent study provides intriguing new information about treatment strategies for controlling lipolysis in ketotic cows. By targeting the inflammatory and hormonal processes of lipolysis,

Researchers looked into using flunixinmeglumine and niacin as possible therapies. With a higher percentage of cows developing normoketonemia than the other treatment groups, the group treated with propylene glycol + niacin + flunixin meglumine demonstrated encouraging outcomes. These cows also showed lower quantities of BHB, acute phase proteins, and NEFA and were more likely to have normal levels of BHB.

Furthermore, they had better metabolic status, as seen by increased blood glucose and insulin concentrations (Chirivi *et al.,* 2023). Without a doubt, this study offers a useful therapeutic strategy that enhances the usage of propylene glycol alone by combining niacin and flunixin meglumine to regulate lipolysis in ketotic cows.

However, preventing ketosis necessitates a thorough strategy that includes adequate cow comfort and management, as well as a logical and planned feeding regimen. Providing diets that are well-balanced in terms of energy, protein, vitamins, and minerals is crucial. Abdominal obesity during calving, high BCS, and BCS losses throughout the dry period can all raise the risk of peripartum diseases, so maintaining an ideal BCS during the dry and transition phase is essential.

Including gluconeogenic precursors in the diet can help avoid ketosis. These precursors lower hepatic TG accumulation and NEFA mobilization, raise circulating insulin levels, and stimulate hepatic gluconeogenesis. Glycerol, calcium propionate, and propylene glycol are examples of additives that have been shown to be effective in both preventing and treating ketosis in dairy cows. Instead of combining these additives with other ingredients, it is advised to administer them as a stop dressing on the complete diet at the feed bunk. The frequency of ketosis in the herd should determine the frequent usage of supplements such as propylene glycol (Melendez & Risco, 2021; Ospina *et al.,* 2013).

Another useful dietary supplement for preventing bovine ketosis is sodium monensin. It can be given as a slow-release ruminal bolus or as a powder. Monensin encourages the rumen to produce propionic acid, a crucial gluconeogenic precursor for ruminants. This increases milk production and fertility while lowering the prevalence of ketosis and related illnesses. In order to ensure that cows with low BCS calve with enough BCS, monensin can also be utilized during the dry period to enhance body reserves (Melendez *et al.,* 2007).

In conclusion, a well-planned feeding program, therapeutic treatments, preventive measures, and appropriate cow comfort and management can all be used to successfully treat ketosis in dairy herds.

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