ABSTRACT

Title of Thesis: The study of hyperketonemia in the dairy cow.

Mario Alberto Barrientos Blanco, Master of Science, 2023.

Thesis Directed By: Ph.D. J. Eduardo Rico, Department of Animal and Avian Sciences.

The metabolic phenomenon of ketosis in dairy cows has remained ambiguous, casting uncertainty over our understanding and its real implications. Ketosis, commonly defined as blood β-hydroxy-butyrate (BHB) ≥ 1.2 mM (*i.e.*, hyperketonemia), has been observationally connected to the onset of peripartal metabolic disorders (*e.g.*, infectious diseases, fatty liver), and reduced milk yield in dairy cows. Although BHB is currently used as standard biomarker for the prediction of negative health and performance outcomes during the peripartum, the nature of this relationship is ambiguous. In contraposition, recent discoveries in mammalian biology indicate BHB as therapeutic metabolite (*e.g.*, alleviation of inflammation and oxidative stress). Our overreaching goal was to study the effects of BHB on dairy cow metabolism and health. In our first study, 6 multiparous (parity = 2.8 ± 0.9) Holstein mid-lactation dairy cows (128 ± 52 days in milk; DIM), were enrolled in a study to evaluate a ketogenic diet using calcium butyrate (—CaBu—; a ruminal ketone precursor) against an un-supplemented control (Control) in a crossover arrangement of

treatments. The CaBu resulted in nutritional ketosis ($P < 0.05$) with blood BHB levels of 0.2 mM higher relative to Control. Although CaBu resulted in reduced dry matter intake (DMI; $P < 0.05$), milk production was not affected ($P > 0.40$), and feed efficiencies were improved ($P < 0.05$) relative to Control. No differences in glucose, NEFA, respiration rates, pain scores, or rectal temperatures were observed between treatments. In the second experiment, 8 multiparous Holstein (2.75 ± 0.89) mid-lactation dairy cows (140 ± 48 DIM), feed *ad libitum,* were enrolled in a in a crossover arrangement of treatments. The aim of the study was to evaluate the effect of ketones by intravenous infusion of either Na-BHB solution (2.5mM; EK) to sustain hyperketonemia —BHB > 1.2 mM and < 3.0 mM—, or NaCl as a control (2.5mM; Control) over a 72h period. A systemic lipopolysaccharide (LPS) challenge (*E. coli* 055:B5; 0,085 μg/kg BW,) was intravenously administered at h 60 from infusion start. Cows sustained hyperketonemia throughout the 72h experimental period (1.4 BHB mM *vs.* 0.7 BHB mM in EK *vs*. Control, respectively). While DMI and milk production were not affected by the BHB infusion, the combination with the LPS challenge resulted in reductions of 20.8% ($P < 0.05$) and 40.1%, ($P = 0.14$) for both measurements in EK *vs*. Control, respectively. No differences were detected in the glucose and NEFA concentrations, but insulin was higher 46.6% ($P < 0.05$) in EK group. Among the immune markers, IL-1β was 30.8% higher ($P < 0.05$) in the EK group, and not differences were detected in TNFα, IL-10, CRP, and caspase-1. As expected, the LPS challenge induced increased respiration rates, temperature, and pain scores over the time course of the evaluation ($P < 0.001$); however, respiration rates tended to be reduced in 8.4% ($P < 0.1$) and rectal temperature increased in 0.3% ($P < 0.05$) by the BHB treatment ($P < 0.05$). Our results are indicative that, in the absence of an immune challenge, hyperketonemia results in no negative impact on cow productivity and health. These data add support to our hypothesis that cofactors other than ketones may be necessary for the development of negative trajectories of health and performance of lactating dairy cows. Future studies will be required to confirm that BHB hyperketonemia metabolic effects could differ from ketosis disorder in dairy cows.

THE STUDY OF HYPERKETONEMIA IN THE DAIRY COW

by

Mario Alberto Barrientos Blanco.

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2023

Advisory Committee:
Professor Ph.D. J. Eduardo Rico, Chair
Ph.D. Nishanth Sunny.
Ph.D. Richard Kohn.

Dedication

This thesis is dedicated to my beloved family. To my wife Natalia, and my children Jose Mario and Julieta, this professional milestone is the result of your energy and motivation. Your unconditional support, love, and commitment were always the running engine that powered me during my master's program. I would like to extend this dedication also to my parents. The years of effort pushing me to move my own boundaries, your counsels encouraging me to pursue bigger goals, and your profound belief in the power of education paved the way for this thesis. With all my love, thank you. This is also yours.

Acknowledgements

I am grateful to the Department of Animal and Avian Sciences at the University of Maryland for their financial support and for giving me the chance to pursue a Master's degree in science.

I would like to express my sincere gratitude to my advisor, Dr. Eduardo Rico, for his excellent mentorship, positive attitude, and love for science. His guidance and support have made my experience as a graduate student truly remarkable.

I want to express my gratitude to Andrea Celemín, Mariana da Silva, Dr. Naghme Bagheri, Dr. Victor Sainz de la Maza, Caitlin Mercado, Nicole Galdos, and Neha Potphode for their great help. Without their support, I wouldn't have been able to complete my experiments successfully. I also want to give a special thanks to Parama Bhattacharjee for your knowledge and willingness to help, which made it possible for us to implement several lab techniques in our research.

I would like to express my sincere gratitude to Brian Spielman and his farm crew for their amazing support during the trials at CMREC dairy farm. My committee members Dr. Richard Kohn and Dr. Nishant Sunny were also fundamental in my master’s program. I am grateful for their guidance when doubts appeared and for all the ideas about how to improve the scientific approaches used in my studies.

Table of Contents

List of Tables

List of Figures.

List of Abbreviations

ATP: ADENOSINE TRIPHOSPHATE

ACAC: ACETOACETATE.

BHB β-HYDROXYBUTYRATE

CACT: CARNITINE ACYL CARNITINE TRANSLOCASE

CK: CLINICAL KETOSIS

CPT II: CARNITINE PALMITOYL TRANSFERASE II

CTP-1: CARNITINE PALMITOYL TRANSFERASE 1

PAMP: PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

DAMP: DAMAGED-ASSOCIATE MOLECULAR PATTERNS

DIM: DAYS IN MILK

DMI: DRY MATTER INTAKE

EU: ENDOTOXIN UNITS

FA: FATTY ACIDS

GH: GROWTH HORMONE

IL-1β: INTERLEUKIN ONE-BETA

IL-6: INTERLEUKIN SIX

IL-10: INTERLEUKIN TEN

IRS-1: INSULIN RECEPTOR SUBSTRATE ONE

KB: KETONE BODIES

LCFA: LONG-CHAIN FATTY ACID

LPS: LYPOPOLYSSACHARIDE

LBP: LIPOPOLYSACCHARIDE-BINDING PROTEIN

LTA: LIPOTEICHOIC ACID

MAT: METHYL ACETO-ACETYL COA THIOLASE

NEFA: NON-ESTERIFIED FATTY ACIDS.

NOD: NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN

NLR: NOD-LIKE RECEPTOR

PKA: PROTEIN KINASE A

PPAR: PEROXISOME PROLIFERATOR–ACTIVATED RECEPTORS.

PRR: PATTERN RECOGNITION RECEPTORS

SCK: SUB-CLINICAL KETOSIS

SCOT: SUCCINYL-COA:3-KETOACID COA TRANSFERASE

TAG: TRIACYLGLYCEROL

TCA: TRICARBOXYLIC ACID CYCLE

TNFα: TUMOR NECROSIS FACTOR ALPHA.

UFA: UNSATURATED FATTY ACID

VFA: VOLATILE FATTY ACIDS

HP: HAPTOGLOBIN

CE: CERULOPLASMIN

SAA: SERUM AMYLOID A

Chapter 1: Literature Review

Energy metabolism of dairy cows

1.1.1. Digestion of fiber and absorption

The energy metabolism in ruminants is supported by the role of microorganisms to segregate the plant fiber components. The action of the microorganisms allows the host to extract the essential nutrients from the forage through anaerobic fermentation (Annison and Bryden, 1998). The enzymatic activity of the microorganisms allows them to break up the carbohydrate complexes cellulose, hemicellulose, and starch into monosaccharides like glucose, xylose, and arabinose (Baldwin and Allison, 1983; Domingues et al., 2016; Beauchemin et al., 2022). These monomers once available for the microorganism will be metabolized to volatile fatty acids, CO_2, CH_4, traces of dihydrogen (H_2), and other compounds (Beauchemin et al., 2022).

The fermentation of the fiber is the primary source of energy used by rumen microbes to supply their maintenance and growth (Baldwin and Allison, 1983). The monomers generated from the fragmentation of the structural and not-structural polysaccharides are used by microorganisms to be glycolyzed to produce pyruvate, the precursor used to produce the main VFA: acetate, propionate, and butyrate. The partition of the monomers through different fermentation pathways depends on the abundance of the microorganisms that encode each pathway, gene expression enzyme activity, and kinetics (Hackmann et al., 2017; Beauchemin et al., 2022).

Volatile fatty acids provide nearly 70% of the metabolizable energy supply in ruminant (Bergman, 1990). Propionate in ruminants acts as the main precursor for glucose synthesis in the liver, supplying from 32% to 73% of glucose demands (Yao et al., 2017). Acetate is identified as the major substrate for the novo synthesis of fats in the mammary gland and adipose tissue. Acetate provides near of 45% of energy from VFA supplied to metabolism in a good feed animal (Urrutia

et al., 2019). Butyrate is the least abundant of the major VFA with a molar proportion lower than 15% (DeFrain et al., 2004; Górka et al., 2018).

The metabolization of VFA depends on different organs to be processed. Propionate is removed mostly by the liver to synthesize glucose (Bergman, 1990). The acetate uptake is mediated through lipogenesis that occurs mainly in peripheral tissues like adipose, mammary gland and skeletal (Hanson and Ballard, 1967; Bergman, 1990; Osorio et al., 2016). A distinctive feature of the butyrate is that 90% of its absorption is converted to ketone bodies (β-hydroxybutyrate -**BHB**- and acetoacetate-**AcAc-**) in the walls of the rumen before entering the portal system to support the cellular activity as an energy source (Bergman, 1990; DeFrain et al., 2004; Baldwin et al., 2018).

1.1.2. Digestion and absorption of dietary fats

The addition of dietary fat has gained interest to improve health and milk fat enrichment with omega 3 fatty acid (Lock and Bauman, 2004; Bernal-Santos et al., 2010; Grossi et al., 2013; Moallem, 2018). The degree of lipid supplementation range between 3 to 4% to a maximum of 6% of DM as total lipid (—including the contained in the forages, grains, or supplements—; (Roche et al., 2013; Bionaz et al., 2020). The inability to implement higher fat concentrations in diets is related to the inhibition of fermentation caused by fatty acids **(FA)** (Jenkins, 1993). The FA can be toxic because of its chemical properties that allow them to bind the bacteria's surface, provoking the disruption of the membrane, and annihilating the microorganisms, especially by unsaturated fatty acids (Jenkins, 1993). The incorporation of dietary fats is a focus of importance nowadays because of human nutrition concerns about consuming saturated fats from animal origin. However fatty acid absorption post rumen fermentation without being hydrolyzed or bio-hydrogenated is hard to succeed in ruminants (Bionaz et al., 2020).

Lipids once entered in the rumen suffer biohydrogenation or lipolysis to be used in *de novo* syntheses by microorganisms. Biohydrogenation is considered a special protection to avoid damage caused by UFA (Jenkins, 1993). The absorption of lipids post ruminal digest is composed mainly of saturated NEFA, being 70% of them from dietary and *de novo* synthesis by microbiota, and small

and variable amounts (10-20%) from microbial phospholipids (Bauchart, 1993). The majority (> 96%) of the medium-chain and long-chain FA present in the diet, or processed by microbiota, are absorbed by the pre-stomachs and abomasum epithelium (Jenkins, 1993). In ruminants, most of the FA flowing through the small intestine is in form of micelles or attached to food particles (Bionaz et al., 2020).

1.1.3. Energy substrates in dairy cows

1.1.3.1. Glucose

Ruminants use mostly VFAs, lipids, ketone bodies, amino acids, and glycerol as the major fuels (Randle, 1998; Abbas et al., 2020). However, as occurs in all mammalians, glucose is the main fuel used by the metabolism and synthesis pathways (Aschenbach et al., 2010). Glucose can be synthesized mainly from propionate, lactate, alanine, isobutyrate, valerate, glycerol, and other amino acids by hepatic gluconeogenesis (Aschenbach et al., 2010), being propionate the main source for the glucose synthesis in the liver (Young; Bell and Bauman, 1997; Yao et al., 2017). Also, the glucose supply could be mediated by glycogen released from muscle and liver tissue (Hui et al., 2020). Gluconeogenesis in the liver provides around 90% of the glucose needs in ruminants, being of great relevance for tissues such as the brain, red blood cells, kidney, and mammary gland (Bell and Bauman, 1997; Murray et al., 2009; Aschenbach et al., 2010; Abbas et al., 2020). The glucose synthesis is closely monitored and controlled by endocrine actions and coordinated according to the physiological states (Bell and Bauman, 1997; Hammon et al., 2009). Glucose is vital for dairy cows not only for the regular metabolic activity but also because it is required for lactogenesis as the main lactose precursor, the primary osmotic determinant in the milk volume (Bell and Bauman, 1997; Abbas et al., 2020; White, 2020).

1.1.3.2. Lipids.

As alternate fuel, fats are also a relevant energy substrate. The major source of lipid metabolism in dairy cows is related to VFA, where acetate and butyrate are the main precursors of

de novo synthesis (Bauman et al., 1970; Bergman, 1990). Acetate contributes between 70% and 80% of acetyl-groups for lipogenesis in adipose tissue, 15-30% in intramuscular reservoirs (Smith & Grouse, 1984; N. L. Urrutia & Harvatine, 2017), and it is the major precursor for *de novo* synthesis in the mammary gland (Osorio et al., 2016). Between 15% to 20% of the acetate absorbed is used by the portal-drained viscera, with low liver uptake. Hepatic low acetate uptake is because of the low liver expression of acetyl Co-A synthetase, hence the liver plays a role in lipogenesis more related to phospholipids and monoglycerides synthesis (Benson & Emery, 1971; Jesse et al., 1986; Wilson et al., 1992). Around 80% of the acetate uptake is used in lipogenesis by the skeletal, mammary gland, and adipose tissue, being last one of the most important in lipid biosynthesis (Bergman, 1990; Wilson et al., 1992). Acetate is a key precursor to stimulate lipid synthesis in mammary gland cells, provides most of the carbon for *de novo* lipogenesis, and supports the electron transport chain with approximately half of the reducing equivalents (NADPH) through the isocitrate pathway in milk synthesis (Bauman et al., 1970; Jacobs et al., 2013).

1.1.4. Energy economy during the cow life cycle

Energy in dairy cows is distributed between growth, maintenance, pregnancy, and milk production (National Research Council, 1980; Dämmgen et al., 2009; Weiss, 2010; Allen, 2020). The amount of energy available to distribute depends on diets, feed intake, digestibility of the diet, and the losses related to the digestion process (Tyrrell and Moe, 1975; Weiss and Tebbe, 2019). Maintenance metabolism represents around 75% of the intake energy distribution during the lactation period (Coppock, 1985), considering all the energy used to compensate for the basal metabolism and heat energy loss-related processes (National Research Council, 1980; Coppock, 1985). Maintenance energy partitioning is influenced by factors like diet, intake, environment, age, activity, diseases, infections, sex, and breed as others (National Research Council, 1980; Coppock, 1985). But, in recent decades, the genetic selection for animals with higher milk production has resulted in bigger animals with higher energy demands, in part, because of the higher weight of internal organs for digestion and metabolism (Gruber et al., 2021).

The energy partitioning can be influenced by the presence of a conceptus, representing an increase of 75% in the energy demand at the end of the gestation for a dairy cow compared to a non-pregnant (Bauman and Bruce Currie, 1980). However, the greatest rate of mass accumulation of the fetus occurs in the late period of pregnancy during the dry period, therefore, being insignificant the competition between milk production and fetal growth (Bauman and Bruce Currie, 1980). Nevertheless, the physiological priorities for metabolizable energy are adjusted along the period of lactation. Lactation energy demand is set as the main priority in the energy partitioning during the first semester of lactation, a feature reverted during the second and third semesters, where reproduction is considered the first physiological priority (Coppock, 1985). Milk synthesis represents around 25% of the flux of net energy used by a dairy cow (Coppock, 1985; Andrew et al., 1991). However, this parameter has been modified as the result of the genetic selection for higher milk yields and higher feed intake capacity. This circumstance has increased the energy intake resulting in higher energy expenditure in milk production but has reduced the energy partition into body tissue in high genetic merit cows (National Research Council (U.S.). Subcommittee on Dairy Cattle Nutrition., 2001; Yan et al., 2006; Gruber et al., 2021).

1.1.4.1. Regulation of nutrient partitioning during lactogenesis

Dairy cow metabolism is orientated to satisfy the lactogenesis energy demand during the first semester of lactation. The energy diversion is mainly modulated by the action of the endocrine system (Hart, 1983; Coppock, 1985). Cows through the transition period exhibit a reduction in the insulin sensitivity in the adipose and muscle tissue, reducing the glucose uptake in those extrahepatic tissues to favor milk production and guarantee the energy supply to the gravid uterus (Zachut et al., 2013; Abuelo et al., 2016; Baumgard et al., 2017; Contreras et al., 2017). Nevertheless, the homeorhetic adjustments toward calving do not induce insulin resistance in the liver (Zachut et al., 2013). Abuelo et al., (2016) associated insulin resistance with alterations in the oxidative status generated from inflammation faced by transient dairy cows. Using mice models le Marchand-Brustel et al., (2003) described mechanisms associating TNFα, cellular stress, and NEFAs as the causal factors of insulin resistance. The low sensitivity response in the insulin action has been described in the reduction in the insulin receptor substrate one **(IRS-1)** phosphorylation provoked by NEFA. This association results from the increasing levels of diacylglycerols and

ceramides and their effects blocking the insulin receptor-activated downstream pathway (Griffin et al., 1999; Chavez et al., 2003).

The reduction in insulin levels during the transition period triggers fat mobilization (Sordillo and Raphael, 2013). Using mice models Choi et al., (2010) described AKT and its role as an antagonist of protein kinase A **(PKA)**, the major lipolytic agonist, which in absence of AKT (because of the low insulin activity) it is allowed to trigger the lipolysis. Aligned with this mechanism, the energy metabolism also is equilibrated allowing the release of glucagon, catecholamines, and growth hormone to increase the rate of gluconeogenesis, lipolysis, and proteolysis (Hart, 1983; Xu et al., 2020).

Growth hormone modulates energy fragmentation in high-yielding dairy cows, diverting glucose and fatty acids away from tissue deposition to be used as a source of energy for milk synthesis (Bell & Bauman, 1997; Etherton & Bauman, 1998). In early lactation, growth hormone affects insulin sensitivity in the liver, skeletal muscle, and adipose to beneficiate the energy partitioning diverted to the mammary gland (Hart, 1983; Bell, 1995; Drackley, 1999; Block et al., 2001). Parallel, it stimulates hepatic gluconeogenesis and lipolysis to supply the energy demand generated under a negative balance (Drackley, 1999). Mohsin et al., (2022) associated growth hormone as the trigger of lipid mobilization from adipose tissue that leads to hyperketonemia post-partum.

Ketosis

1.1.5. Ketosis

Ketosis is currently considered as a metabolic disease caused by high BHB blood concentration resulting from the negative energy balance faced by dairy cows in early lactation (White, 2015; Zhang et al., 2016; Song et al., 2021). The energy deficiency is compensated by the ramping up of NEFA mobilization to be oxidized by the liver, resulting not only in an ATP

production increase but also carrying out ketone bodies synthesis when the TCA cycle capacity is exceeded (White, 2015).

Ketosis also referred to as hyperketonemia, is differentiated into two conditions (LeBlanc, 2015). Animals detected with BHB levels higher than 3 mmol/L are classified as suffering from clinical ketosis **(CK),** while cows with levels in the range of 1.2 mmol/L and 3.0 mmol/L of BHB are classified within subclinical ketosis (SCK Ospina et al., 2010d; McArt et al., 2012a). Clinical ketosis is characterized by an increase in blood, urine, or milk ketone bodies in conjunction with decreased appetite, weight loss, rapid body condition loss, dry manure, and decreased milk production (Gordon et al., 2013). In exceptionally severe cases neurological signs such as leaning or walking against the stall front, licking, or chewing (Gordon et al., 2013; McArt et al., 2013; LeBlanc, 2015). However, despite CK having a concentration established that classifies the severity of the affection, the threshold at which clinical signs occur is unclear because animals registering CK levels may not show clinical signs (Gordon et al., 2013; LeBlanc, 2015). Therefore, this metabolic disorder may be best described as hyperketonemia rather than trying to distinguish clinical from subclinical (Gordon et al., 2013).

The prevalence of CK in the farms is lower compared to SCK, ranging between 3,7% to 11,6% (Vanholder et al., 2015). Meanwhile, SCK appearance in dairy farms has a prevalence in herds between 11,2% and 47,2% (Benedet et al., 2019). Hyperketonemia has been detected worldwide with a prevalence average of >20% (Overton et al., 2017), but in some cases can reach as high as 80% in some dairy herds in North America (Duffield, 2000). Several researchers have associated the buildup of BHB to a higher risk of peripartal disorders, lower reproduction activity, and milk production (Chapinal et al., 2011, 2012a; McArt et al., 2012a, 2013; Suthar et al., 2013; Overton et al., 2017). The elevated BHB concentrations around parturition is used in the farms as a proxy for an increased risk of peripartal disorders like metritis, endometritis, retained placenta, displaced abomasum, mastitis, high culling rates, lameness, and lower fertility (Drackley, 1999; Dann et al., 2005; Dubuc et al., 2012; McArt et al., 2012a, 2013; Suthar et al., 2013; LeBlanc, 2015; Benedet et al., 2019). Although much is known about the pathobiology of ketosis, there is still a need to better understand the etiology and pathogenesis of the disease (Zhang et al., 2016).

1.1.6. History of ketosis

Ketosis has been deemed as a concern for the dairy industry for more than 170 years (Shaw, 1956). Back in 1849, ketosis was first described as "*mania puerperalis"* resulting from nervous disturbances (Steffen, 1917; Udall, 1943). However, the disease was first time documented as a high concentration of ketone bodies in blood and urine by Sjollema & van der Zande (1923). Their report identified animals with acetone odor, lack of appetite, dry feces reduced milk yield, and exited state (at lower odds) exhibiting a high concentration of ketones, especially BHB as the major one. They ended up hypothesizing the etiology of ketosis resulting from functional changes in the liver caused by some intoxication. Shaw, (1943) discussed hypoglycemic metabolism and linked it to ketosis development as the explanation of the ramping milk production under low caloric intake during early lactation. A physiological adaptation that later was elegantly defined as *homeorhetic* changes by Bauman & Bruce Currie, (1980). According to Krebs (1966), this limited glucose condition (e.g., starvation or diabetes disease) is subsequently followed by the rise in gluconeogenesis activity and plasma-free fatty acids mobilization and paralleled by ramping ketone bodies levels. This physiological adaptation to fuel tissues under low glucose conditions can differ from pathological when the ketone bodies grossly surpassed requirements (Krebs, 1966). This *biochemical sign* (Bergman, 1971) was questioned by Baird et al., (1968) appointing oxalacetate availability as the missing metabolite diverting the fate of the energy flux towards ketogenesis (**figure 1**).

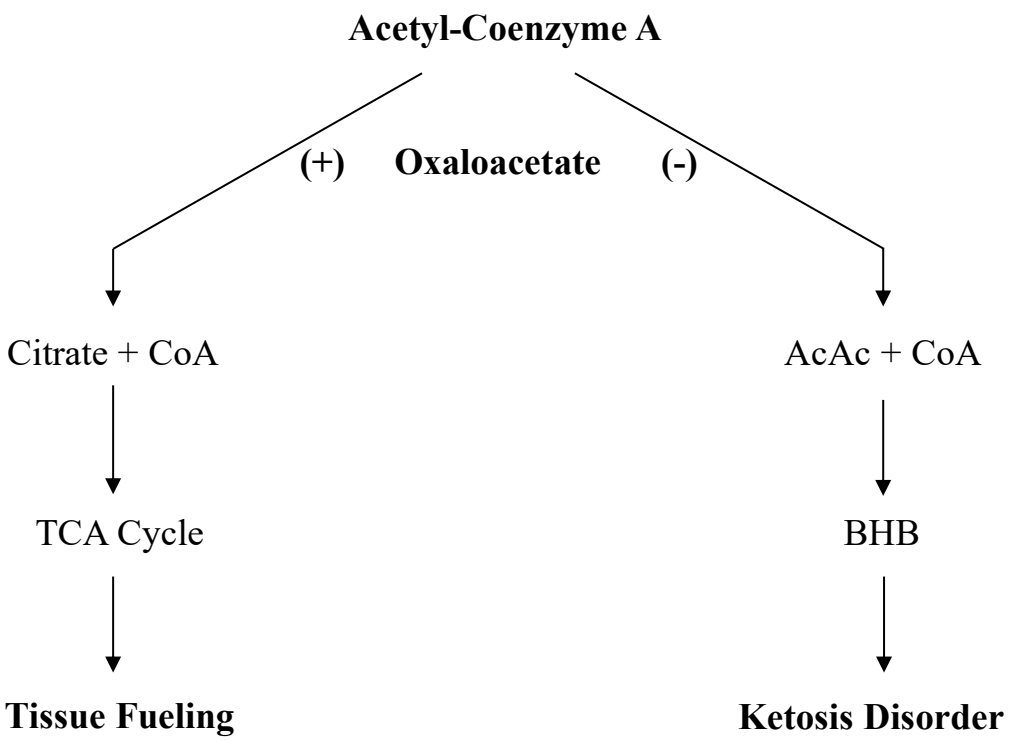


Figure 1. Acetyl-Coenzyme A metabolic fates under regular and low physiological mitochondrial levels of oxaloacetate.

**Adapted from Krebs, (1966)

Shaw, (1943) reported in his review that increasing ketones levels were frequently accompanied by reproductive complications, which were later associated with early lactation by scientists from Cornell University (Ospina et al., 2010a; b, 2013; McArt et al., 2013). During these first twenty years of the twentieth century, several researchers have advocated their investigations to elucidate the etiology and pathophysiology of the disease. In the last two decades, important advances in knowledge were added establishing the cow-level cut-off points (CK and SCK levels), the associations with postpartum health events affecting reproduction and milk performance, and the cost-related implications (Overton et al., 2017). Also, efforts were made to introduce new technologies related to expanding constituents' measurements in blood and milk (Overton et al., 2017). Handheld ketone body meters are broadly available in the markets and used in farms to detect blood concentrations as a routine monitoring tool for treatment decisions (Overton et al., 2017). However, despite the research focused on the transition period over the last two decades, a

great challenge remained to elucidate about the potential cause of ketosis (Zhang and Ametaj, 2020; Horst et al., 2021).

1.1.7. Negative energy balance (NEB) in peripartal dairy cows.

High-yielding dairy cows experience a negative energy balance in early lactation because feed intake can't support the required energy for milk production and maintenance (Bell, 1995; de Vries and Veerkamp, 2000). At the onset of lactation, the copious milk production increases the glucose demand necessary for lactose synthesis, increasing the total energy requirements approximately four-fold (Bauman and Bruce Currie, 1980) meanwhile the dry matter intake **(DMI)** is reduced by one-third in the first week's post-calving (McArt et al., 2013). In a study to detect changes in milk composition associated with energy balance, de Vries & Veerkamp, (2000) found that cows returned to positive energy balance until 41.5 days in milk **(DIM)**. In a time set description, cows reach the milking peak around week 3 or 4, whereas the net energy intake did not peak until several weeks later, achieving zero net energy balance until the reduction in the milk production dropped to less than 80% of the peak of production (Bauman and Bruce Currie, 1980).

To balance the energy deficit, cow's metabolism orchestrates series of homeorhetic changes necessary to support the physiological state (Bell and Bauman, 1997). In late pregnancy and early lactation, cows face a reduction in glucose and insulin concentration, while the hepatic glucose output is approximately increased two-fold, accompanied by an increasing concentration of essential amino acids, L-lactate and glycerol in the blood (Aschenbach et al., 2010). Also, at the onset of lactation, cows exhibit a reduction in insulin sensitivity, restricting the glucose uptake in insulin-sensitive tissues to compensate for the energy deficiency (Bell, 1995; Zachut et al., 2013; Bradford et al., 2015; Abuelo et al., 2016; Baumgard et al., 2017; Horst et al., 2021). The reduced insulin status after parturition knocks off the glucose uptake into lipogenesis and favors glucose uptake into the mammary gland, which is not affected by insulin action (Aschenbach et al., 2010) and triggers the fat mobilization through lipolysis (Sordillo and Raphael, 2013). However, Bradford et al., (2015) mentioned that exaggerated insulin resistance in transition cows may exacerbate lipid mobilization and impair metabolic health.

Several homeorhetic adaptations take place to release glucagon, catecholamines, and growth hormone to increase the rate of gluconeogenesis, lipolysis, and proteolysis (Hart, 1983; Bell, 1995; Xu et al., 2020). These homeorhetic controls alter the adipose tissue metabolism to increase lipid mobilization to support a new physiological state (Bauman and Bruce Currie, 1980). During late pregnancy and early lactation, catecholamines sensitivity increases several folds coinciding with the increase in plasma NEFA concentration (Bauman and Bruce Currie, 1980). Also, bovine somatotropin (growth hormone) promotes NEFA export from adipose tissue by increasing the lipolytic response to β-adrenergic signals and reducing the antilipolytic responses to insulin (Baumgard et al., 2017). The hepatic gluconeogenesis and oxidation of fatty acids are crucial to support the energy supply and milk synthesis in transition dairy cows (White, 2020). Block et al., (2001) estimated that the mobilization of endogenous lipids met approximately one-third of the cow's energy requirements between parturition and the third week of lactation.

At the onset of the NEB, triglycerides are mobilized from the adipose tissue to the liver to be oxidized to produce ATP. The severity of NEB is positively associated with circulating NEFA levels (Bauman et al., 1988). When the lipids flux exceeds the TCA cycle capacity, a different fate turns out, diverting the free fatty acids to be incompletely oxidated, generating ketone bodies or re-esterified lipids (Li et al., 2012a; White, 2020). Added to this condition, the oxalacetate availability regulates the synthesis of BHB, therefore under shortage conditions of this TCA cycle precursor, the ketone bodies concentration ramps up (Ringseis et al., 2015). As a result, ketone bodies are exported into circulation increasing the pool available to be metabolized (White, 2015). The increasing AcAc and BHB levels in plasma and milk are correlated negatively to energy balance in transition dairy cows (Xu et al., 2020). These ketone bodies can be used as an alternative fuel source by extrahepatic tissues (Peterson et al., 2012), coinciding with the body fat mobilization and high glucose requirements for lactose synthesis in early lactation (Xu et al., 2020).

Although increasing levels of NEFA and BHB in circulation are part of the necessary adaptations to lactation, as it was mentioned before, the elevated levels have been associated with a higher risk of negative health (Overton et al., 2017). Also, extreme lipid mobilization leads to increased uptake of NEFAs triglyceride accumulation by the liver, which can be associated with ketosis susceptibility (Drackley, 1999).

1.1.8. Pathologies associated with ketosis

1.1.8.1. Endotoxicosis

According to Horst et al., (2019), intestinal permeability can influence inflammation, rumen acidosis, decreased feed intake, and psychological stress in periparturient dairy cows. This condition could result from rumen tissue remodeling and dietary switches faced by transition cows (Goff and Horst, 1997; Mezzetti et al., 2021). During the transition period cows face a reduction of rumen papillae length and volume because of the high forage diet and fetus growth (Dirksen and Mayer, 1985; Kuhla, 2020b; Mezzetti et al., 2021). This condition reduces the rumen epithelia's capacity to absorb the VFAs, especially the lactate, which induces a pH drop (Goff and Horst, 1997; Eckel and Ametaj, 2016). Also, after parturition cows are moved from high fiber to high concentrates diet, a switch which is associated with negative gastrointestinal tract integrity because of the abrupt pH change (Martens et al., 2012). These conditions are associated with rumen acidosis and rumen epithelium damage that allows endotoxins to leak from the rumen lumen into circulation (Abaker et al., 2017; Guo et al., 2017), facilitating the development of periparturient diseases (Khafipour et al., 2009; Bradford et al., 2015; Eckel and Ametaj, 2016).

Cows under rumen acidosis caused by high-concentrate diets exhibit higher plasma LPS concentration, higher levels of pro-inflammatory cytokines and acute phase proteins (Khafipour et al., 2009; Dong et al., 2014; Guo et al., 2017; Wu et al., 2021). Liver infiltration of LPS derived from the digestive tract during rumen acidosis is associated with hepatocyte impairment, liver mitochondrial disruption by high oxidative stress, enhanced hepatic immune gene expression, activated inflammatory signal pathways, and development of liver abscess (Nagaraja and Chengappa, 1996; Abaker et al., 2017; Guo et al., 2017). However, these connections under acute acidosis are difficult to detect (Guo et al., 2017) because free LPS translocated into hepatic portal circulation can be detoxified by the liver before reaching the peripheral blood by the Kupffer cells and its related immune response (Gozho et al., 2007; Vels et al., 2008).

Rumen acidosis does not have a direct association with NEFA and BHB levels (Oetzel, 2004). However, according to Mensching et al., (2021) the connection between rumen acidosis and

energy metabolism is possible because ruminitis affects lower feed intake, aggravating the energy balance and therefore increasing the mobilization of free fatty acids. LeBlanc et al., (2005) found that the timing and magnitude of circulating concentrations of NEFA and BHB are related to a higher risk of abomasal displacement during the transition period. Dairy cows with NEFA > 0.5 mEq/L are associated with the development of displacement abomasum during the transition period (Chapinal et al., 2011), and can present adipose tissue macrophage M1 infiltration resulting in an inflammatory response (Contreras et al., 2015). Also, animals suffering SCK, have 3 to 8 times increased odds of displacement abomasum (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010a). According to Liang et al., (2017), left displacement abomasum has the greatest cost associated with health disorders ($432-$639).

Endotoxicosis may also contribute to liver pathophysiology (Eckel and Ametaj, 2020). Cows experiencing fatty liver have increased concentrations of tumor necrosis alpha (**TNFα**) serum amyloid A (**SAA**), haptoglobin (**Hp**), NEFA, TG, cortisol, and cholesterol in the plasma (Ametaj et al., 2005).

1.1.8.2. The associated effects of NEB on reproduction in peripartal dairy cows

Bacterial infections are common pathologies in early lactation (LeBlanc et al., 2011) and are ranked as one of the most expensive health issues in the dairy industry (Liang et al., 2017; Wankhade et al., 2017; Benedet et al., 2019). Lactating dairy cows with more than 28 kg of body weight lost post-parturition, and facing metabolic, reproductive, or digestion disorders are correlated with low cyclicity (Vercouteren et al., 2015). Negative energy balance faced during the transition period is associated with decreased cyclicity as a result of changes in glucose, free fatty acids, haptoglobin, insulin, insulin growth factor 1 (**IGF-1**), and luteinizing hormone (Butler, 2003; Dubuc et al., 2012; Furukawa et al., 2021).

According to LeBlanc, (2015), ketosis has a profound negative impact on the farms' reproduction performance. Cows experiencing ketosis during the first two weeks of lactation had a reduced probability of pregnancy at the first insemination (Chapinal et al., 2012a; McArt et al., 2012b; LeBlanc, 2015). Animals suffering SCK, have a 3-times greater risk of metritis (Duffield et

al., 2009), increased chances of endometritis during week four of lactation (Hammon et al., 2006a; Dubuc et al., 2010), 1,5-fold increased odds of endometritis at 35 DIM (Dubuc et al., 2012) and 1.8-times increased odds of culling before 60 DIM (Roberts et al., 2012). Oetzel, (2013) reported reduced pregnancy rates by 150 DIM for cows that first developed their SCK earlier in lactation.

Nevertheless, (Rodriguez et al., (2022). determined that reproductive performance is not associated with hyperketonemia unless cows are grouped in stratified milk production levels. In their study, they exposed that cows with hyperketonemia and low milk production were the only ones associated with reduced reproductive activity, meanwhile, cows with hyperketonemia and high milk yields did not present reproductive performance differences compared to high milk yielding non-hyperketonemic (Rodriguez et al., 2022).

1.1.9. Economic impact of hyperketonemia

The economic impact of hyperketonemia in dairy cows has been widely monitored in different scenarios. According to (Benedet et al., 2019), many models from Europe and the United States have integrated cost variables like milk production, health disorders related, to reproductive performance, treatments, and some other values such labor and slaughter. In all the results exists a negative impact, with average costs varying from $77 to $289 per case a year. Also, there is a higher negative impact on the costs for primiparous than multiparous and the most important cost is related to the negative effect on reproduction.

1.1.10. Treatment of ketosis in dairy cows.

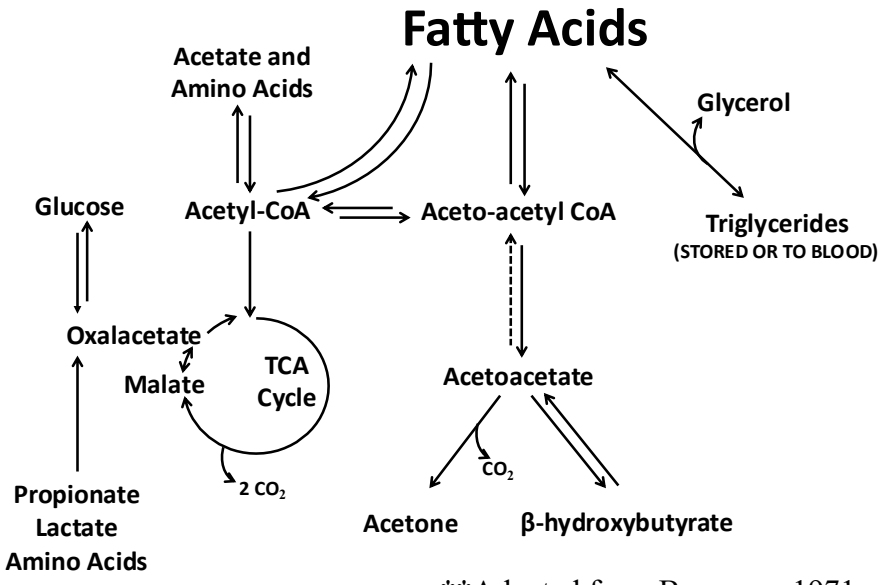
Another important fact to add to the economic impact is the lack of options to be used for treatment or even strategies to prevent this metabolic disorder (Mann et al., 2018; Capel et al., 2021). Polypropylene glycol (PG) is the only available therapy well-documented to work positively to attenuate the effect of hyperketonemia in the animal (McArt et al., 2011). The positive effects of PG are related to improving hepatic gluconeogenesis, insulin sensitivity, and reduced NEFA

oxidation. The supplementation of PG causes stimulus of gluconeogenesis by the metabolization of PG to pyruvate through different reactions, which finally is used by the liver to synthesize oxaloacetate and then to be moved in the TCA cycle to replenish the lack of this precursor in the media (Zhang et al., 2020). Then can be hypothesized that the inclusion of PG in the system could cause an alleviation in the lack of oxaloacetate allowing it to oxidize the acetyl-CoA originating from FA into the TCA cycle. Dextrose is considered a second-line treatment for ketosis because animals with severe affections with concurrent hypoglycemia can be beneficiated from the blood glucose increasing concentrations after an intravenous bolus (Gordon et al., 2013). The decrease in blood BHB levels caused by dextrose treatment works for less than 24h and must be repeated or followed with another treatment for lasting effect (Wagner and Schimek, 2010).

Energy metabolism

1.1.11. Energy metabolism in the transition period

In a series of changes, the marked reduction in glucose concentration during the transition period influences the lipids mobilization to compensate the energy imbalance (Bell and Bauman, 1997). As a result of this homeorhetic adaptation, free fatty acids (**FFA**) levels in the bloodstream and liver content increase (Bergman, 1971; Luo et al., 2019). Within the liver, fatty acids are β-oxidized to acetyl-CoA units to follow four possible fates (**figure 2**): (1) complete oxidation through tricarboxylic acid cycle **(TCA)**, (2) incomplete oxidation (ketogenesis), (3) TAG synthesis, packing in very-low-density lipoprotein and exportation out of the liver (*de novo* synthesis), or (4) TAG synthesis for storage as liver lipids (Bergman, 1971; Grummer, 1993). The FFA flux entering the liver in transitioning cows can exceed TCA-cycle oxidation capacity during the lipid mobilization peak. This condition favors the ramp-up in ketogenesis and TG deposition observed in transitioning dairy cows (White, 2015).



**Adapted from Bergman, 1971

Figure 2. Major pathways of ketone body, free fatty acid metabolism and hepatic gluconeogenesis. *The reaction shown by the dotted line does not occur in the liver.

1.1.12. Lipolysis

Hepatic FA oxidation provides the metabolism of three main functions: provision of acetyl-CoA to be oxidized in the TCA, provision of alternative energy source in the form of ketone bodies to the peripheral liver tissues and maximize the gluconeogenesis rates during glucose shortages (Jesse et al., 1986). Mobilization of FFA from the adipose tissue stores is mediated by the activation of hormone-sensitive lipase (Urrutia and Harvatine, 2017) while the glucagon to insulin ratio is responsible for regulating the hepatic fatty acid oxidation in dairy cows (Jesse et al., 1986; Drackley et al., 2001). To be oxidized, the fatty acids are activated in the hepatocyte cytoplasm by the addition of a molecule of coenzyme A (Qi et al., 2022) by the membrane-bound fatty acid acyl-CoA synthetases (Vanitallie and Nufert, 2003). These activated fatty acid esters are transported into mitochondrial matrix via carnitine palmitoyltransferase-1 **(CPT1)** and cleaved by the β-oxidation pathway. The end products of this pathway will be acetyl-CoA and reduced electron carriers that can be used in ATP synthesis (Li et al., 2012a).

1.1.13. Ketogenesis

Hyperketonemia may occur in all mammalian species and can be brought forth by starvation, low carbohydrate diet, cold exposure, anesthesia, impaired liver function, hypoglycemia, and endocrine and metabolic disorders (Bergman, 1971; Laffel, 1999). The ketone bodies, Acetoacetate **(AcAc)**, β-hydroxybutyrate **(BHB)**, and Acetone, are compounds rich in energy primarily synthesized in the rumen and liver of dairy cows (Krebs, 1966; Remond et al., 1995). Non-hepatic peripheral tissues cannot directly utilize the FFAs because of the lack of expression of the key mitochondrial enzyme: 3-hydroxymethylglutaryl-coenzyme A synthase (Newman and Verdin, 2017; Qi et al., 2022). The BHB is the main ketone body circulating in the blood (Qi et al., 2022).

1.1.13.1. Hepatic ketogenesis

Early lactation dairy cows deal with the increasing demand for gluconeogenesis associated with copious milk synthesis (Bauman and Bruce Currie, 1980). This feature limits the availability of glucose precursors and affects oxaloacetate production (Krebs, 1966; Baird et al., 1968). The reduction in the oxalacetate pool generates an acetyl-CoA *bottleneck* at the entrance of the TCA. This condition results because oxaloacetate, which in addition to acetyl-CoA, is one of the two substrates required for the citrate synthase to produce citrate at the entrance of the TCA. The reduced TCA activity favors the diversion of the excessive acetyl-CoA toward ketone synthesis (Bergman, 1971; White, 2015).

Fatty acid oxidation (**figure 3**) can be mediated by the β-oxidation pathway in the mitochondria to generate acetyl-CoA to be oxidized in the TCA cycle (Vanitallie and Nufert, 2003). However, the fate of this metabolite can be diverted to generate aceto-acetyl CoA by the action of 3-keto thiolase (**3-KT**), which is the first step in ketogenesis. Downstream in the chain of reactions, HMG-CoA is generated from aceto-acetyl CoA by mitochondrial HMG-CoA synthase (**mHS**). The enzymatic activity of mHS can be stimulated in humans by starvation and high-fat diets, but reduced by insulin (Serra et al., 1993). HMG-CoA also can be produced from ketogenic amino acids such

as leucine, lysine, and tryptophan in a separate enzymatic process (Laffel, 1999). Subsequently, HMG CoA is cleaved to release AcAc in a step mediated by HMG CoA lyase (**HL**).

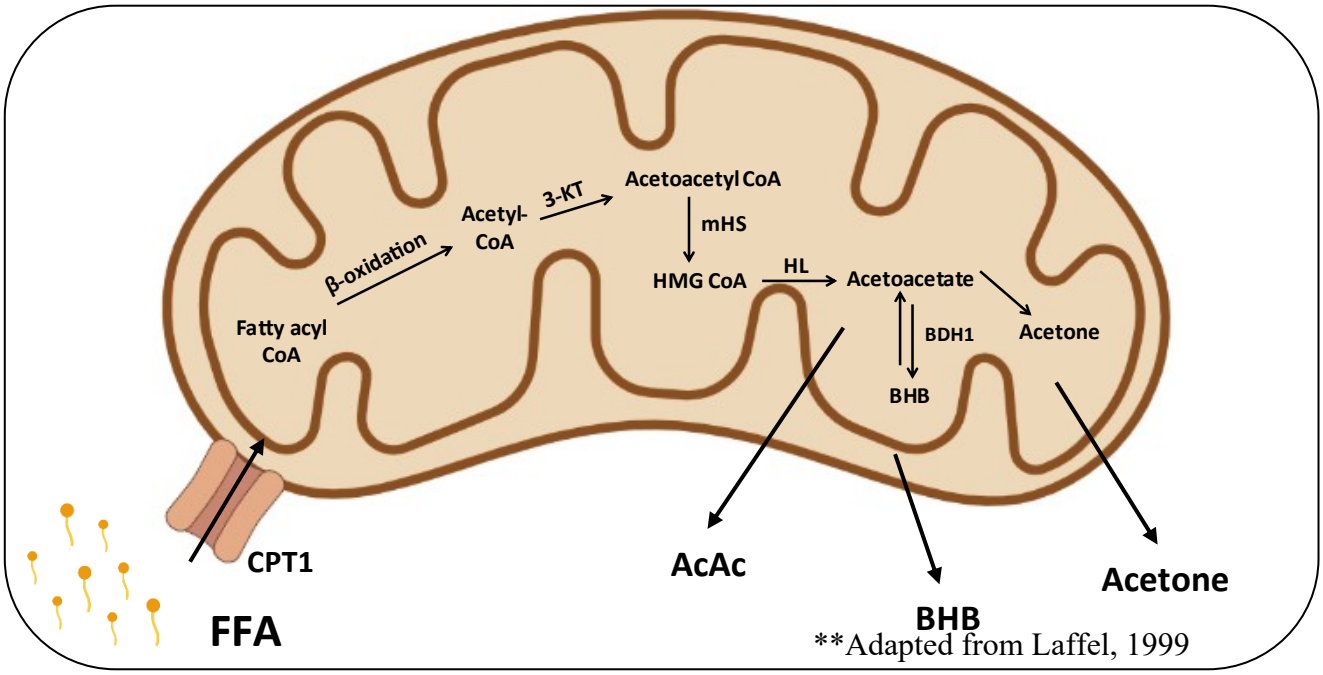


Figure 3. Ketone body synthesis in mitochondria. 3-ketothiolase (3-KT), HMGCoA synthase (mHS), 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA), HMG CoA lyase (HL), β-hydroxy-butyrate dehydrogenase-1 (BDH1).

The AcAc production inside mitochondria can have three fates: (1) enter the bloodstream, (2) being spontaneously decarboxylated into CO_2 and acetone (both fat-soluble molecules defuse out hepatocytes and removed out through ventilation), (3) or be reduced by β-hydroxybutyrate dehydrogenase-1 (**BDH-1**) into BHB (Vanitallie and Nufert, 2003). Ruminant liver, in contrast to non-ruminants, contains 80%-95% of BDH-1 located in the cytosol, whereas, in the rumen epithelial, BDH-1 is 95% located in the mitochondria (Heitmann et al., 1987). The BDH-1 activity modulates the reaction to reduce AcAc to BHB, requiring NADH in the process (Laffel, 1999). Therefore, the ratio BHB to AcAc is dependent on the redox potential of the NADH to NAD^+ ratio (Laffel, 1999) and can be used as a measurement to determine the redox state (Newman and Verdin, 2017). The BHB is a nonvolatile and chemically stable metabolite whose only metabolic fate is the interconversion with AcAc (White & Venkatesh, 2011).

$$(R) - 3 - Hydroxybutyrate + NAD^{+} \rightleftharpoons Acetoacetate + NADH + H^{+}$$

Figure 4. β-hydroxybutyrate dehydrogenase-1 ketone body synthesis reaction.

Ketogenesis is affected by different factors like glucose levels, BHB : AcAc, redox state in hepatic and extrahepatic tissues, NEFA concentration, insulin : glucagon, and enzymatic expression (Bergman, 1971; Heitmann et al., 1987; Newman and Verdin, 2017). The BHB is the most predominant ketone body in all species but, its ratio BHB to AcAc differs between blood and liver and becomes lower as the animal gets more ketotic (Bergman, 1971). In sheep, Bergman & Kon (1964) reported that insulin, glucose, and propionic acid injections reduced AcAc production rates and plasma AcAc concentrations.

1.1.13.2.Ruminal ketogenesis

In the rumen, butyrate uptake is mostly converted to BHB by the epithelial cells before being released into the portal circulation (Weigand et al., 1972; Remond et al., 1995; Baldwin and Jesse, 1996). According to Baldwin et al., (2018), this process is mediated by the butyrate activation of the peroxisome proliferator–activated receptors **(PPAR)** in the epithelial cells of the rumen. This activation causes the metabolization of the butyrate by the acid β-oxidation pathway (Baldwin et al., 2018). The synthesis of ketone bodies in the epithelial cells of the rumen is allowed by the expression of HMGCS2 (Novak et al., 2019), a required enzyme for ketogenesis (Qi et al., 2022). Between 33% to 78% of the rumen's butyrate is converted to ketone bodies (Weigand et al., 1972). Under fed conditions, the gut is accounted as the major source of ketone bodies in ruminants (Heitmann et al., 1987). Seely et al., (2021) reported the peak in the BHB blood concentrations after four hours post-feeding. Nonetheless, during hyperketonemia, hepatic ketogenesis accounts for the bulk of ketone body production, particularly in animals with reduced DMI (Bergman, 1971).

1.1.14. Ketolysis

Most of the tissues can use ketone bodies as an energy source, or in the case of the mammary gland for fat synthesis (Bergman, 1971). However, BHB cannot be used as energy fuel

by the liver (Bergman, 1971). Also, innate immune cells can use ketone bodies as an energy fuel Puchalska et al., 2019). Using mice macrophages Puchalska et al., (2019), reported that these innate cells do not express BHD-1, feature that impedes the BHB ketolysis but not the use of AcAc. Due to the mass covered by the skeletal muscle, in humans, this tissue is considered the organ with the highest fraction of total ketone body metabolism (Balasse & Féry, 1989; Laffel, 1999). It is possible to hypothesize this concept in dairy cows since the skeletal muscle is also the largest organ (Sadri et al., 2023). Using *in vivo* mice models, Hui et al., (2020) determined that skeletal muscle, heart, small intestine, brain, and kidneys are the most BHB substrate consumers under ketogenic conditions.

The rates of BDH-1 utilization and AcAc production are proportional to BDH-1 concentration (Kerner and Hoppel, 2004). The reduction of BHB to AcAc **(figure 4)** also depends on the state of the cellular pool of NAD^+ (Williamson et al., 1967). Under reduced glucose conditions, because the liver is oxidizing free fatty acids, the mitochondrial NADH : NAD^+ matrix ratio increases. This condition generates a positive difference over the NADH : NAD^+ cytosol ratio (Heitmann et al., 1987). Because ruminant liver BDH-1 is expressed mostly cytosolic, the reduced NADH : NAD^+ ratio (higher concentration of NAD^+) favors the AcAc production (Heitmann et al., 1987). In the final steps, AcAc is converted to aceto-acetyl-CoA, by the enzyme succinyl-CoA:3-ketoacid CoA transferase (**SCOT**), and subsequently by methyl aceto-acetyl CoA thiolase (**MAT**). This last step yields the cleavage of the acetyl group from the aceto-acetyl CoA releasing the acetyl CoA (Laffel, 1999). The enzymatic activity of SCOT in mice can be downregulated by AcAc when its concentrations are elevated conditions, a phenomenon responsible for increasing levels of BHB despite constant rates of hepatic ketogenesis (Laffel, 1999).

1.1.15. The regulation of gluconeogenesis by BHB

Through human and mouse models has been determined that BHB inhibits gluconeogenesis (Stubbs et al., 2017). Gluconeogenesis and glucose homeostasis is mainly regulated by insulin and glucagon hormones (Aschenbach et al., 2010; Oh et al., 2013). During the fed state, insulin decreases hepatic gluconeogenesis by activating the AKT Ser/Thr kinase and

subsequent phosphorylation of forkhead box protein O1 **(FOXO1**). This action inhibits the FOXO1 nuclear translocation, resulting in reduced gluconeogenesis (Petersen and Shulman, 2018). On the other hand, during the fasted state, glucagon promotes gluconeogenesis by activating peroxisome proliferator-activated receptor gamma coactivator-1α (**PGC1α**). This last activation interacts downstream with FOXO1, promoting a genomic expression for the enzymatic synthesis of glucose 6-phosphatase (**G6P**) and phosphoenolpyruvate carboxykinase (**PCK1**). These metabolic modulations result in the stimulation of gluconeogenesis in the liver (Petersen and Shulman, 2018). In addition to this regulatory pathway, glucagon also induces dephosphorylation of the class II histone deacetylases (**HDACs**). This dephosphorylation causes the translocation and deacetylation of nuclear FOXO1, thereby beneficiating the promotion of gluconeogenesis (Mihaylova et al., 2011).

The metabolic activity of BHB causes the inhibition of class I and II HDACs (Bradshaw et al., 2020; Hartman & Rho, 2014; B. Li et al., 2021). The HDAC inhibition increases the acetylation of FOXO1, thereby reduces its activity promoting gluconeogenic enzymes in the liver. As a result of this process, BHB is associated with decreasing blood glucose levels (Newman and Verdin, 2017; Bradshaw et al., 2020; Pan et al., 2022b). By inducing hyperketonemia in dairy cows with BHB infusions, Zarrin et al., (2017) reported decreases plasma glucose concentrations. Interestingly, in mice, the reduction in gluconeogenesis occurs only by signaling modulation of BHB, but not by AcAc (Pan et al., 2022b).

Metabolic alterations in dairy cows affected by hyperketonemia

1.1.16. Nutritional immunomodulation

In immune cells, transitioning from an inactive state to an inflammatory and then post-inflammatory state is accompanied by metabolic reprogramming (Bradshaw et al., 2020). The metabolic switch is required to assure the cells have access to adequate energy and redox potential (Bradshaw et al., 2020). Several authors have associated NEB in early lactation, higher NEFA and

BHB, with a higher risk of negative health affections (McArt et al., 2013; Overton et al., 2017). However, as it is discussed by Horst et al., (2021), this connection may fall into inadequacies of rationale because this condition could also reflect normal metabolic adaptations or the metabolic consequences from immune activations. Nevertheless, as was stated by Krebs, (1966), bovine ketosis could be the shift from a "physiological" to a "pathological" state.

The tight connection interplayed between the immune system, nutrition, and metabolism is necessary to balance the interventions mediated to protect the health during disease affections (Bradshaw et al., 2020). The sentinel functions of the immune system not only consist of mechanisms to protect against pathological conditions but also contribute to the regulation of energy supply in the metabolism. Using mice models Loft et al., (2022) revealed how the glucocorticoid receptor (GR) activation in the Kupffer cells (liver macrophage residents) can regulate the ketogenesis in the liver by suppressing the expression of the cytokine TNFα. According to Loft et al., (2022), this condition highlights how a local-derived signal from an immune cell can facilitate a switch from an anabolic to a catabolic condition. The mechanisms in the sentinel function of the immune system are not only balanced by the action of pro- and anti-inflammatory cytokines, but also by the nutritional status (Omenetti and Pizarro, 2015; Okawa et al., 2021). Macrophages inflammatory-related M1 type utilizes mainly glycolysis, while immunosuppressive M2 macrophages use fatty acid oxidization (Kelly and O'Neill, 2015).

1.1.17. Ketone bodies as immunomodulators of NRLP3

As was mentioned before, nutritional metabolic conditions can influence the immune response tone. The BHB, besides being an alternative fuel, also is directly implicated in anti-inflammatory immunomodulation. The activation of the NLRP3, a sensor presented mainly in macrophages (later reviewed in extension) is affected by the nutritional metabolic status. It has been reported that in human and mouse macrophages, BHB, but not AcAc, downregulates the activation of this sensor (Youm et al., 2015; Kim et al., 2020). Kim et al., (2020) reported that in human macrophages after being challenged with LPS *in vitro*, the high glucose conditions favored the pro-inflammatory activation of the NLRP3. However, Kim et al., (2020) not only demonstrated the anti-

inflammatory action of BHB, but they also reported that this BHB effect can be overridden by the action of insulin.

1.1.18. The antioxidant effects of ketone bodies

The BHB has also been associated with anti-oxidative effects. High oxidative stress has been reported in transition dairy cows (Sahoo et al., 2009; Sun et al., 2021) and is associated with inflammation during this period (Sordillo and Mavangira, 2014). During ketogenesis, BHB synthesis results in increasing cytoplasmic NADPH (Kashiwaya et al., 1997). This increase in NADPH favors in the cytoplasm the reactive oxygen species (**ROS**) scavenging. Therefore, the increasing levels of BHB modulate positively the antioxidant mediation (Stubbs et al., 2020a). Another mechanism of cyto-protection to mitigate oxidative stress is mediated by BHB activating the transcription factor Nrf2, and the inhibition of HDACs (Hartman & Rho, 2014; Meroni et al., 2018). This feature induces the gene expression of heme oxygenase-1 (**HO-1**) conferring cellular protection against antioxidative stress (Hartman and Rho, 2014; Meroni et al., 2018). However, Jain & Mcvie, (1999) reported increasing levels of oxygen radicals and lipid peroxidation in human red blood cells cultivated with AcAc, but not with BHB. Interestingly, the presence of BHB together with AcAc, in equivalent proportions, did not influence the AcAc capacity to induce ROS and lipid peroxidation (Jain & Mcvie, 1999).

1.1.19. Ketone bodies and HCAR2-mediated immunomodulation

Hydroxycarboxylic acid receptor 2 (**HCAR2**) also known as G-protein coupled receptor 109A (**GPR109A**) can be activated by BHB (Kyoung Lee et al., 2020). GPR109A possesses the ability to modulate the inflammatory tone reaction by inhibiting the activation of the transcriptional regulator nuclear factor-kappa B (—**NF-κB**—; Thangaraju et al., 2009). The NF-κB is a pro-inflammatory transcription factor that can induce the synthesis of TNFα, IL-6, IL-1β, activation of the inflammasomes, and apoptosis signaling (Bradshaw et al., 2020; Stubbs et al., 2020a). The BHB inhibits the NF-κB expression-mediated inflammation by binding GPR109A presented in

macrophages (Bradshaw et al., 2020). Modulating the immune response after an LPS insult, Swartz et al., (2021a) determined that mouse macrophages continuously exposed to BHB in cell culture were polarized towards an anti-inflammatory tone even after receiving an *S. uberis* challenge. Interestingly, GPR109A activation, mediated by an SCK concentration of BHB (1.2 mM) in bovine neutrophils, modulated an increase in the chemotaxis activities in these cells (Carretta et al., 2020).

1.1.20. Immunomodulation in hyperketonemic dairy cows

Elevated BHB concentration is a frequent feature of early lactation in dairy cows (Overton et al., 2017; Benedet et al., 2019). In recent research Swartz et al., (2021b), implementing BHB infusions in dairy cows, they reported impairment in the immune defenses post mammary LPS insult. In their results, the animals infused with BHB turned out with higher milk bacterial count, reduced DMI, and milk yield. Zarrin et al., (2014) also reported that animals infused with BHB had lower somatic cell counts (**SCC**), diminished influx of leukocytes into milk, and higher levels of IL-10 mRNA post-LPS mammary challenge. Onizawa et al., (2022) reported that peripheral blood mononuclear cells (PBMC) from dairy cattle, cultured *in vitro,* treated with BHB exhibited anti-inflammatory immunomodulation after an LPS insult, while the ones treated with AcAc expressed a pro-inflammatory modulation. Neutrophils isolated from periparturient cows under NEB with elevated NEFA and BHB exhibited reduced chemotaxis and myeloperoxidase activity (Hammon et al., 2006). Hoeben et al., (1997) reported a reduction in the oxidative burst capacity in polymorphonuclear leucocytes (PMNL) from dairy cows after being exposed *in vitro* to SCK metabolic doses. Using a different approach, Grinberg et al., (2008) isolated neutrophils from producing dairy cows and pre-incubated them with BHB. After challenging these cells with *E. coli*, they reported a reduction in the bactericidal capacities because of the diminished phagocytic and extracellular trap capacities.

1.1.21. Inflammation

Inflammation is an adaptive response triggered by noxious stimuli and conditions such as infections, tissue injury, or signals of cellular stress (Medzhitov, 2008). The main task of innate immunity is to generate a protective reaction against pathogen-associated molecular patterns (**PAMPs**) from infectious agents such as bacteria and viruses (Roh and Sohn, 2018). Also, inflammation can be triggered by damaged-associate molecular patterns (**DAMPs**) identified in cellular stress factors (—e.g., reactive oxygen or nitrogen species—; (Goldsby et al., 2001; Roh and Sohn, 2018). These specialized cells will do recognition, scavenging, and amplification of the inflammatory response (Goldsby et al., 2001). The innate immune cells also will present, through their major histocompatibility complex (**MHC**), the antigen structure to the adaptive immune cells. In the case of B cells, their recognition of the antigen will be used to design the unique antibody for future affections, while the T cells will improve the removal capacity of the antigen out the body (Goldsby et al., 2001; Fischer et al., 2010).

1.1.21.1. Cytokines associated with inflammation

Among the pro-inflammatory chemical mediators released by the immune cells are cytokines such as tumor necrosis factor-alpha (**TNFα**) and interleukin one-beta (—**IL-1β**—; Bradford et al., 2015). Cytokine IL-1β has biological functions like leukocytic pyrogen, fever mediator, anorexigenic, and an acute-phase response activator factor (Van Miert, 2011; Kaneko et al., 2019). This cytokine is also a potent inflammatory activator of neutrophils, monocytes, and macrophages which can then produce other pro-inflammatory cytokines (Goldsby et al., 2001; Bolon et al., 2004). Another pyrogenic pro-inflammatory cytokine is TNFα, primarily produced by macrophages (Dinarello, 1999). This cytokine plays a role in fever response, inflammation, anorexia, cell apoptosis, and regulates the switch from ‘‘antigen mode’’ to ‘‘inflammation mode’’ in the T cell differentiation, (Huang et al., 2000; Clark et al., 2005; Van Miert, 2011; You et al., 2021). The TNFα and IL-1β are crucial for the induction of other cytokines (IL-6 and IL-8) and agents such as platelet-activating factor, prostaglandins, leukotrienes, and nitric oxide (Van Miert, 2011).

Both cytokines IL-1β and TNFα have also metabolic modulatory roles. Cytokine IL-1β inhibits glucose transporter GLUT-4 translocation to the plasma membrane, and reduces insulin-stimulated glucose uptake and lipogenesis (Matsuki et al., 2003; Shi et al., 2019). On the other hand, TNFα is a mediator of insulin resistance, a lipolysis stimulator, negative regulation of peroxisome PPAR-γ (a vital insulin-sensitizing nuclear receptor), and an inhibitor of lipoprotein lipase (**LPL**). This cytokine also can inhibit the expression of fatty acid synthase, acetyl-CoA carboxylase (**ACC**), glycerol 3-phosphate dehydrogenase (**GPDH**), and glucose receptor GLUT-4 (Popko et al., 2010; Shi et al., 2019).

1.1.21.2. Acute phase proteins associated with inflammation

Cytokines IL-1β and TNFα along with interferon-γ are the principal mediators in the release of acute phase proteins (APP) from the liver (Gruys et al., 2005; Van Miert, 2011). The APP includes haptoglobin (**Hp**), lipopolysaccharide-binding protein (**LBP**), serum amyloid A (**SAA**), and C-reactive protein (**CRP**) among others (Ramadori and Christ, 1999; Goldsby et al., 2001). In the case of Hp, its synthesis is associated with the expression of CD136 on the cell surface of hepatocytes, Kupffer cells, and tissue macrophages (MacKellar and Vigerust, 2016). The synthesis of Hp is also induced by IL-1β and IL-6, but downregulated by TNF-α, IL-4, and interferon γ (MacKellar and Vigerust, 2016). The Hp is bacteriostatic in nature by its ability to bind free hemoglobin, a condition that causes iron levels to decrease leading to an inhibition of bacterial growth (Eaton et al., 1982).

The LBP is a lipid-binding protein stimulated in response to the presence of LPS. This protein can increase the sensitivity of PPR toll-like receptors (**TLR**) expressing cells (e.g., monocytes, macrophages, dendritic cells) by its affinity to bind the lipopolysaccharide membrane of gram-negative bacteria (Tobias et al., 1989; Schroedl et al., 2001). A key step in the LPB immune mediation is the presentation of the LBP-LPS complex to the CD14 protein (Meng et al., 2021). CD14 protein is expressed on the cell membrane of the innate immune cells and contributes to the recognition by the TLR of the LBP-LPS complex, triggering pro-inflammatory reactions (Meng et al., 2021). The LBP-LPS complex also enhances phagocytosis and clearance of LPS from blood (Le Roy et al., 2001). The SAA is described to bind lipopolysaccharide comparable to LBP

(Schroedl et al., 2001). The SAA influences the immune response by acting as a chemoattractant and mediates the migration of neutrophils and monocytes to the site of infection (Abdallah et al., 2016).

The CRP was the first documented APP and was identified by being bound to the C-terminal polysaccharide of a pneumococci bacteria (Tillett and Francis, 1930). It binds directly to several microorganisms, degenerating cells, and cell remnants (Gruys et al., 2005). The CRP activates the complement system by the classical C1q pathway, and acts as an opsonin (Gruys et al., 2005). The APPs perform different acute phase responses among the different species. Particularly, CRP is a constitutive serum protein in cattle without considerable changes in response to injury while in dogs is the major APP whose concentration can increase 100-fold (Schroedl et al., 2001).

1.1.21.3. Cytokines under kyperketonemic conditions

Hyperketonemic cows can present higher concentrations of IL-6 and TNFα 4 weeks before calving and during the week of the disease (Zhang et al., 2016). Trevisi et al., (2012) found that animals after calving can exhibit a peak in IL-6 concentrations, with higher levels with low liver function index. Using recombinant bovine TNFα, injected subcutaneously, Bradford et al., (2009) detected evidence of alterations in the liver by promoting fatty acid uptake and storage and decreasing fatty acid oxidation. Using a similar approach, Yuan et al., (2013) reported that TNFα injection 7 days postpartum decreased milk yield and components by 15%-18%. (Bradford et al., 2009) also found that injecting TNFα in late lactation dairy cows resulted in a promotion of TAG accumulation in the liver. Hepatic lipidosis in cows is associated with ketosis, anorexia, reduced rumen motility, displaced abomasum, weight loss, predisposition to infections, and diminished fertility (Imhasly et al., 2014). Also, increasing levels of TNF-α is related to lower glucose-6-phosphatase and phosphoenolpyruvate carboxykinase-1 gene expression, two genes required for gluconeogenesis (Bradford et al., 2009).

1.1.21.4. Acute phase proteins under kyperketonemic conditions

Cows under hyperketonemia can exhibit higher concentrations of LBP, Hp, and SAA in serum before parturition and post-calving (Abuajamieh et al., 2016; Zhang et al., 2016). Kováč et al., (2009) established that NEFA and BHB have a significant correlation with Hp, meanwhile, SAA correlates to NEFA levels in dairy cows. The SAA can increase up to 4.2 folds in hyperketonemic cows compared to their healthy counterparts, while Hp can increase between 4 to 6-folds (Abuajamieh et al., 2016). Also, it is being documented that increasing levels of Hp during the first week postpartum is a common risk for dairy cows (Alsemgeest et al., 1994; Dubuc et al., 2010). The settlement of the acute phase reaction is described by SAA raising concentrations prior eight weeks before calving, which continues elevated four weeks post-partum (Zhang et al., 2016). Trevisi et al., (2012) reported not only an increase of Hp in all the cows post-calving but also determined that animals with low liver functional index presented a marked increase. Animals under hyperketonemic conditions also can manifest an increase ranging from 45% to 66% in LBP concentrations compared to healthy cows after calving (Abuajamieh et al., 2016). These results have associated the development of hyperketonemia is tied to systemic inflammation (Abuajamieh et al., 2016; Zhang et al., 2016).

1.1.21.5. Inflammasome and pro-inflammatory signals.

A challenge to the health's homeostasis may elicit an inflammatory response at the local or systemic levels (Medzhitov, 2008). The innate immune system is the mechanism in charge to protect health against signals of danger, including both pathogenic microorganisms and sterile incursions such as metabolic perturbations (Tweedell et al., 2020; Zheng et al., 2020). The recognition of these risk factors can be mediated not only by PRRs like toll-like receptors (e.g., TLR4), but also can be detected by the multiprotein complex called inflammasome (Malik and Kanneganti, 2017; Lebreton et al., 2018; Zheng et al., 2020). Inflammasomes oligomerize to form a platform in response to the sensing DAMPs or PAMPs (Sharma and Kanneganti, 2016; Kelley et al., 2019). Five members of PRRs have been identified as NOD-like receptors (**NLRs**) (Kelley et al., 2019). These sensors after being activated induce the conformation of a multicomplex protein to serve as the platform required to activate caspase-1 protein (Malik and Kanneganti, 2017).

Further, in the chain of events, caspase-1 induces the maturation of IL-1β and IL-18, which will function as amplifiers of the inflammatory cascade (Malik and Kanneganti, 2017; Tweedell et al., 2020). Activated caspase-1 can also cleave gasdermin-D, as a substrate that after being activated forms pores in the cell membrane to execute pyroptosis, a form of cell apoptosis induction (Kelley et al., 2019). The pores in the death cell also serve to spill the IL-1β and IL-18 to continue exerting the inflammatory reaction (Tweedell et al., 2020).

The NLRP3 is a canonical inflammasome composed of a tripartite protein confirmed by an amino-terminal pyrin domain (**PYD**), a central nucleotide-binding and oligomerization domain (**NOD**), and a C-terminal leucine-rich repeat (**LRR**) domain (Franchi et al., 2009). The main function of NLRP3 inflammasome is the maturation and the production of IL-1β via toll-like receptor-nuclear factor kappa (Lebreton et al., 2018). The NLRP3 inflammasome can be assembled in response to a vast range of distress signals like microbial cell wall components, nucleic acids, pore-forming toxins, and endogenous danger signals like SAA, ATP, and uric acid crystals (Liang et al., 2021; Sharma & Kanneganti, 2016). Cholesterol crystals, palmitic acid, and oxidized low-density lipoprotein also are known to trigger NLRP3 in mouse primary macrophages and human macrophage cell lines (Liang et al., 2021). NLRP3 activation has been connected to multiple classes of lipids, including saturated fatty acids (SFAs), sphingolipids, ceramides, and bile acids (Liang et al., 2021; Vandanmagsar et al., 2011). Nevertheless, monounsaturated and polyunsaturated fatty acids can act as NLRP3 inhibitors in mice (Finucane et al., 2015; Shen et al., 2017).

The NLRP3 inflammasome is also an important mediator of diseases linked to insulin resistance (Liang et al., 2021). In diet and genetically induced obese mice Stienstra et al., (2010) found increased levels of caspase-1 and IL-1β activity in the adipose tissue. They also reported that NLRP3 knockout in mice generated higher metabolic activity in the adipose cells. On the other hand, they also reported that while using a caspase-1 antagonist, the animals presented higher insulin sensitivity and fat oxidation capacities. Elevated mRNA expression of NLRP3 also correlates with insulin resistance in human adipose tissue and with non-alcoholic fatty liver disease (**NAFLD**) progression in humans (Vandanmagsar et al., 2011). The NLRP3 activation by lipids is slow, requiring between 6 and 24 hours to reach maximal activity in mouse primary macrophages and human THP-1 macrophage cell lines (Liang et al., 2021).

1.1.22. Inflammasome signaling under hyperketonemic conditions

Onizawa et al., (2022) studied the effect of BHB and AcAc over the NLRP3 inflammasome in dairy cow peripheral blood mononuclear cells **(PBMC)**. They reported that the cells primmed with LPS and treated with AcAc resulted in increasing pro-inflammatory expression of NLRP3 and IL-1β. However, when these cells were treated with BHB, the pro-inflammatory expression was suppressed. These results are in accordance with the results reported by Jain et al., (2003) who incubated U937 monocytes (human immune cells line) with AcAc and BHB. They reported that exogenous addition of AcAc, but not BHB, increases IL-6 secretion and ROS generation.

Shen et al., (2019) found that dairy cows under hyperketonemia exhibit a state of systemic inflammation. They detected in the first week after calving of those cows elevated BHB, higher TAG accumulation in the liver, higher expresion of pro-inflammatory cytokines (IL-18, IL-1β, and TNFα), and lower levels of interleukin 10 **(IL-10)**. Shen et al., (2019) also reported that hyperketonemic cows expressed higher mRNA abundance of NLRP3 and caspase-1 along with higher caspase-1 activity in the liver. The AcAc cultured bovine hepatocytes caused an increased the secretion of IL-1β, IL-6, and TNFα in a dose-dependent manner (Li et al., 2016). The inflammasome is not constitutively active in macrophages and requires a stimulant (DAMP or PAMP) plus the specific efflux of potassium to trigger the protein complex activation (Gattorno et al., 2007). Youm et al., (2015) reported that BHB inhibition of the NLRP3 activity can be mediated mechanistically by preventing the K^+ efflux and protein complex oligomerization. Rahman et al., (2014) concluded that BHB can polarize neutrophils and macrophages to an anti-inflammatory state after bounding the receptor GPR109A in mice (Lukasova et al., 2011).

Behavioral indicators in dairy cows

1.1.23. Feed intake in hyperketonemic dairy cows

In dairy cows feed intake is dramatically reduced before parturition and requires several weeks to reach positive energy balance in high-yielding animals (Bauman and Bruce Currie, 1980; Bell, 1995; de Vries and Veerkamp, 2000; Pérez-Báez et al., 2019). In addition to this negative condition, before and after parturition, animals suffering hyperketonemia, are also impacted by a reduction in the DMI (Duffield et al., 2009; Goldhawk et al., 2009; Zhang et al., 2016; Sun et al., 2021). Goldhawk et al., (2009) determined that animals under SCK exhibited an alteration in their feeding behavior with fewer visits and less time spent compared to the healthy animals. Pérez-Báez et al., (2019) also determined that in the last three days before calving, for each 0.1% point decreased in DMI, and for every Mcal decreased, the odds of having ketosis increased by 8% and 5%, respectively. Goldhawk et al., (2009) reported that for every ten minutes decreased in the time spent at the feeder during one week before calving, the odds to develop SCK increased 1,9 times, meanwhile for every kg reduced in DMI, the odds to develop SCK increased 2,2 times.

However, Zarrin et al., (2013) using continuous intravenous infusion of BHB did not impact the DMI in the mid-lactating dairy cows. Rico et al., (2021) using calcium butyrate as a ketogenic precursor reported a reduction in the DMI until BHB reached 5 mM blood levels.

1.1.23.1. Appetite under hyperketonemic conditions.

The reduction in DMI, energy imbalance, and high levels of BHB early in lactation are the key features of transitioning dairy cows. The improvement in the feed intake of cows at the onset of lactation is a crucial step to decrease the risk of metabolic disorders. However, the mechanisms which cause dysregulation in the energy balance during the transition period are not clearly understood (Kuhla, 2020; Brown and Bradford, 2021).

Appetite and satiety are in control over the central nervous system by signals from the central and peripheral nervous systems converging to regulate food intake and energy expenditure (Bradford et al., 2022; Masi et al., 2022). Because hunger and satiety are influenced by nutrients, it has been hypothesized that hyperketonemic conditions (humans and rodents) could have a specific role in the regulation of appetite (Masi et al., 2022). In rodents, Langhans et al., (1983) reported that a subcutaneous BHB infusion generates an acute and sustained decrease in food intake, but not when they were injected with AcAc. Also, an infusion of BHB in the third ventricle of rodents' brain cause a reduction in appetite (Davis et al., 1981). In humans, the use of exogenous sources of BHB has been associated with lower appetite, delaying hunger and the desire to eat (Stubbs et al., 2018).

Roekenes & Martins, (2021) mentioned that studies aimed to measure hunger feelings in humans through different questionary methods have demonstrated that ketogenic diets are associated with the diminished or absent feeling of hunger, reduced desire to eat, and decreased appetite. The anorexigenic effect of BHB has been associated with several hypotheses, but the reduction in ghrelin levels is the most likely explanation up to date (Stubbs et al., 2018; Deemer et al., 2020; Masi et al., 2022). Nevertheless, the mechanism that leads to appetite suppression under ketogenic conditions remains to be elucidated (Deemer et al., 2020; Roekenes and Martins, 2021; Masi et al., 2022).

1.1.24. Milk production of hyperketonemic dairy cows.

The association of hyperketonemia with milk production is a paradox unresolved. Several studies have reported reductions in milk production associated with hyperketonemia (Duffield et al., 2009; McArt et al., 2013; Zhang et al., 2016; Sun et al., 2021). However, this is an inconsistent result since several research have reported increasing milk yields in hyperketonemic animals (Vanholder et al., 2015; Belay et al., 2017; Rathbun et al., 2017; Piñeiro et al., 2019). The ramping NEFA flux in response to the NEB is associated not only with hepatic lipidosis but also with a reduction in milk production and changes in the composition (de Vries and Veerkamp, 2000; McArt et al., 2014; Kayano and Kataoka, 2015; White, 2015; Rodriguez et al., 2022). Pralle et al., (2021)

reported that hyperketonemic animals not only had a reduction in milk production and protein concentrations, but also increased fat concentrations and somatic cell count. The NEB is a risk factor that increases its negative impact with the number of lactations (Ospina et al., 2010b; a; Chapinal et al., 2012a; Kayano and Kataoka, 2015; Santschi et al., 2016). Also Rodriguez et al., (2022a) reported that the negative affection is associated with the week of detection. They reported that only the animals detected with hyperketonemia during the first-week post-calving had a reduction in the milk yield but not during the second week. Vanholder et al., (2015) suggested that the higher milk-yielding cows observed in hyperketonemic may result because of the higher energy demand for milk production.

The reduction in the milk production in hyperketonemic cows could be associated to high oxidative stress in their mammary gland. Sun et al., 2021) reported high oxidative stress and pro-inflammatory conditions in the mammary epithelium of hyperketonemic dairy cows. According to (Sun et al., 2021), cows facing hyperketonemia displayed activation of NF-κB signaling, NLRP3 inflammasome, and the Bcl-2/caspase 9 apoptotic pathway. Also, they reported greater proinflammatory cytokines (TNF-α, IL-6, and IL-1β) in the mammary gland. These relevant findings complement previous reports where hyperketonemic cows exhibit systemic oxidative stress (Sahoo et al., 2009). Oxidative stress in high-yielding dairy cows causes inflammation of the mammary gland and can cause a reduction of milk yield and negative effects on the milk composition (Polawska et al., 2012).

Chapter 2: The induction of nutritional ketosis in lactating dairy cows using calcium butyrate: effects on lactation performance and health

Introduction

Ketones are a secondary fuel synthesized in the rumen and liver in dairy cows (Krebs, 1966; Remond et al., 1995). During prolonged glucose shortages, ketone bodies fuel vital organs like the brain and heart (Hui et al., 2020b), the epithelial cells of the rumen's walls (Krehbiel et al., 1992a; Remond et al., 1995), and sums to the pool of substrates used in milk lipogenesis (Bionaz et al., 2020). Ruminal ketogenesis is a fed state condition resulting from the conversion of butyrate into ketones bodies by the epithelial cells of the rumen's walls (Krehbiel et al., 1992b; Remond et al., 1995). On the contrary, liver ketogenesis is a homeostatic mechanism triggered to support the lack of energy under prolonged negative energy balance (Bergman, 1971; Bauman and Bruce Currie, 1980). Liver ketogenesis in dairy cows results from the partial lipid oxidation during high-fat fluxes and depleted glucose conditions (Baird et al., 1968; Bergman, 1971; Laffel, 1999).

In addition to their roles as energy substrates, research in non-ruminants suggests that ketones of dietary origin may exert health-promoting effects (Shaw et al., 2019; Poff et al., 2020; Yurista et al., 2021). Mammalian studies providing exogenous β-hydroxybutyrate (BHB) have found a pleiotropy in the metabolic effects such as anti-inflammatory (Youm et al., 2015; Yamanashi et al., 2017; Kim et al., 2020; Thio et al., 2022), antioxidant (Haces et al., 2008; Meroni et al., 2018; Stubbs et al., 2020a), lipolysis attenuation (Taggart et al., 2005), muscle glucose uptake reduction (Maizels et al., 1977; Yamada et al., 2010), insulin sensitization (Park et al., 2011; Myette-Côté et al., 2018), and the reduction of hepatic lipid accumulation reduction and reversal of inflammation-driven kidney damage (Anders et al., 2018; Lee et al., 2020). Despite these metabolite attributes, at the farm level, ramping levels of BHB in transitioning cows are considered a proxy for reduced fertility and milk production, as well as the development of metabolic disorders and infectious diseases (Chapinal et al., 2011, 2012a; McArt et al., 2012a, 2013; Roberts et al.,

2012; Suthar et al., 2013; Abuajamieh et al., 2016). In practice, BHB is currently used as a major predictor of poor performance and impaired health in transitioning dairy cows (Overton et al., 2017). Nevertheless, its usefulness as a marker is limited, as evidence by the inability of BHB to consistently predict negative outcomes (—i.e, no correlation or inverse association—; LeBlanc, 2015; Vanholder et al., 2015; Rodriguez et al., 2022). The observational entanglement of ketosis with other concurrent peripartal metabolic conditions (e.g., hyperlipidemia, fatty liver, hypocalcemia) undermines our ability to fully understand the roles of ketones on metabolism and health.

Different *in vitro* studies point to BHB as an immunosuppressant factor at the cellular level (Grinberg et al., 2008; Swartz et al., 2021a; Dong et al., 2022). However, the lack of *in vivo* models to study BHB role *per se* has been the major obstacle in studying hyperketonemia (Drackley et al., 1991). Previous studies using a ketogenic precursor in combination with feed restriction proved to effectively induce ketogenesis (Mills et al., 1986; Drackley et al., 1991) but failed to isolate BHB as the causal factor. Recent randomized controlled experimentation achieved the dissection of hyperketonemia out of the peripartal homeorhetic adaptations (Zarrin et al., 2013, 2014; Swartz et al., 2021b). In their research, negative trajectories in health and productivity were reported when hyperketonemia was combined with an immune challenge, but not under hyperketonemic status. These conditions suggest the necessity of external cofactors other than ketones to cause an affection in the cow's performance and health. By such circumstances, a window of the speculation opens the possibility to explore the benefits associated to ketogenic diets observed in humans to the dairy cows.

Nutritional ketosis (NK) is defined as a nutritionally induced metabolic state resulting in BHB increasing concentrations regardless of the endogenous or exogenous induction (Poff et al., 2020; Shaw et al., 2020). At the rumen epithelial cell level, butyrate is a common substrate for ketogenesis, accounting for most BHB levels under non-negative energy balance conditions (Krehbiel et al., 1992a; Remond et al., 1995). Based on this principle, our group has recently evaluated the effects of intraruminal infusion of butyrate boluses in lactating dairy cows (Rico et al., 2021; Ruiz-González et al., 2022), and effectively increased BHB concentrations to the levels commonly associated with subclinical and clinical ketosis (McArt et al., 2011). Such models may

be a useful alternative to currently available ones, as they overcome the limitations that confounding factors (e.g., negative energy balance and fatty liver) pose to the specific study of ketosis and hyperketonemia. Following a continuous progression into practical use, the necessity to evaluate the induction of nutritional ketosis and its effects has emerged. Therefore, herein we aim to induce ketosis using a butyrate-based ketogenic diet, and to evaluate the implications of acutely inducing nutritional ketosis on dairy cow performance, milk quality, and acute markers of health.

Materials and methods

2.1.1. Animals, feeding, and husbandry

The experiment was conducted at University of Maryland Central Maryland Research and Education Center (CMREC) dairy farm facility in Clarksville, MD. All procedures involving the use of animals were approved in accordance with the regulations and guidelines established by the University of Maryland Animal Care and Use Committee (Protocol 1830894-3). Six, mid-lactation (128 ± 52 DIM), multiparous (2.8 ± 0.9 parity) Holstein cows (n = 6) were enrolled in this study. Animals were moved to a tied stall barn 2 weeks before the beginning of the experiment for proper acclimation to feeding and housing conditions. Cows were weighed (615.9 ± 86.5 kg) and body condition score (BCS) was assessed (3.0 ± 0.3; scale, 1-5) at the end of the acclimation period. Cows were milked twice daily at 630 and 1530 h, and were fed at 110% of nutrient recommendations once daily (830 h) **(Table 1)**. During the period of housing, the tie-stall barn was continuously illuminated, and water was offered *ad libitum*.

Blood samples (10 mL) were collected by coccygeal venipuncture at 0, 2, 4, 8, 10, 12, 16, 24, and 48 h related to the first bolus. Samples collected at h 0, 24, and 48 were taken before feeding the cows. Blood β-hydroxybutyrate (BHB) levels were measured with a ketone strip handheld meter (Precision Xtra, Abbott Laboratories, Abbott Park, IL). Blood was kept on ice for 30 min until centrifugation at 2,800 *g* for 15 min at 4°C. Plasma containing EDTA was removed, immediately snap-frozen in dry ice, and stored at -80°C until being analyzed. Plasma

was analyzed for glucose (Wako Chemicals, Richmond, VA) and NEFAs (Wako Chemicals). Intra-and interassay coefficient of variation (CV) were 4.1% and 4.2%, 2.8% and 3.4% for glucose and NEFA, respectively.

Table 1. Ingredient and nutrient composition of treatment diets.

Ingredient (% of DM)	**Amount**
Corn silage	32.7%
Triticale silage	8.6%
Alfalfa haylage	9.3%
Ground corn	23.3%
Grain mix[1]	17.3%
Soybean meal	8.3%
Fat supplement[2]	0.4%
Chemical composition (% of DM)	
Dry matter (%)	40.9
Crude protein	15.4
Acid detergent fiber	0.8
Amylase-treated neutral detergent fiber	1.1
Lignin	19.2
Crude fat	33.4
Ash	3.0

[1]UMD CRMEC grain mix is composed of crude protein 29.7%, NDF 24.0%, digestible neutral detergent fiber 18.3%, rumen digestible starch 3.12%, rumen soluble sugar 2.32%, fat, 4.0%, ash 20.6%, calcium 2.7%, phosphorus 0.3%, magnesium 1.1%, potassium 0.9%, sulfur 0.5%, sodium 2.6%, chloride 1.6%, Met, 4.6%, Lys, 8.3%, manganese 182.133 mg/kg, zinc 182.1 mg/kg, copper 70.8 mg/kg, iron 225.7 mg/kg, selenium 1.81 mg/kg, vitamin A 30.5 IU/g, vitamin E 239.5 iu/kg, monensin 59.6 g/ton (Cargill Animal Nutrition, Hagerstown, MD).

[2] Megalac ® (Church & Dwight Co., Inc., Princeton, NJ). Blood sampling and plasma analyses

2.1.2. Clinical Outcomes

Feed intake was recorded daily subtracting the refusals from daily feeding TMR. Milk yield (MY) was recorded at the parlor at each milking and summed by day. Milk quality (MQ) was evaluated by collecting milk samples at every milking and mixed in a tube provided with a preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA). Samples were stored at 4°C and inverted twice a day. All samples were shipped to a commercial laboratory (Lancaster DHIA Laboratory, Manheim, PA) to quantify protein, fat, lactose, MUN, and somatic cell count (SCC). Two days before the calcium butyrate bolus feeding started, DMI, MY, and MQ were measured to be used as covariates in the statistical analysis. During the period of study, rectal temperature (RT) and respiration rates (RR) were recorded within 1h post-CB1 and CB2. The pain score was estimated using Gleerup et al., (2015) method. Briefly described, this score calculates pain conditions by considering attention towards the surroundings, head position, ear position, facial expression, response to approach, and back position of the dairy cow.

2.1.3. Calculations

Energy-corrected milk (ECM) was calculated as described by (Hall, 2023) where ECM (kg/d) = (0.25 x kg milk) + (12.2 x kg fat) + (7.7 x kg protein). 3.5% fat-corrected milk (FCM) was calculated as: 3.5% FCM (kg/d) = (0.432 x kg milk) + (16.23 x kg fat) as described by (Linn, 2006). Feed efficiency was calculated as: daily milk (kg) / DMI (kg). Fat-corrected milk efficiency was estimated as: FCM (kg) / DMI (kg). Energy-corrected milk efficiency was calculated as: ECM (kg) / DMI (DMI). To calculate pain, all pain scores were transformed into categorical binary data using a pain score of 3 as the cut-off point suggested by Gleerup et al., (2015) as the painful condition for a dairy cow.

2.1.4. Statistical analyses

All data were analyzed with SAS (version 9.3; SAS Institute Inc., Cary, NC). Analysis of repeated measures for continuous variables was performed with the use of PROC-MIXED. The model included the random effect of cow, the fixed effects of time relative to boluses, period, treatment, and their interaction. Covariance structures for modeling repeated autoregressive one (AR-1), unstructured (UN), variable components (VC), compound symmetry (CS), and Toepliz (TOEP) were assigned and selected by the lowest Goodness-of-fit test results. All data collected prior to the period of evaluations were averaged and tested as a covariate to account for individual differences between cows. Backward elimination was used to remove non-significant interactions when values in the model resulted in $P > 0.25$. Treatment, time, time × treatment, and covariate were used in the model regardless of significance. In all models, residuals were assessed for normality and outliers (PROC UNIVARIATE). Least square means (LSM) test was used to calculate differences between the effects on the model. All results are expressed as LSM and their standard errors unless stated otherwise. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Results

2.1.5. Nutritional ketosis and BHB blood levels

The nutritional ketogenic effect of calcium butyrate resulted in a significant increase in blood BHB levels ($P = 0.03$). The CaBu-treated group blood BHB registered a higher concentration with a difference of 0.2 ± 0.03 mM compared to Control (**figure 5A,** $P = 0.03$). The CaBu-treated animals reached peaks in the BHB levels 4 h post-CB1 (1.1 ± 0.1, mM, $P = 0.01$) and 8 h post-CB2 (0.9 ± 0.1 mM, $P = 0.05$). During the period of study, no differences were detected in the plasma glucose concentrations between the treatments (**figure 5B** $P = 0.32$). Plasma glucose concentrations were 65.0 ± 1.7 mg/dL and 67.6 ± 1.7 mg/dL for CaBu and Control, respectively (**figure 5C**). Plasma NEFAs concentrations did not

differ between the groups (**figure 5D**, $P = 0.70$) and were 76.1 ± 6.8 µmol and 74.7 ± 6.8 µmol for CaBu and Control, respectively (**figure 5E)**.

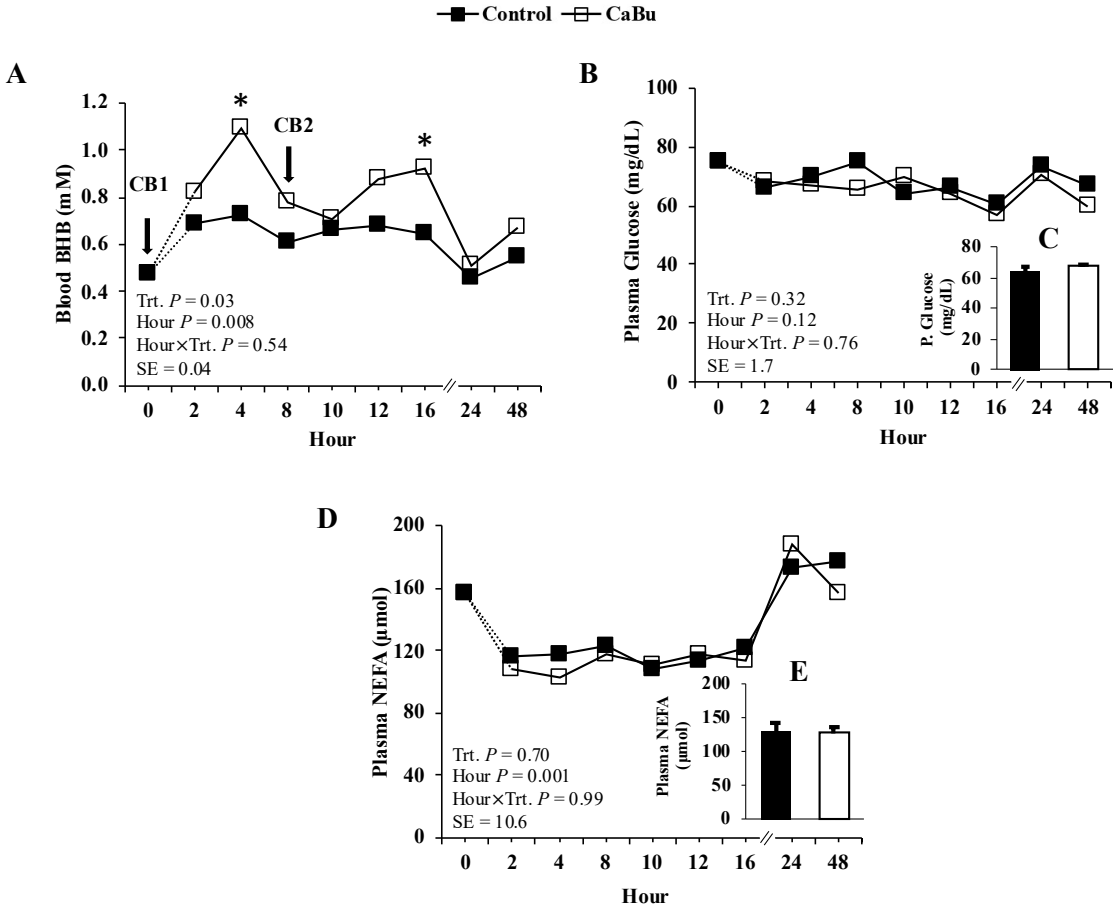


Figure 5. Nutritional ketosis is stimulated by feeding calcium butyrate. **(A)** Blood-BHB concentrations, **(B)** Plasma glucose, **(C)** Plasma glucose overall treatment effect, **(D)** Plasma NEFA, **(E)** Plasma NEFA overall treatment effect. At hours 0 and 8, calcium butyrate bolus (4%DMI) CB1 and CB2 were fed, respectively. The calcium butyrate dose was calculated daily after calculating the previous day DMI. Measurements at hours 24 and 48 were taken before feeding the cows. Data presented as LSM. CaBu, Calcium Butyrate; SE, Standard error; Trt., Treatment; (*) *P*-value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

2.1.6. Feed intake, milk production, feed efficiency, and milk quality.

The CaBu-group had -2.9 ± 1.1 kg/d of DMI ($P = 0.01$) compared to Control. The DMI was 23.3 ± 0.7 kg/d and 26.2 ± 0.8 kg/d for CaBu and Control, respectively (**figure 6A**). No

differences were detected between the groups in milk production, adjusted 3.5%FCM or ECM (**figure 6B**, **6C, 6D**, $P > 0.25$). All calculated energy efficiency resulted higher for the cows under nutritional ketosis ($P < 0.05$). Energy efficiencies were 1.7 ± 0.05 and 1.5 ± 0.05, 1.8 ± 0.06 and 1.6 ± 0.06, 1.6 ± 0.05 and 1.4 ± 0.05 for CaBu and Control in Milk /DMI, 3.5%FCM/DMI, and ECM/DMI respectively. (**figure 6E**, **6F, 6G**, $P < 0.05$).

No differences were detected in milk components between groups (**Table 2**; $P > 0.15$), but a significant difference of 0.9 ± 0.25 of higher somatic cell score (—SCS—) was detected in the CaBu group ($P = 0.01$).

Table 2. Milk composition of Holstein cows receiving ketogenic precursor calcium butyrate (CaBu) or un-supplemented (Control).

	LSM			*P* value				
Variable	**CaBu**	**Control**	**SEM**	**Treatment**	**Period**	**Day**	**Trt.×Day**	**Cov.**
Fat (Kg)	1.54	1.54	0.02	0.95	<0.01	0.64	0.86	<0.01
Fat (%)	4.03	3.94	0.11	0.16	<0.01	0.38	0.69	0.27
Protein (Kg)	1.19	1.22	0.02	0.31	0.35	0.44	0.60	<0.05
Protein (%)	3.13	3.14	0.08	0.77	<0.01	0.06	0.59	0.99
Lactose (Kg)	1.86	1.91	0.02	0.22	0.57	0.28	0.66	<0.01
Lactose (%)	4.86	4.88	0.03	0.16	<0.05	0.07	0.14	0.42
SCS	16.4	15.5	0.40	<0.05	<0.05	0.91	0.30	<0.05
MUN (mg/dL)	10.6	10.5	0.28	0.67	<0.01	<0.01	0.49	0.21

*SCS. Somatic cell score.

**MUN. Milk urea nitrogen.

2.1.7. Health parameters.

No differences were detected in RR and RT between groups ($P > 0.30$). The RR were 36.9 ± 1.9 and 39.7 ± 1.9 breaths per minute (BPM) for CaBu and Control, respectively. The RT were 100.6 ± 0.1 and 100.6 ± 0.1 °F for CaBu and Control, respectively. None of the records detected painful results after Gleerup et al., (2015) methodology was followed.

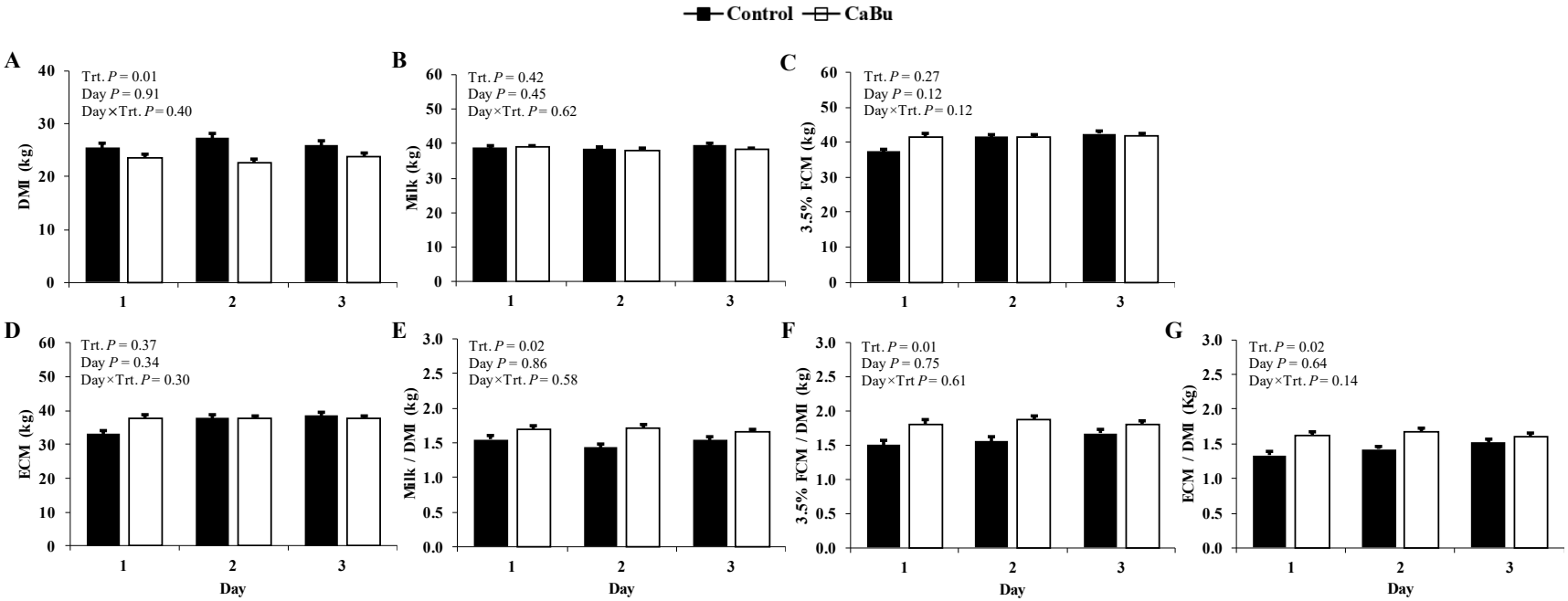


Figure 6. Nutritional ketosis effect on dairy cow's performance. **(A)** Dry matter intake (DMI), **(B)** Milk production, **(C)** 3.5% fact-corrected milk (3.5%FCM), **(D)** Energy corrected milk, **(E)** Feed efficiency milk over DMI, **(F)** Feed efficiency 3.5%FCM over DMI, **(G)** Feed efficiency ECM over DMI. 3.5%FCM was calculated as 3.5% FCM (kg/d) = (0.432 x kg milk) + (16.23 x kg fat). ECM was calculated as ECM (kg/d) = (0.25 x kg milk) + (12.2 x kg fat) + (7.7 x kg protein) Data presented as LSM. CaBu, Calcium Butyrate treatment; SE, Standard error; Trt., Treatment; (*) P -value <0.05, (†) P-value $0.05 < P \leq 0.10$.

Discussion

The hyperketonemia mediated by high BHB concentration, and the sick health status of ketosis are two distinct conditions. Several observational studies have associated high BHB concentrations with reduced productivity and impaired health in dairy cows (Overton et al., 2017). However, emerging knowledge supports BHB as a beneficial metabolite to improve energy use (—i.e., marathons—; Poff et al., 2020), and as a therapeutic treatment of diseases associated with inflammation and oxidative stress (—i.e., alzheimer, epilepsy—; Zhu et al., 2022). Under this dichotomy, our study proposed the use of nutritional ketosis as a model to test, in controlled randomized conditions, the acute effects of increasing BHB levels in mid-lactating dairy cows. The supplementation of calcium butyrate promoted the ketogenic action of butyrate on the epithelial cells of the rumen's walls (Remond et al., 1995) increasing the BHB concentration. This dietary modification allowed us to modulate the expected nutritional ketosis (NK). The hallmark of NK in humans is set in a range of plasma ketone levels between 0.5 and 3.0 mM (Athinarayanan et al., 2019). However, for dairy cows, to the best of our knowledge, there is not a parameter established for nutritional ketosis. Rather, all thresholds are set to predict only negative outcomes (—>1.2 mM—; McArt et al., 2012a).

Ketosis disease in dairy cows is also categorized in an analog comparison to *diabetes mellitus* types (Guliński, 2021). Ketosis type I describes a glucose shortage, hypoinsulinemia, and homeostatic conditions of active lipolysis and ketogenesis granting the spare of glucose for milk synthesis (Zhang and Ametaj, 2020). Meanwhile, ketosis type II disease is concurrent with fatty liver disease, featured by hyperglycemia, hyperinsulinemia, and some degree of insulin resistance (Zhang and Ametaj, 2020). Nevertheless, none of these categories considers ruminal ketogenesis and its increasing BHB levels as part of a pathological state. Not having recorded impaired health, nervous ketone symptoms, painful conditions, or acute reduction in milk production confirms our expected no detrimental effects associated with the increasing BHB concentration recorded.

Reduced glucose concentrations are associated with nutritional ketosis in humans (Poff et al., 2020) and is likely explained by the action of BHB on gluconeogenesis (Stubbs et al., 2017). Nevertheless, we did not detect any difference between the treatments despite of the increasing BHB levels. Different studies have reported that increasing butyrate either intravenous, ruminal, abomasal infused, or diet-supplemented can cause a reduction in the glucose levels (Herrick et al., 2017, 2018; Urrutia et al., 2019; Engelking et al., 2022), nevertheless, this is not consistent result (Herrick et al., 2018; Izumi et al., 2019). Demigné et al., (1986) reported *in vitro* the inhibitory effect of butyrate on the propionate uptake in the liver. Propionate is the major glucose regulator in the cows' metabolism (Aschenbach et al., 2010), therefore its reduced liver uptake it is expected to directly impact glucose concentrations. However, in our conditions, the net butyrate intake and the BHB levels, thereby their modulatory effect on the glucose concentration.

Lipolysis can be inhibited through the AMPK pathway by BHB agonist action on the free fatty acid receptor GPR109A (Taggart et al., 2005; Lee et al., 2020). Nevertheless, plasma NEFA concentrations were not altered by our ketogenic diet. NEFA concentrations also can also be influenced by butyrate supplementation. Inconsistent results in butyrate research have reported negative or no changes in NEFA concentration in either diet-supplemented or ruminally infused evaluations (Herrick et al., 2017, 2018; Izumi et al., 2019; Urrutia et al., 2019). So, the interaction of butyrate with lipid metabolism modulation in dairy cows remains unclear (Engelking et al., 2022) and future studies are required to better understand the implications of nutritional ketosis in plasma lipid activity.

Ketogenic diets in mammals also associate the increasing levels of BHB with reduced appetite (Davis et al., 1981; Langhans et al., 1983; Stubbs et al., 2018). Roekenes & Martins, (2021) reported fewer hunger feelings, reduced desire to eat, and decreased appetite from humans under nutritional ketosis. Although we recorded increasing levels of BHB and a reduction in DMI, Urrutia et al., (2019), did not observe the same effect in DMI after fed calcium butyrate at 2.5% DM and reported 0.9 mM plasma BHB concentration. The reduction in DMI could also be explained by the low palatability link to butyrate (Rook

et al., 1965; Bedford and Gong, 2018; Urrutia et al., 2019). Izumi et al., (2019) and Engelking et al., (2022) did not report effects on DMI when calcium butyrate was supplemented at doses < 2.0% DM. Therefore, it is possible to associate our higher dose with implications in the TMR palatability.

Another factor to be considered in the reduction of DMI is the higher energy density supplemented in non-isocaloric conditions by our ketogenic diet. The increasing energy supplementation sourced from butyrate could have acted as a satiety signal in the regulation of food intake (Simkins et al., 1965). However, the reduced DMI did not affected milk production, therefore higher energy efficiencies were calculated in the CaBu-treated group. In this regard, the modulatory effect mediated by the higher energy provided by our ketogenic diet most likely affected the cow's satiety and therefore DMI. Even though the non-isocaloric condition is an undesirable design, it was a necessary step to evaluate the capacities of our model to increase BHB.

The milk production responses from butyrate infusion studies vary (Rook et al., 1965; Herrick et al., 2018). In this study, the unaffected milk production could have been influenced by the use of mid-lactating cows. Conditions, like expected positive energy balance and non-limiting glucose for milk synthesis could have confounded the increasing butyrate effects on milk yield (Huhtanen et al., 1993). However, milk fat is component commonly reported higher in hyperketonemic ruminants (Lisuzzo et al., 2022; Rodriguez et al., 2022) and in cows ruminally infused with butyrate (Rook et al., 1965; Huhtanen et al., 1993). But, we did not registered this change. Inconsistent results in milk fat have been reported when butyrate is part of the TMR (Izumi et al., 2019; Urrutia et al., 2019), suggesting a possible link to the method of supplementation. However, the increased energy efficiencies in the cows under nutritional ketosis most likely explain the unaltered milk fat in this study. The non-isocaloric ketogenic diet with a higher energy density might have supplemented the requirements to maintain milk yield, and quality despite the reduced DMI.

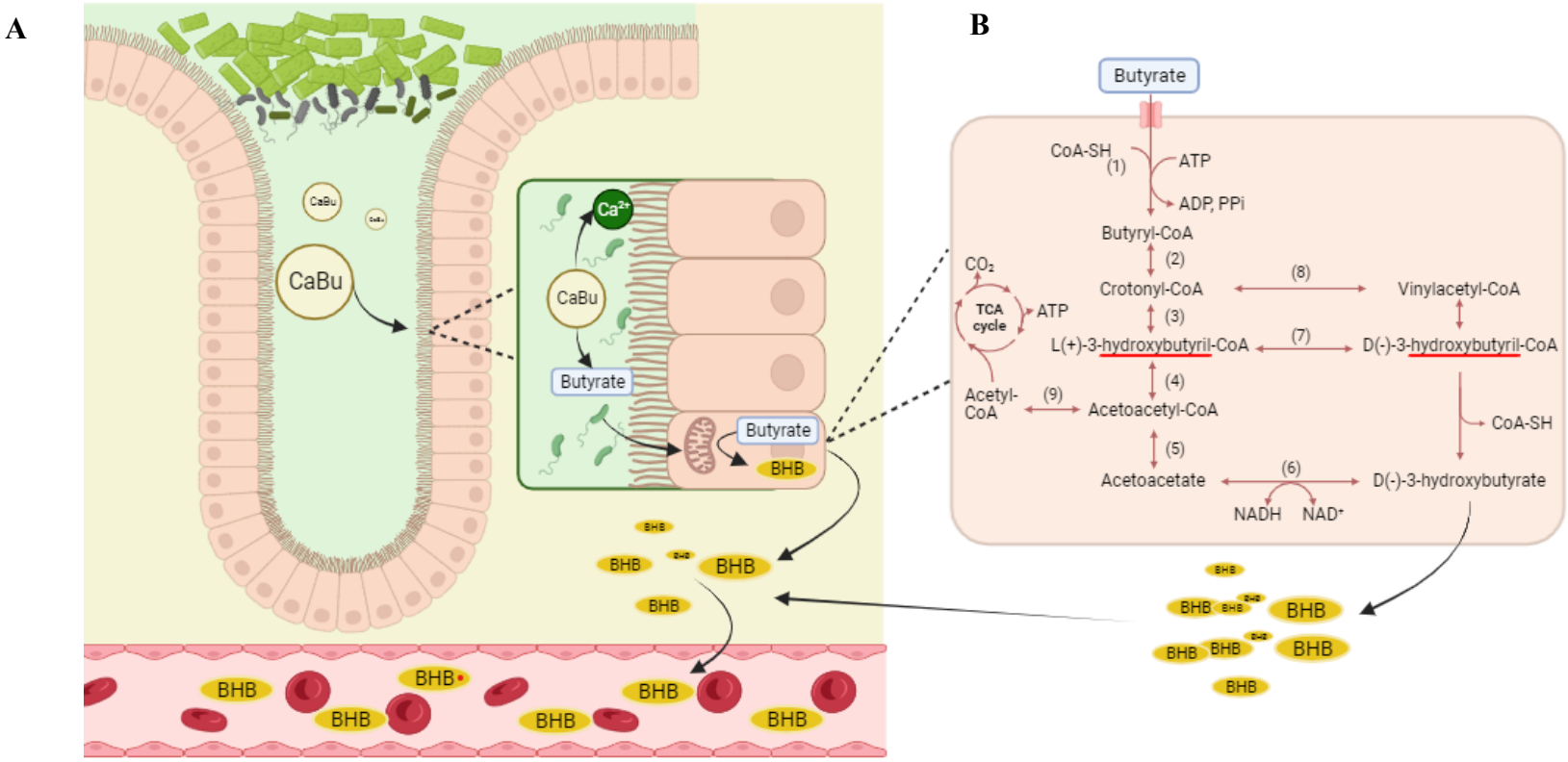


Figure 7. Nutritional ketosis model in dairy cows. **(A)** Calcium butyrate salt dissociates in the rumen matrix to be uptake in the walls of the rumen. **(B)** Butyrate is transported into the epithelial cells of the rumen walls following total oxidation in the TCA or ketogenesis by different enzymatic routes. (1) Acyl-CoA synthetase (EC 6.2.1.); (2) acetyl-CoA thiolase (EC 2.3.1.9); (3) butyryl-CoA dehydrogenase (EC 1.3.99.2); (4) crotonase (EC 4.2.1.17); (5) deacylase (EC 3.1.2.11); (6) D (-)-3- hydroxybutyrate dehydrogenase (EC 1.1.1.30; (7) 3-hydroxybutyryl-CoA racemase (EC 5.1.2.3; (8) vinylacetyl-CoA isomerase (EC 5.3.3.3); (9) L (+)-3-hydroxybutyryI-CoA dehydrogenase (EC 1.1.1.35). Designed with Biorender and adapted from Remond et al., (1995) review

Different aspects of our nutritional ketosis model in dairy cows have to be analyzed for future studies. Ruminal microbiota gets perturbated by the infusion of butyrate (Li et al., 2012b), and is highly likely that was affected by the dietary supplementation of CB in our ketogenic diet. The volatile fatty acids contribute with approximately 70% to the caloric requirement of ruminants (Bergman, 1990). So, their perturbation in our model could influence cow's productivity and health (Bedford and Gong, 2018). Also, butyrate has an offensive odor that limits its palatability (Bedford and Gong, 2018; Urrutia et al., 2019). Although we used a coated source of calcium butyrate, this condition could have also limited our model.

Conclusion

The absence of aberrant health, unchanged milk production, and higher energy efficiency suggest no detrimental effects associated with the induced nutritional ketosis. In this regard, the nutritional ketosis model guaranteed the conditions necessary to study, under controlled experimentation, the metabolic implications of BHB effects. The unaffected performance outcomes also suggest a discrepancy between the BHB hyperketonemia effects and its negative implications described in ketosis disorder. Future evaluations with prolonged periods of study and isocaloric dietary conditions are going to be required to establish nutritional ketosis as a model to study hyperketonemia in dairy cows.

Chapter 3: The effects of a sustained intravenous β-hydroxy-butyrate infusion in combination with a systemic immune challenge in lactating dairy cows

Introduction

Although ketosis is a metabolic disorder first time described 100 years ago, its metabolic implications and definitive treatment remain to be elucidated (Shaw, 1943; LeBlanc, 2015; Mann et al., 2018; Capel et al., 2021; Horst et al., 2021). Ketosis is a health affection related to high concentrations of ketone bodies (Sjollema and Van Der Zande, 1923) with hundreds of million-dollar toll only in the United States (McArt and Oetzel, 2015). From a physiological perspective, the increasing concentration of BHB can be seen as a *metabolic sign* of active fat and ketone bodies oxidation under depleted glucose conditions (Krebs, 1966; Bergman, 1971; Bauman and Bruce Currie, 1980; Gröhn et al., 1983). However, increased BHB levels are associated with impaired health and decreased performance in transitioning dairy cows (Overton et al., 2017). Despite of this connection, the demarcation between the homeorhetic mechanism aimed at counterbalancing the energy imbalance and the development of a metabolic disorder in early lactation has not been elucidated (Krebs, 1966; Bergman, 1971; Zhang and Ametaj, 2020). Given our current limited understanding of the mechanisms triggering this metabolic disorder, may be beyond our current knowledge to judge whether BHB is a real affection, to propose solutions, or even to calculate the costs associated.

Hyperketonemia is recognized at the farm level as a proxy for a higher incidence of metabolic disorders like metritis, endometritis, retained placenta, displaced abomasum, poor fertility, low milk production, and generalized inflammation (Drackley, 1999; Dann et al., 2005; Chapinal et al., 2011, 2012a; McArt et al., 2012a, 2013; Roberts et al., 2012; Suthar et al., 2013; LeBlanc, 2015; Abuajamieh et al., 2016; Overton et al., 2017; Benedet et al., 2019). However, these associations might be full of inaccuracies (Horst et al., 2021a). The existence of paradoxes

like animals with BHB levels considered with clinical ketosis without manifesting symptoms (Gordon et al., 2013; LeBlanc, 2015), hyperketonemic cows producing more milk (Vanholder et al., 2015; Belay et al., 2017; Rathbun et al., 2017; Piñeiro et al., 2019), inconsistent associations with reduced fertility (Gross et al., 2011; Chapinal et al., 2012a; McArt et al., 2012a), and broad evidence supporting BHB as a pleiotropic metabolite with anti-inflammatory and antioxidant properties (Youm et al., 2015; Yamanashi et al., 2017; Kim et al., 2020) make clear the lack of understanding about the role of BHB *per se*.

Undeniably, the ramping concentration of NEFA and BHB occurs concomitantly when 75% of the metabolic disorders and infectious diseases take place in the transitioning cows (LeBlanc et al., 2006; Chapinal et al., 2011, 2012a). *In vitro* models have demonstrated a direct immunosuppressant effect of BHB on the immune cells' cytokine release, chemotactic and phagocytic activity (Hoeben et al., 1997; Hammon et al., 2006; Grinberg et al., 2008; Youm et al., 2015; Swartz et al., 2021a; Dong et al., 2022; Onizawa et al., 2022a). Also *In vivo* studies with isolated hyperketonemic conditions have reported immunosuppressant effects of BHB in the immune response after being challenged in the mammary gland (Zarrin et al., 2014; Swartz et al., 2021b). However, the role of BHB on lactating dairy cows' performance and immune response to systemic infectious agents under long-term hyperketonemic conditions remains unclear. In this regard, we aimed in this study to elucidate the role of ketone bodies in lactating dairy cows' metabolism and its implications on health, productivity, and behavior under regular and systemic immunologically challenging conditions.

Materials and methods

3.1.1. Animals, feeding, and husbandry

The animal experiment was conducted at the University of Maryland Central Maryland Research and Education Center (CMREC) dairy farm facility in Clarksville. All procedures involving the use of animals were approved in accordance with the regulations and guidelines established by the University of Maryland Animal Care and Use Committee (Protocol 1830894-3). Six, mid-lactation (140 ± 48 DIM), multiparous (2.75 ± 0.89 parity) Holstein cows (n = 8)

were enrolled in this study. Animals were moved into the tied stall barn 5 weeks before the beginning of the experiment to guarantee optimal adaptation to the housing and experimental feeding conditions. The day prior to the experimental period starts, all cows were weighed (643.6 ± 81.5 kg) and the body condition score (BCS) was evaluated (2.9 ± 0.3; scale, 1-5). Cows were taken to the parlor to be milked twice daily at 630 and 1530 h. Fed TMR was provided fresh once daily (800 h) using a diet balanced to meet the requirements of a late lactation cow producing 34.6 kg/d of milk and consuming 24.3 kg/d of dry matter **(Table 3)**. Using MAX TM System for Dairy software the TMR was formulated (Cargill ®, Minneapolis, MN). The amount fed was adjusted for a minimum of 10% refusals. Samples of the TMR were taken daily and stored at -20°C. A compilation of samples was prepared and shipped to a commercial laboratory to be analyzed (Cumberland Valley Analytical Services **—CVAS—**, Waynesboro, PA). Water was available *ad libitum* and during the entire period of evaluations the housing barn was illuminated.

3.1.2. Experimental design and solution preparation

Cows were blocked by DIM and lactation, and then randomly assigned to one of the 2 infusion treatments in a 2 x 2 Latin square design. Treatments included intravenous infusion of 2.5M 3-hydroxybutyric acid sodium salt (**EK**; BoxNutra™, Akron, OH; n=6) or 2.5M NaCl solution (**Control**; Fisher Scientific, Pittsburgh, PA; n = 6). Periods of evaluations were 72 h in length and separated by 14-d washout intervals. The saline solutions we prepared following Swartz et al., (2021b) method. The EK solution was prepared by mixing 315.2g of BHB salt per total volume of 1.0 L of distilled water to achieve 2.5M. Control solution was prepared by mixing 146.1g of NaCl per total volume of 1.0 L of distilled water to achieve 2.5M. Both solutions were stirred, pH corrected to 7.4, filtered with a 20µm mesh paper filter, and autoclaved at 121°C, 15 psi for 1h. The solutions were stored in 20 L sterile Nalgene jugs at 8°C until the trial started.

Table 3. Ingredient and nutrient composition of treatment diets.

Ingredient (% of DM)	**Amount**
Corn silage	32.7%
Triticale silage	8.6%
Alfalfa haylage	9.3%
Ground corn	23.3%
Grain mix[1]	17.3%
Soybean meal	8.3%
Fat supplement[2]	0.4%
Chemical composition (% of DM)	
Dry matter (%)	43.3
Crude protein	17.4
Acid detergent fiber	0.7
Amylase-treated neutral detergent fiber	1.4
Lignin	18.4
Crude fat	31.3
Ash	2.7

[1]UMD CRMEC grain mix is composed of crude protein 29.7%, NDF 24.0%, digestible neutral detergent fiber 18.3%, rumen digestible starch 3.12%, rumen soluble sugar 2.32%, fat, 4.0%, ash 20.6%, calcium 2.7%, phosphorus 0.3%, magnesium 1.1%, potassium 0.9%, sulfur 0.5%, sodium 2.6%, chloride 1.6%, Met, 4.6%, Lys, 8.3%, manganese 182.133 mg/kg, zinc 182.1 mg/kg, copper 70.8 mg/kg, iron 225.7 mg/kg, selenium 1.81 mg/kg, vitamin A 30.5 IU/g, vitamin E 239.5 iu/kg, monensin 59.6 g/ton (Cargill Animal Nutrition, Hagerstown, MD).

[2]Megalac ® (Church & Dwight Co., Inc., Princeton, NJ).

3.1.3. Catheterization

The day before the infusions started, cows were contralateral-jugular catheterized. One catheter was used for the continuous infusion and the second was for blood sampling. Cows

were restrained in a chute and haltered to be immobilized. The area of installation was clipped and cleaned with 70% ethanol once. Wearing sterile gloves, the jugular vein was occluded and a 12G and 2-inch needle introducer (Neogen, Lexington, KY) was used to fill 25 cm of EPO gas-sterilized Tygon tubing (Saint-Gobain Performance Plastics, Akron, OH). The introducer was removed and fitted with an 18G Luer-Lok™ needle adapter (Exel International Inc, Tysons Boulevard, VA). Flux was tested by drawing 1 mL of blood to confirm correct placement. The catheter was flushed with 5 mL of 0.9% saline solution (Dechra Veterinary, Overland Park, KS) and 2 mL of 1:10 heparinized saline solution (100 U/mL, Sagent, Schaumburg, IL) to avoid clotting. The catheter was capped with an injectable plug Luer-Lok™cap (Exel International Inc, Tysons Boulevard, VA). The external section of the catheter was fixed to the cow's skin with 3M elastic adhesive tape (3M, Saint Paul, MN) and glued with Tag Cement (W.J. Ruscoe Company, Akron, OH). Installed catheters were secured by wrapping them with Powerflex® tape (Andover Health Care, Spartanburg, SC) around the neck. This procedure was repeated with the contralateral jugular vein. To avoid any catheter removal, cows were restrained with halters instead of collars for the period of evaluations.

3.1.4. Infusion, and blood sampling

Cows were continuously infused using Abbott XL Plump pumps (Abbott Laboratories, Chicago, IL) with an adapted cassette connected with a stainless-steel tip barbed adaptor Bulkhead Fitting 6,5 mm (Fischer Scientific, Hampton, NH). Adaptor was designed to connect an EPO sterilized silicon tube line placed inside the Nalgene jug filled with the infusates. In the beginning blood samples were taken every 15 min during the first hour of infusion and EK-treated cows' infusion rates were regulated targeting the BHB concentration between 1.2 and 1.8 mM. To maintain osmolar concentrations, Control infusion rates were paired with EK average rates. After h 2, blood BHB concentration was tested every 4 h. To collect blood, 3 mL were initially extracted and discarded to remove the heparin contained in the catheter. After sample collection, to avoid clotting the catheter line was flushed with 5 mL of 0.9% saline solution (Dechra Veterinary, Overland Park, KS) and filled with 1 mL of 1:1000 heparinized 0.9% saline solution (solution 10 U/mL; Sagent, Schaumburg, IL).

To monitor blood BHB levels, blood was collected (10 mL) from the not infused indwelled catheter at regular intervals and evaluated using a ketone strip handheld meter (Precision Xtra, Abbott Laboratories, Abbott Park, IL). Blood samples were collected at -24, 0, 0.5, 1.5, 2, 6, 10, 24, 36, 48, 60, 60.1, 60.4, 61.5, 62, 66, and 72 h after the start of the infusions. Blood was kept on ice and stored in tubes containing EDTA until centrifugation at 2,800 × *g* for 15 min at 4°C. Plasma was removed, immediately snap frozen in dry ice, and stored at -80°C until being analyzed.

3.1.5. Liver Biopsies

Liver biopsies were collected following Hughes (1962) procedure. In brief, liver biopsies were collected from the 10th intercostal space. The biopsy site was clipped and rinsed using water and a brush from caudal to ventral. The incision area was prepared using alternating betadine surgical scrub 3 times, starting from the center of the surgical site to the outside of the prepared area in a circular manner. The area was then rinsed following the previous procedure but using 70% ethanol. Lidocaine (12 mL; PHOENIX, St Josepth, MO) was subcutaneously administered at the center of the incision site. After 5 min, a 0.5-cm incision was made through the skin with a scalpel. Using a custom trocar, approximately 1g of liver tissue was collected and snap-frozen in liquid nitrogen immediately after collection. Biopsy sites were cleaned with hydrogen peroxide, stapled, and sprayed with antiseptic (Alushield®, Neogen). Banamine® was i.v. administered via the jugular catheter at 1ml/100 lb of BW. Staples were removed 10 days after the biopsies. All samples were stored at -80°C.

3.1.6. Plasma analyses

Plasma was analyzed for glucose (Wako Chemicals, Richmond, VA), NEFAs (Wako Chemicals) to determine concentrations using enzymatic colorimetric procedures. Insulin (Mercodia, Uppsala, Sweden), C reactive protein (CRP; MyBioSource, San Diego, CA), tumor necrotic alpha (TNFα; MyBioSource), and IL-1β (Fisher Scientific), interleukin 10 (IL-10; Antibodies Online, Limerick, PA), were quantified using bovine-specific ELISA.

Spectrophotometric measurements were conducted using a SpectraMax Plus 384 Microplate Reader (Molecular Devices, San Jose, CA). The intra-and interassay coefficient of variation (CV) were 5.0% and 4.0%, 4.0% and 7.1%, 3.3% and 3.2%, 3.5% and 3.5%, 3.7% and 3.7%, 5.1% and 5.0%, 4.4% and 4.2% for glucose, NEFAs, insulin, CRP, TNFα, IL-1β, IL-10 respectively.

3.1.7. Liver Caspase-1 analysis

Liver protein caspase-1 levels were also quantified using bovine-specific ELISA (Creative BioMart, Shirley, NY). Liver samples (25 µg) were homogenized and lysed using bead mill homogenizer (VWR, Radnor, PA) in 500 µL of cell lysis buffer (Creative BioMart, Shirley, NY). Samples were centrifugated at 15 000 rpm for 15 min at 4°C. Protein content was determined with bicinchoninic acid (BCA) assay (Fisher Scientific). Then, 200µg of protein were incubated with 4 mM of Ac-YVAD-pNA substrate (2 µM final concentration) at 37°C for 2 h. Optical density was determined at 405 nm absorbance with a spectrophotometer (Molecular Devices, San Jose, CA). Caspase-1 is reported as the relative activity of EK (n= 5) versus control (n= 4).

3.1.8. LPS challenge.

At h 60 of infusion, all animals were subjected to an i.v. LPS challenge of *Escherichia coli* O55:B5 (Sigma Aldrich, St. Louis, MO). LPS was dissolved in sterile 0.9% saline solution to a stock solution of 2 mg/mL 6 hours previous to be used, and stored at 4°C. All doses were prepared in single syringes at a concentration of 0.085 µg/kg of BW. This solution was infused in a single bolus dose through the jugular catheter. After the bolus infusion, 5mL of 0.9% saline solution was used to flush the catheter and 1mL of 1:1000 heparin was applied.

3.1.9. Clinical Outcomes

Feed intake was recorded daily subtracting the refusals from daily feeding TMR and adjusted to dry matter (DMI). Milk yield (MY) was recorded at the parlor at each milking and summed by day. Milk quality (MQ) was evaluated by collecting milk samples at every milking and mixed in a tube provided with a preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA). Post-LPS challenge MY and MQ were only recorded during the 630h milking. Samples were stored at 4°C and inverted twice a day. All samples were shipped to a commercial laboratory (Lancaster DHIA Laboratory, Manheim, PA) to quantify protein, fat, lactose, MUN, and somatic cell count (SCC). Two days before the infusion started, DMI, MY, and MQ were measured to be used as covariates in the statistical analysis. At the beginning of the infusion, rectal temperature (RT) and respiration rates (RR) were recorded every 6 h. After the LPS challenge, RT and RR were evaluated every hour until complete the 72h period. The pain score was calculated following Gleerup et al., (2015) method. Briefly described, this is a score calculated by considering attention towards the surroundings, head position, ear position, facial expression, response to approach, and back position.

3.1.10. Calculations

Energy-corrected milk (ECM) was calculated as described by (Hall, 2023) where ECM (kg/d) = (0.25 x kg milk) + (12.2 x kg fat) + (7.7 x kg protein). 3.5% fat-corrected milk (FCM) was calculated as: 3.5% FCM (kg/d) = (0.432 x kg milk) + (16.23 x kg fat) as described by (Linn, 2006). Feed efficiency was calculated as: daily milk (kg) / DMI (kg). Fat-corrected milk efficiency was estimated as: FCM (kg) / DMI (kg). Energy-corrected milk efficiency was calculated as: ECM (kg) / DMI (DMI). To calculate pain, all pain scores were transformed into categorical binary data using a pain score of 3 as the cut-off point suggested by Gleerup et al., (2015) as the painful condition for a dairy cow.

3.1.11. Statistical analyses

All data were analyzed with SAS (version 9.3; SAS Institute Inc., Cary, NC). Analysis of repeated measures for continuous variables was performed with the use of PROC-MIXED. The mixed model included the random effect of cow, the fixed effects of time relative to infusions, period, treatment, and their interaction. Analysis of repeated measures for the categorical variables was performed with the use of PROC-GLIMMIX model including the random effect of cow, the fixed effects of time relative to infusions, period, treatment, and their interaction. Covariance structures for modeling repeated autoregressive one (AR-1), unstructured (UN), variable components (VC), compound symmetry (CS), and Toepliz (TOEP) were assigned and selected by the lowest Goodness-of-fit test results. All data collected prior to the period of evaluations were averaged and tested as a covariate to account for individual differences between cows. Backward elimination was used to remove non-significant interactions when resulted values in the model were $P > 0.25$. Treatment, time, treatment × time, and covariate were included into the model regardless of significance. In all models, residuals were assessed for normality and outliers (PROC UNIVARIATE). To improve residual distribution a transformation was made on SCC to somatic cell score (SCS = $\log_2$ (SCC/100,000) + 3) according to (Ali and Shook, 1980). Least square means (LSM) test was used to calculate differences between the effects on the model. All results are expressed as LSM and their standard errors unless stated otherwise. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Results

3.1.12. Hyperketonemia induction, BHB blood levels and infusion rate

The intravenous infusion of sodium-β-hydroxybutyrate resulted in a rapid and sustained elevation of blood BHB levels relative to control (**Figure 8**, $P < 0.001$). During the period of study, blood BHB levels were 1.4 ± 0.03 mM and 0.7 ± 0.03 mM for EK and Control, respectively ($P < 0.001$). Hyperketonemia was sustained at the subclinical threshold for ketosis prior to the LPS challenge, but interrupted following the LPS bolus

($P < 0.001$). In the EK group, blood BHB levels decreased by 33% 1.5 h after receiving the LPS bolus (0.9 ± 0.09 mM, $P < 0.001$) and remained low until h 62 (0.9 ± 0.09 mM, $P < 0.001$). Infusion rates (IR) were ramped up 30% after h 61.5 ($P<0.001$) and kept higher until the end of the study ($P<0.001$). Hyperketonemic levels were re-established until h 66 (1.6 ± 0.10 mM, $P = 0.02$). Similar to EK group, Control treatment blood BHB levels tended to drop 1.5 h post-LPS bolus (0.5 ± 0.09 mM, $P = 0.09$), were lower at hour 62 (0.4 ± 0.09 mM, $P < 0.001$), and ended at 0.5 ± 0.09 mM at h 72 ($P = 0.15$).

3.1.13. Feed intake, milk production, milk quality, and feed efficiency

Dry matter intake (DMI) was not affected by the induced hyperketonemia until the LPS challenge was performed. The DMI pre-LPS challenge was 21.4 ± 0.9 kg/d and 21.3 ± 0.9 kg/d for EK and Control, respectively (**figure 9A**). However, after the LPS challenge, not only the DMI decreased for both treatments ($P < 0.001$) but also was 21% lower in the hyperketonemic cows versus their counterparts (**figure 9A**, $P = 0.09$). No differences were detected between the treatments in the milk production previous ($P = 0.79$) and after the immune challenge ($P = 0.14$). Previous to the LPS challenge (**figure 9B**), milk production was 36.0 ± 0.7 kg/d versus 35.7 ± 0.7 kg/d, for EK and Control, respectively. Post-LPS challenge, milk production (only recorded during the AM milking time) decreased to 9.0 ± 2.4 kg in EK group compared to 15.5 ± 2.0 kg for the Control cows (**figure 9B**). Milk composition was not affected by hyperketonemia before the LPS challenge (**figure 9C, 9D, 9E, 9F, 9G, 9H**) but differed in the fat and lactose proportion post-LPS challenge (**figure 9C, 9G**). Fat % post-LPS challenge was 2.3 ± 0.5 and 3.7±0.5 for EK and Control, respectively ($P = 0.10$). In the case of lactose %, the levels post-LPS challenge were 6.1 ± 0.4 and 3.8 ± 0.4 for EK and Control, respectively ($P = 0.02$). Previous to the immune challenge, the adjusted cow's milk production was not different between treatments ($P > 0.55$). However, post-LPS challenge, 3.5%-fat-corrected milk (3.5% FCM) and energy-corrected milk (ECM) were reduced by 18% in the EK compared to their counterparts (**figure 10A, 10B**; $P < 0.03$). There were no differences detected in the energy efficiencies between the treatments for feed efficiency, 3.5% FCM efficiency, and ECM efficiency, before or post-LPS challenge (**figure 10C, 10D, 10E**).

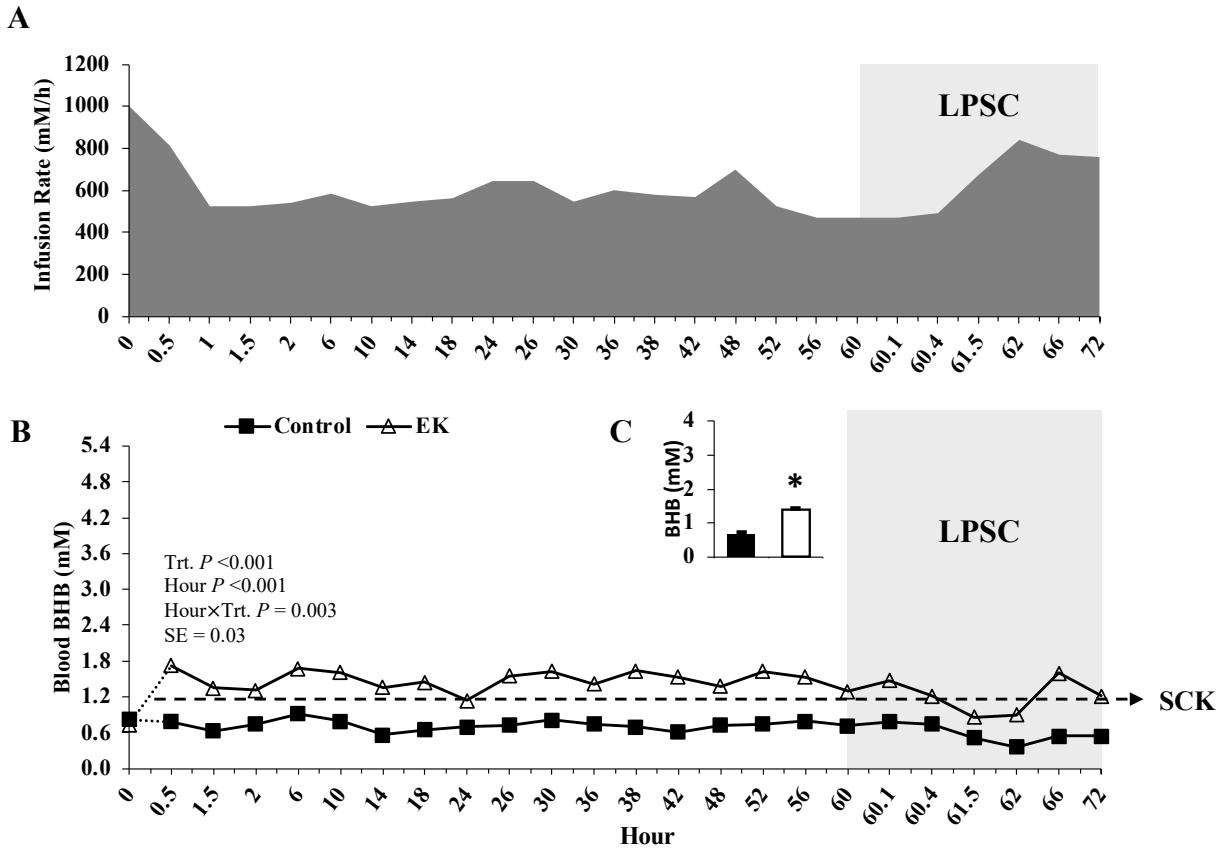


Figure 8. Hyperketonemia induction model in dairy cows. **(A)** Intravenous infusion rate, **(B)** blood BHB levels, and **(C)** overall treatment effect. At h 60 i.v. E. coli LPS challenge (LPSC) was performed. The light grey zone after h60 refers to the period of LPSC evaluation. Intravenous infusion of Na-β-hydroxybutyric acid (EK, 2.5 M) or NaCl (Control, 2.5M) was continuously administered for 72h. Blood BHB was measured in an hourly time set and infusion rates (IR) were adjusted to maintain concentrations between 1.2 and 1.8 mM blood BHB levels. During the period of study, the IR were 245.7±5.4 mL/h and 243.6±5.4 mL/h for EK and Control, respectively. Control and EK infusion rates were paired. Cows were i.v. challenged with Escherichia coli O55:B5 lipopolysaccharide, 0.085 µg/kg of BW. The dotted line represents SCK blood BHB threshold. Data presented as LSM. EK, exogenous ketones; SCK, subclinical ketosis, (*) *P*-value <0.05.

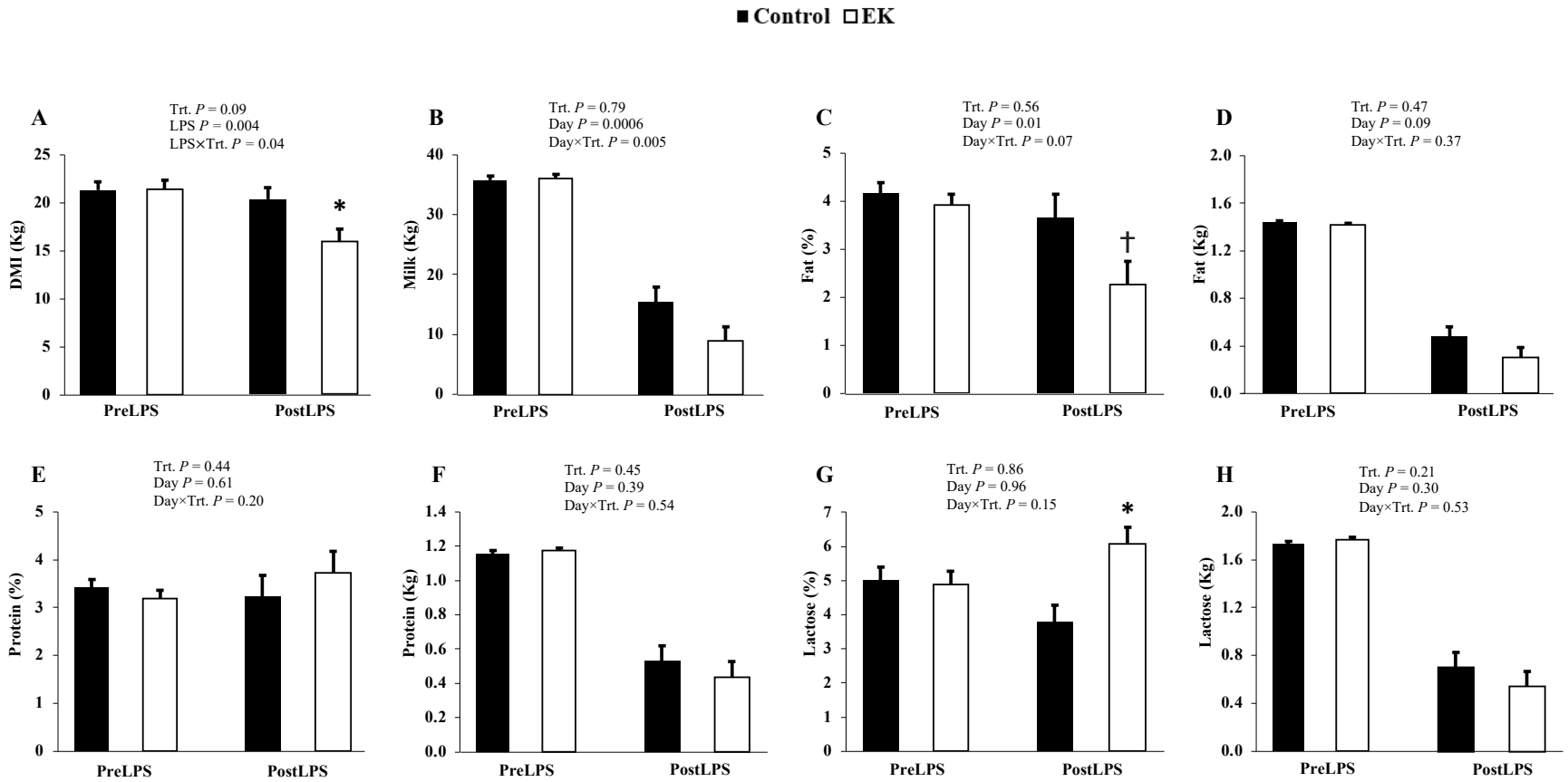


Figure 9. Induced hyperketonemia on dairy cow's production performance and milk quality. **(A)** DMI (Kg), **(B)** Milk production (Kg), **(C)** Fat (%), **(D)** Fat (Kg), **(E)** Protein (%), **(F)** Protein (Kg), **(G)** Lactose (%), **(H)** Lactose (Kg). Cows were disconnected from the infusion and taken to the parlor twice a day (630 h and 1500h). DMI, dry matter intake. Data presented as LSM. EK, exogenous ketones; (*) *P* -value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

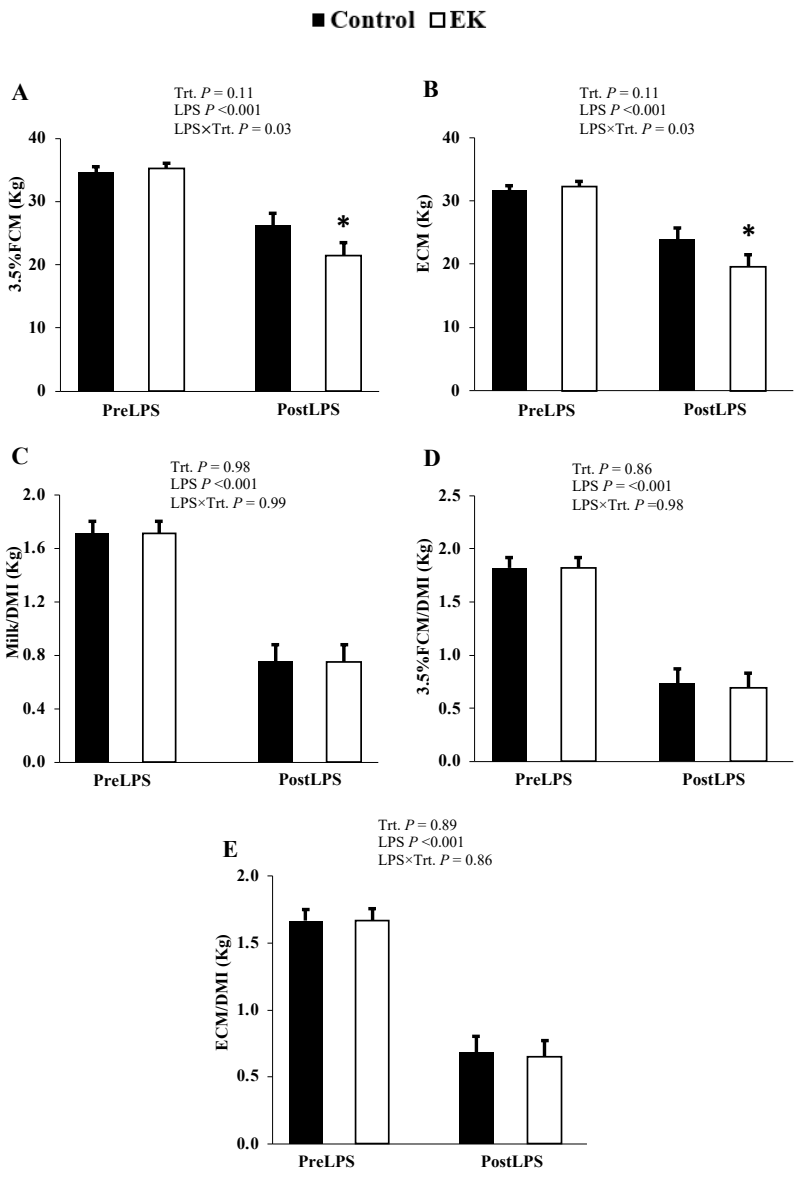


Figure 10. Induced hyperketonemia on dairy cow's energy efficiency performance. **(A)** 3.5% FCM (Kg), **(B)** ECM (Kg), **(C)** Feed efficiency (Kg), **(D)** 3.5% FCM efficiency (Kg), **(E)** ECM efficiency (Kg). 3.5% FCM, 3.5% fat-corrected milk; ECM, energy-corrected milk; DMI, dry matter intake, EK, exogenous ketones. 3.5%FCM was calculated as 3.5% FCM (kg/d) = (0.432 x kg milk) + (16.23 x kg fat). ECM was calculated as ECM (kg/d) = (0.25 x kg milk) + (12.2 x kg fat) + (7.7 x kg protein). Data presented as LSM, (*) *P* -value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

3.1.14. Plasma metabolites

Hyperketonemic conditions did not affect plasma glucose concentrations but those had a tendency to differ over time ($P = 0.10$). Only at h 61.5 was the only time where higher glucose plasma concentrations were detected in the EK group ($P < 0.001$). The LPS bolus caused a depletion in the plasma glucose concentration for both groups. Glucose plasma concentration pre-LPS challenge was 76.2 ± 1.7 mg/dL and 76.0 ± 1.7 mg/dL for EK and Control, respectively (**figure 11A**, $P = 0.91$). Glucose plasma concentration, after the LPS challenge (**figure 11**) decreased to 69.1 ± 2.2 mg/dL and 65.1 ± 2.2 mg/dL for EK and Control respectively ($P < 0.001$) but were not different between the treatments ($P = 0.20$).

Like glucose, plasma NEFA concentrations were not different between the groups (**figure 11C,** $P = 0.32$) but tended to be influenced by the time relative to the LPS challenge ($P = 0.10$). Plasma NEFA concentration pre-LPS challenge were higher for EK group (92.5 ± 5.1 µmol EK versus 81.7 ± 5.3 µmol Control, $P = 0.05$), however after the LPS bolus, both groups had a reduction in the NEFA plasma circulation ($P <0.001$), but was not different between the treatments (71.3 ± 5.6 µmol EK versus 72.2 ± 5.7 µmol Control, $P = 0.89$).

Hyperketonemic animals experienced higher concentrations of insulin during the course of infusions (**figure 11E**, *P*-value = 0.04). The area under the curve (AUC) estimated for plasma insulin concentration was also higher for the EK-treated group compared to their counterparts (**figure 11F**, $P = 0.02$). Plasma insulin concentration was not different between the groups pre-LPS challenge (1.06 ± 0.2 µg/L EK versus 0.9 ± 0.2 µg/L Control, $P = 0.46$), but post-LPS challenge plasma insulin concentration increased in the EK group (1.43 ± 0.2 µg/L EK versus 0.9 ± 0.2 µg/L Control, $P = 0.04$)

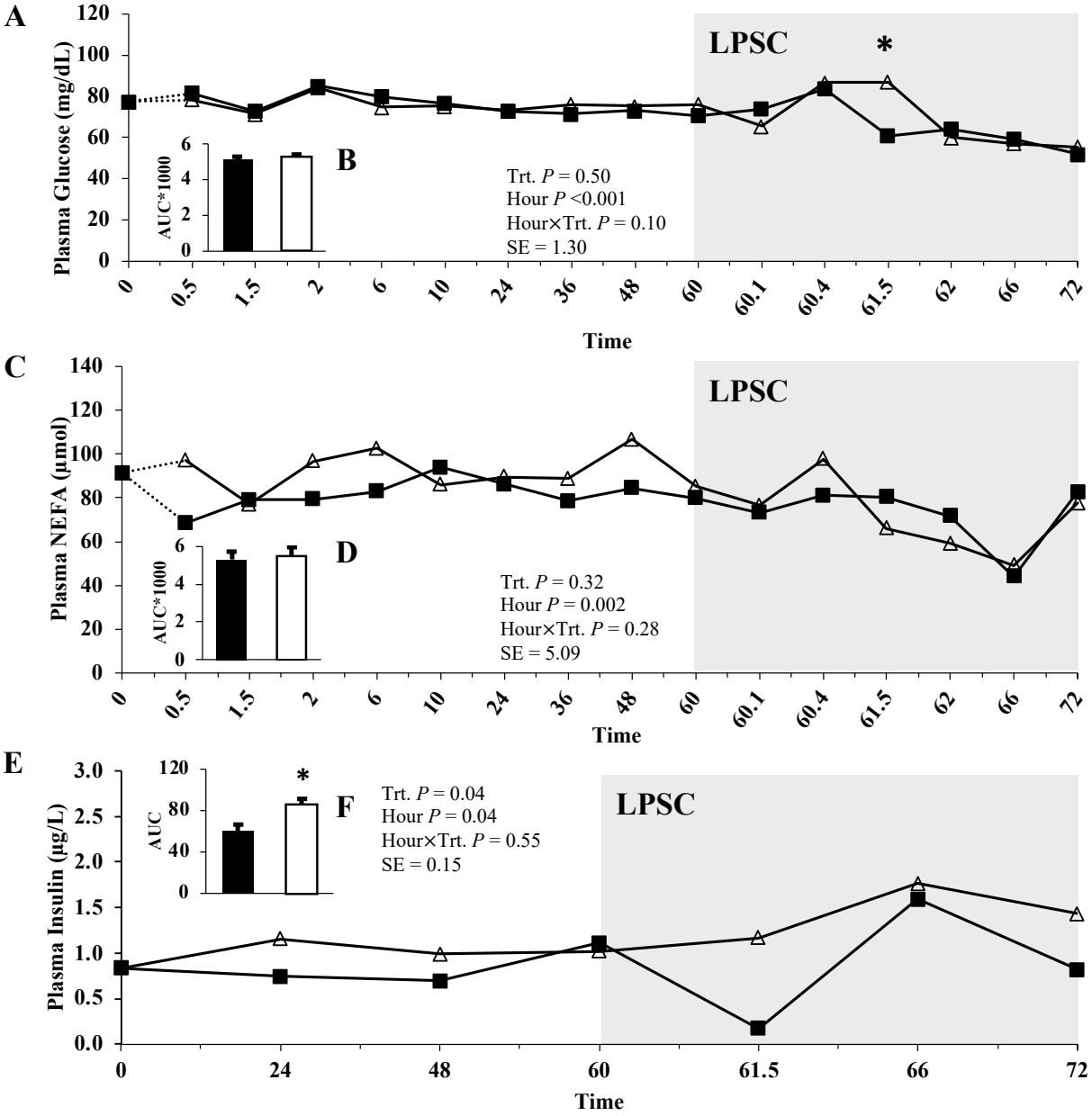


Figure 11. Induced hyperketonemia on dairy cow's energy metabolism. **(A)** Plasma glucose, **(B)** Plasma glucose area under the curve (AUC), **(C)** Plasma NEFA, **(D)** NEFA AUC, **(E)** Plasma insulin, **(F)** Plasma insulin AUC. The light grey zone after h 60 refers to the period of LPSC evaluation. EK, exogenous ketones; LPSC, lipopolysaccharide challenge; NEFA, non-esterified fatty acids. Data presented as LSM, (*) *P* -value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

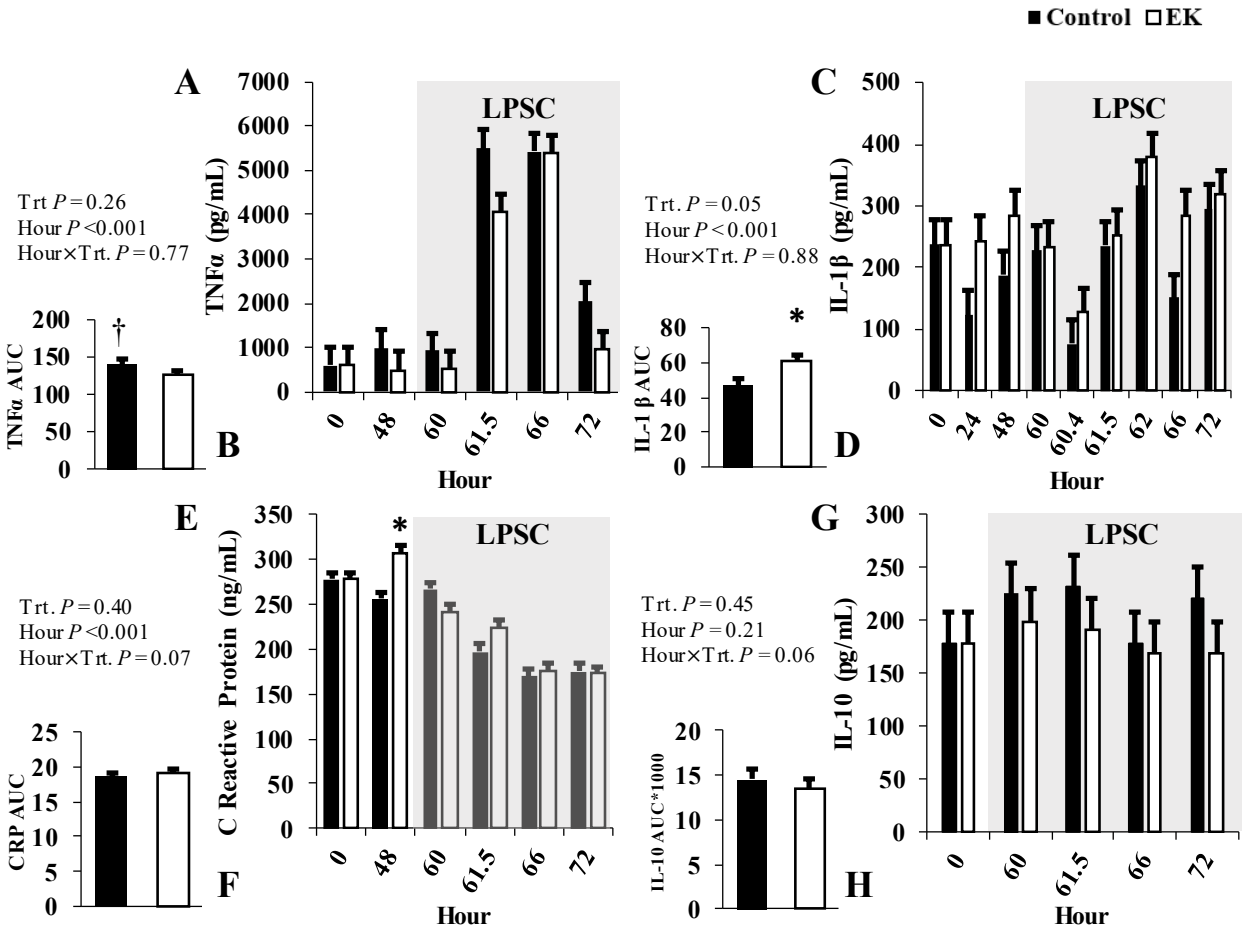


Figure 12. Induced hyperketonemia on dairy cow's immune activity. **(A)** Plasma tumor necrosis factor alpha (TNFα), **(B)** Plasma TNFα area under the curve (AUC), **(C)** Plasma interleukin one beta (IL-1β), **(D)** Plasma IL-1β AUC, **(E)** Plasma C reactive protein (CRP), **(F)** Plasma CRP AUC, **(G)** Plasma interleukin 10 (IL-10), **(H)** Plasma IL-10 AUC. The light grey zone after h 60 refers to the period of LPSC evaluation. EK, exogenous ketones, LPSC, lipopolysaccharide challenge. Data presented as LSM, (*) *P* -value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

3.1.15. Immune system action under hyperketonemic conditions

No differences were detected between treatments in plasma tumor necrosis factor-alpha (TNFα) concentrations ($P = 0.26$), but the AUC tended to be higher in the Control group ($P = 0.07$). Plasma TNFα had an increase in response to the immune challenge, going from pre-LPS

challenge concentrations of 496.9 ± 682.0 pg/mL versus 978.9 ± 682.7 pg/mL to 3462.7 ± 566.8 pg/mL versus 4345.4 ± 5 67.6 pg/mL post-LPS challenge for EK and Control, respectively (**figure 12A,** $P < 0.001$).

Higher IL-1β plasma concentrations and higher AUC was estimated in the EK group (**figure 12C, 12D**, $P < 0.05$). On average IL-1β plasma concentrations were 266.1 ± 23.7 pg/mL versus 203.5 ± 23.4 pg/mL for EK and Control, respectively ($P = 0.05$). No differences in the IL-1β plasma concentrations were detected between treatments pre versus post-LPS challenge ($P = 0.36$). Plasma IL-1β concentrations pre-LPS challenge were 254.0 ± 36.5 pg/mL versus 177.4 ± 36.4 pg/mL for EK and Control, respectively ($P = 0.13$). Post-LPS challenge IL-1β plasma concentrations were 272.7 ± 29.4 pg/mL versus 215.9 ± 29.2 pg/mL for EK and Control, respectively ($P = 0.16$). The highest IL-1β plasma value was detected 2h post-LPS bolus in both treatments ($P = 0.02$). Plasma IL-1β plasma concentrations at 62 h were 379.4 ± 54.7 pg/mL versus 333.3 ± 54.6 pg/mL for EK and Control, respectively.

Plasma C-reactive protein (CRP) concentrations were not different between EK and Control (**figure 12E**, P value = 0.40) but were depleted post-LPS challenge ($P < 0.001$). Plasma CRP concentrations pre-LPS challenge were 273.7 ± 11.6 pg/mL versus 262.3 ± 11.6 pg/mL for EK and Control, respectively (P value = 0.51). Post-LPS challenge CRP plasma concentrations decreased ($P < 0.001$) to 190.4 ± 9.7 pg/mL versus 181.5 ± 9.7 pg/mL for EK and Control, respectively ($P = 0.55$).

No differences were detected in IL-10 plasma concentrations between the groups (**figure 12G**, $P = 0.45$). Plasma IL-10 pre-LPS challenge was 195.4 ± 32.6 pg/mL versus 215 ± 33.0 pg/mL for EK and Control, respectively ($P = 0.58$). Post-LPS challenge IL-10 plasma concentrations were 172.3 ± 26.5 pg/mL versus 202.2 ± 27.0 pg/mL for EK and Control, respectively ($P = 0.23$).

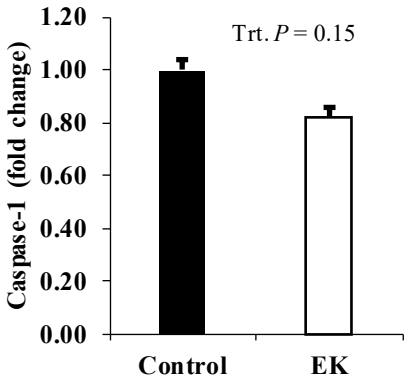


Figure 13. Induced hyper-ketonemia on caspase-1 liver protein expression. Data presented as EK LSM normalized to fold change versus CON, (*) P-value <0.05, (†) P-value $0.05 < P \leq 0.10$.

In liver tissue, at the end of the study, no differences were detected in the fold changes of caspase-1 between the groups (**figure 13**, *P* value = 0.15).

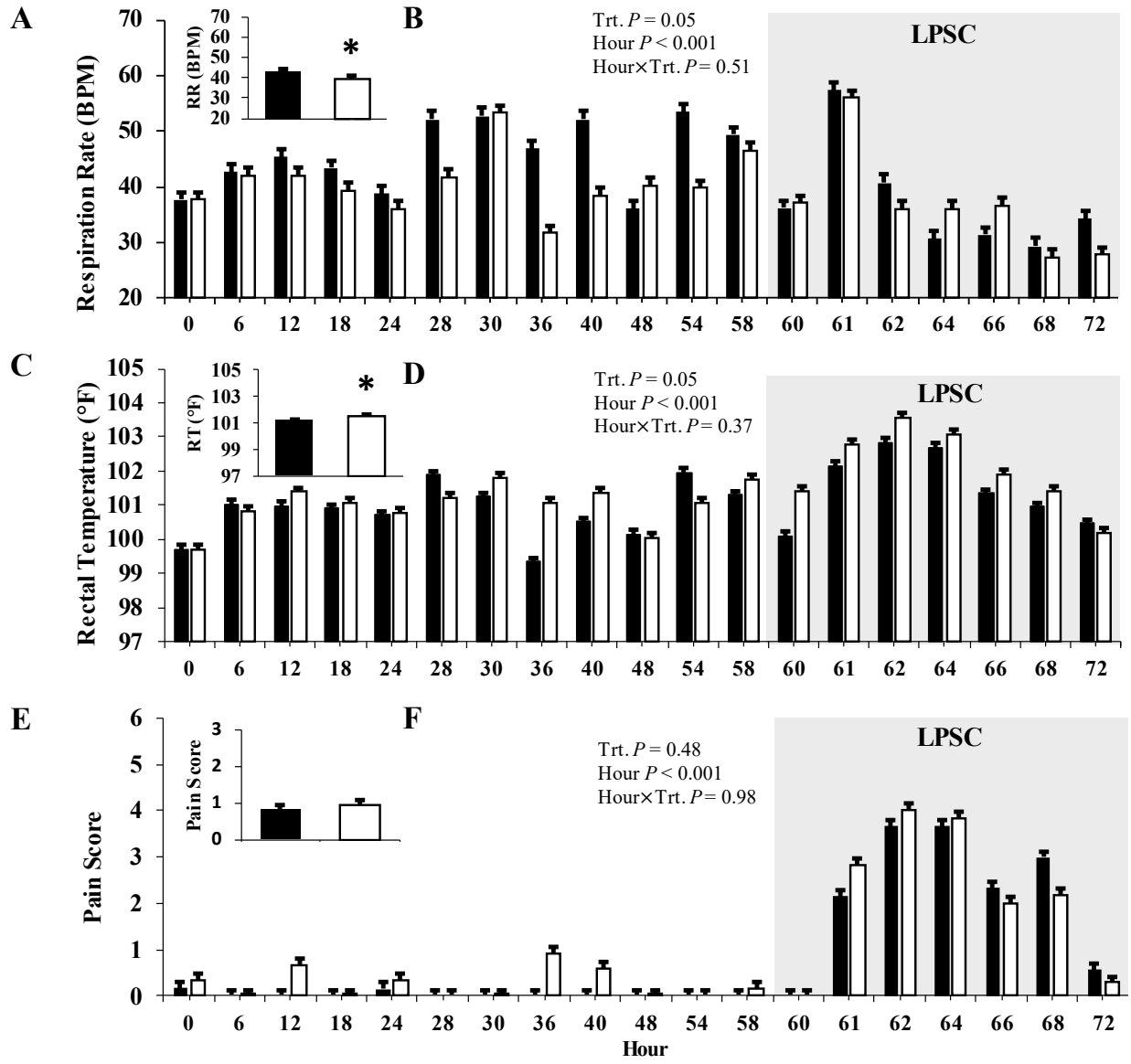


Figure 14. Induced hyperketonemia effects on dairy cow's health. **(A)** Respiration rates, **(B)** Overall treatment effect on respiration rate, **(C)** Rectal temperature, **(D)** Overall treatment effect on rectal temperature, **(E)** Pain Score, **(F)** Overall treatment effect on pain score. EK, exogenous ketones, LPSC, lipopolysaccharide challenge. Data presented as LSM, (*) *P* -value <0.05, (†) *P*-value $0.05 < P \leq 0.10$.

3.1.16. Health performance in hyperketonemic cows

During the period of study, the respiration rates (RR) tended to be lower in the hyperketonemic group (**figure 14A, 14B**, $P = 0.06$). The registered RR was 39.3 ± 1.4 BPM versus 43.0 ± 1.4 BPM for EK and Control, respectively. Rectal temperature (RT) also was higher in the EK-treated group compared to control (**figure 14C, 14D**, $P = 0.05$). The RT was 101.5 ± 0.1°F versus 101.1 ± 0.1°F for EK and Control, respectively. The subjective estimation of pain revealed no differences in the pain scores (PS) between the groups (**figure 14E, 14F**, $P = 0.05$). The PS were 0.95 ± 0.1 versus 0.8 ± 0.1 for EK and Control, respectively. Categorical analysis after transforming the PS results to a binary output using PS > 3 cut-off revealed no differences between the treatments ($P = 0.81$).

Three EK-treated cows die within the first 5 hours post liver biopsies. Two animals died in the first period and one in the second. Two animals had difficulty breathing, intense teeth grinding, bloated ruminal cavity, and one case presented nose bleeding. Two animals assigned to the control group in the second period also presented the same bloating but did not die. The third animal did not show any of the previous symptoms and partial *in situ* necropsy in this cow revealed no evident tissue damage or organ failure.

Discussion

Over the last two decades, several observational studies have pointed to BHB as the standard to predict reduced performance and impaired health in peripartal dairy cows (Overton et al., 2017; Benedet et al., 2019). Nevertheless, the simultaneous alterations in several aspects of cow physiology complicate the interpretation of this relationship. Physiological conditions related to energy insufficiency (e.g., high NEFA, low glucose and insulin), tissue-level adaptations (e.g., rumen papillae modification, uterine involution), inflammation (e.g., parturition), and a rapid increase in milk synthesis, are components of

the metabolic transformation undergone by peripartal dairy cows (Bauman and Bruce Currie, 1980; LeBlanc, 2008; Laarman et al., 2015; Hansen et al., 2017; Kuhla, 2020). Coupled with these conditions, the ramping ketogenesis takes place concomitantly with the occurrence of 75% of diseases in dairy cows (LeBlanc et al., 2006; Roberts et al., 2012). So, under such circumstances, analysis aiming to determine the role of BHB with the associated negative outcomes may result in misguided conclusions (Horst et al., 2021). Our historic inability to dissect the contributions of factors inextricably related to ketone synthesis has likely clouded our understanding of the actual effects of hyperketonemia (Drackley et al., 1991; Horst et al., 2021). Therefore, the elucidation of whether the BHB is a mere metabolic homeorhetic coincidence or a certain negative factor remains as one of the major frontiers in the dairy industry.

Our model of study for 72 h allowed us to construct the necessary conditions to evaluate the role of BHB in an approximated term to the resolution of a regular ketosis affection (McArt et al., 2011). This scenario gave us the opportunity to test the ability of the model to replicate the negative trajectories in health and milk production commonly associated with BHB (Ospina et al., 2010c; b, 2013; McArt et al., 2013). The absence of aberrant behavior in RR, RT, and PS previous to the LPS challenge, suggests a non-existent deterioration in health that could be linked to hyperketonemia. Nevertheless, a tendency to have lower RR and a significantly higher RT were recorded in hyperketonemic cows. Such results can be inferred as not indicative of impaired health since the outputs were not abnormal respiration rates (e.g., 24 - 56 BPM; Garner et al., 2017) or febrile (e.g., >103°F; Benzaquen et al., 2007). Results on RR, RT, and PS post-challenge confirmed the effectiveness of the LPS bolus triggering the immunomodulation but not evidenced an alteration affected the hyperketonemia previous to the immune activation (Kvidera et al., 2017; Chandler et al., 2022). However, given the absence of impaired health outcomes, it seems likely that the effects associated with BHB may depend on the inflammatory status rather than explicitly high BHB levels.

While this study did no recorded any aberrant health behavior during the 12 h of evaluation period of LPS challenge, determining the cause of the death of the three hyperketonemic animals is beyond the scope of this investigation. However, the observed difficulty in breathing may be associated with pulmonary dysfunction, resembling the characteristics of acute respiratory distress syndrome (ARDS) or acute respiratory injury (ALI). Previous studies have identified LPS as the principal causative factor of ARDS and ALI, which induce pathologic changes such as alveolar edema, marked infiltration by neutrophils and macrophages, and destruction of pulmonary architecture (Brigham and Meyrick, 1986; Yokoi et al., 1997). The increased release of reactive oxygen species (ROS) within pulmonary endothelial cells, stimulated by the LPS, has been recognized as the main cause of the disruption of the pulmonary endothelial barrier (Grinnell et al., 2012). Immunosuppressed is a common feature observed in human cohorts with ARDS (Cortegiani et al., 2018). It is plausible to speculate that hyperketonemic cows may have had a reduced ability to resolve the acute inflammatory response triggered by the LPS bolus, however future studies are required to evaluate this association.

The no detection of differences in DMI and milk production previous to the LPS bolus defies the constant association of increasing BHB concentrations with decreased feed intake and milk production (Chapinal et al., 2012a; b; McArt et al., 2012a). In contrast, once the endotoxemia was triggered by the LPS bolus, the expected hypophagia and reduced milk production (Kvidera et al., 2017; Horst et al., 2019a; Brown and Bradford, 2021) revealed an exacerbated effect on the DMI and MY in the hyperketonemic cows. Despite the post-LPS challenge a difference of 42% in milk yield was recorded in the hyperketonemic cows compared to the Control, the lack of statistical power could have affected our statistical analysis. These results could be explained by the relatively small sample size used in our experimental design.

The effect of hyperketonemia in DMI and MY recorded in the hyperketonemic group suggests a connection between energy metabolic status and inflammation. In agreement with this hypothesis, Swartz et al., (2021b) reported an aggravated DMI and MY

in cows receiving a sustained BHB infusion combined with an intramammary challenge. Also, when Gross et al., (2020) grouped transitioning cows by glucose levels, they reported elevated BHB in the low-glucose group, and slower DMI and MY recovery after administering an intramammary challenge. Although BHB has been described as an appetite suppressor (Langhans et al., 1983; Stubbs et al., 2018; Masi et al., 2022), the unaltered DMI during the first two days of sustained hyperketonemia suggest no hypophagic effect exerted by BHB post-challenge. The degree of reduction in milk production and appetite can also be associated with the magnitude of the inflammatory conditions (Yuan et al., 2013; Bradford and Swartz, 2020). During an endotoxemia modeled by LPS, non-ruminant studies have suggested an immune-to-brain signaling on anorexic regulators mediated by cytokines, prostaglandins, fever; and nutritional state (Elander et al., 2007; Lyons and Roche, 2018). Therefore, a preconditioned inflammatory tone influenced by high BHB could explain the acute DMI and MY reduction triggered by the LPS. But, future studies are required to understand how these associations could be mediated.

In our results, we did not observe any difference between the treatments in plasma glucose levels following the start of infusions. This finding opposes the expected decreasing glucose observed in dairy cows (Zarrin et al., 2013; Swartz et al., 2021b) and other non-ruminant species (Ari et al., 2019; Poff et al., 2020; Soto-Mota et al., 2022) after the administration of exogenous BHB. It is unclear the cause for this result, but non-ruminant studies have reported that whether the increasing BHB levels result from a ketogenic diet or exogenous supplementation, the glucose levels are depleted in a BHB dose-dependent condition (Kesl et al., 2016; Ma et al., 2018; Poff et al., 2020; Falkenhain et al., 2022). The major effect of BHB in glucose regulation in non-ruminants is linked to reduced gluconeogenesis (Poff et al., 2020; Falkenhain et al., 2022). This metabolic condition is mostly explained by the signaling action of BHB selectively binding histone deacetylase-7 (HDAC7). This is a modulation that preserves downstream forkhead transcription factor (FOXO1) inactivated in the hepatocytes (Pan et al., 2022b). Activated FOXO1 promotes the gene induction of Pck1 and G6pc responsible for gluconeogenesis activity (Puigserver

et al., 2003). Nevertheless, despite recording a reduction in G6pc gene, Zarrin et al., (2013) did not detect a statistical difference in the hyperketonemic cows after infusing BHB.

Having not recorded a reduction in glucose could be explained by the blood BHB concentration reached in our model. The depleted glucose concentration reported by Swartz et al., (2021b) was in conditions where BHB levels were approximately 30% above our results. Therefore, it is possible to hypothesize that our BHB-infused levels could have not impacted the liver gluconeogenic activity in a sufficient concentration to generate a depletion in the glucose concentration. However, research about how dose dependent BHB affects glucose levels or gluconeogenesis in dairy cows is scarce. So, future studies will need to better understand the role of BHB in glucose homeostasis.

When exogenous ketone bodies are supplemented in non-ruminant species, reduced lipolysis has been reported (Madison et al., 1964; Stubbs et al., 2017; Myette-Côté et al., 2018; Evans et al., 2022; Høgild et al., 2023). In non-ruminants, the incorporation of exogenous BHB is expected to cause an acute action on lipolysis (Poff et al., 2020) modulating a homeostatic prevention of excessive ketogenesis (Taggart et al., 2005). The anti-lipolytic effect of BHB is mediated after binding to the G protein-coupled receptor 109A (—GPR109A—; Newman and Verdin, 2017; Kurtz et al., 2021), reducing cAMP concentrations, and decreased PKA-mediated activation of triglyceride lipases (Wanders and Judd, 2011; Bendridi et al., 2022). Not having recorded the antilipolytic effect of BHB is likely explained in the use of mid-lactation dairy cows because these animals were not expected to undergo extensive lipolysis while being in a positive energy status. In similar studies, using mid-lactation cows, Zarrin et al., (2013) did not detect differences in the NEFA concentrations, but Swartz et al., (2021b) reported a tendency to be reduced in the hyperketonemic cows. Using ketogenic intra-ruminal boluses (Ruiz-González et al., 2022), did not find differences in post-prandial NEFA concentrations, despite the increasing BHB concentration. Therefore, it is unclear to what extent BHB can attenuate lipolysis in dairy cows.

The increasing BHB resulted in higher insulin concentration in hyperketonemic cows and exacerbated in both treatments after the LPS endotoxemic action. Increasing exogenous BHB concentrations promote insulin secretion in a wide variety of non-ruminant species (Jenkins et al., 1970; Biden and Taylor, 1983; Holdsworth et al., 2017) and in dairy cows (Swartz et al., 2021b; Ruiz-González et al., 2022). The proposed mechanism of action by BHB is linked to an alteration in the β-cells causing an insulin secretion stimulus (Madison et al., 1964). Under such conditions, the increased insulin concentration can cause the attenuation of NEFA release and thereby avoid increasing ketone concentrations as a compensatory mechanism (Madison et al., 1964). Post-LPS challenge, we observed a spike in insulin concentration at h 66 coinciding with the nadir in NEFA concentrations. This outcome is likely explained by the effective anti-lipolytic capacity of insulin (Froesch, 1967), and seconded by the documented increasing concentrations during endotoxemia modulated with LPS in dairy cows (Kvidera et al., 2017).

The decreasing concentrations of BHB observed in both treatments post-LPS challenge is a constant and unclear output found in the LPS endotoxemia modeled in dairy cows (Burgos, 2000; Zarrin et al., 2013; Kvidera et al., 2017; Swartz et al., 2021b). Our results indicated higher blood BHB removal in the hyperketonemic cows explained by the increased infusion rates modified to retrieve the hyperketonemic conditions post-LPS bolus. Kupffer cells are macrophages cells, residing in the liver with roles like sentinel screening, immune response priming, and antigen's clearance (Su et al., 2002; Ametaj et al., 2005; Pérez-Hernández et al., 2021). Since macrophages can oxidize acetoacetate but not BHB (Puchalska et al., 2019) it is possible to hypothesize that under the high energy demand of the modeled endotoxemia, BHB can fuel these cells. The highly demanding energy conditions triggered by the LPS bolus (Kvidera et al., 2017) could prime the liver to play a pivotal role in converting the exogenous BHB into AcAc (Cuenoud et al., 2020). A study presented by Cox et al., (2016) reported a substrate preference by the skeletal-muscle cells for higher BHB oxidation over glucose during high energy demand conditions. This pattern of preference is associated with the higher BHB efficiency to fuel more ATP over glucose

(Cox et al., 2016). However, future studies will be required to evaluate the role of BHB in the immune cells fueling, especially under challenging conditions in dairy cows.

The use of LPS is a well-established experimental model of endotoxemia, characterized for inducing inflammation in dairy cows (Burgos, 2000; Horst et al., 2019a; Chandler et al., 2022). The increasing concentrations of TNFα after the LPS infusion reflect a systemic and local activation of the innate and non-innate cells expressing pattern recognition receptors (Eckel and Ametaj, 2016; Mohammad and Thiemermann, 2021). Increasing TNFα concentrations were observed followed by temporal changes in the febrile response post-LPS bolus, evidenced by the pyrogenic role of TNFα in dairy cows and partially explained the increasing RT (Dinarello, 1999; You et al., 2021; Chandler et al., 2022). However, no differences were detected in the RT between the treatments pre- or post-LPS challenge, suggesting no apparent implications of BHB in the acute release of TNFα and its modulation.

Previous literature has suggested BHB as an anti-inflammatory metabolite (Youm et al., 2015; Yamanashi et al., 2017; Swartz et al., 2021a). However, we were not able to detect these effects modulated in the hyperketonemic group. Increasing concentrations of IL-1β are described as a pro-inflammatory tone in non-ruminants (Dinarello, 1999; Kaneko et al., 2019b) and dairy cows (Trevisi et al., 2016; Kuhla, 2020). The higher IL-1β concentrations registered over the time course of evaluations suggest increasing inflammatory conditions linked to the higher BHB levels. A possible explanation is associated with the predominant type of ketone body in the media. While BHB can attenuate the inflammatory reaction, AcAc is viewed as a pro-inflammatory modulator (Onizawa et al., 2022b). However, this association depends on the oxidation of BHB to AcAc, which is regulated by the metabolic redox state, therefore link to the metabolic energy status (Laffel, 1999). The high energy demand triggered by the modeled endotoxemia could have primmed the liver to actively oxidize BHB to AcAc, thereby turning the anti-inflammatory tone into pro-inflammatory conditions. Future studies will be required to establish the possible

associations between the type of ketone body and the immunomodulatory tone under challenging conditions.

In addition to our findings, the higher IL-1β also could be linked to higher insulin concentration. Dalmas, (2019) reported *in vitro* an acute insulin secretory stimulus in the pancreatic β-cells mediated by exposure to IL-1β. Therefore, the increased the higher insulin concentrations in the hyperketonemic cows could be associated with increasing pro-inflammatory conditions, especially post-LPS bolus. Also, after recognizing a foreign agent, NOD-like receptor-3 (NLRP3) activates the protein caspase-1 (Molla et al., 2020). This activation in turn induces the maturation of IL-1β and its release (Lebreton et al., 2018; Tweedell et al., 2020; Onizawa et al., 2022a). This mechanism of action is attenuated by the BHB blocking the NLRP3 activation (Youm et al., 2015; Kim et al., 2020). However, this anti-inflammatory action of BHB can be overridden by insulin (Kim et al., 2020). So, despite having registered increased sustained hyperketonemia, the expected lower caspase-1 from the liver was likely affected by the increased insulin post-LPS challenge.

The recorded CRP concentrations in our study were reduced progressively post-LPS-bolus opposing our expectations from this positive APP (Gulhar et al., 2022). The triggered action by the pro-inflammatory IL-1β and TNF-α stimulates the CRP release from the liver (Gabay and Kushner, 1999; Saco and Bassols, 2023). Both cytokines were increased in response to the LPS bolus in both treatments in this study, therefore a positive CRP expression might have occurred. In humans, CRP concentration starts to rise after 4 h to 6 h and peaks between h 36 h and h 50 (Gulhar et al., 2022). Studies, implementing acute ruminal acidosis to modulate an LPS leaky-gut reported increasing CRP along several days of evaluations, but not within an hour change (Emmanuel et al., 2008; Zebeli and Ametaj, 2009; Dong et al., 2011). During the initial hours of endotoxemia mediated by the LPS leukopenia is a common feature (Kvidera et al., 2017; Chandler et al., 2022). Tavares et al., (2005) used a leukopenic-induced rat model to report decreased CRP concentration during the initial 12 h post-LPS bolus reproducing a similar trend to our results. Therefore, the expected increasing concentrations may have happened within a time range different from

our time set of evaluations. Nevertheless, future studies will be required to document the CRP concentration manifested under endotoxemia conditions.

One limitation of our study was the hypertonic confounding effect of the saline infusions. Studies have suggested changes in the immune tone affected by infused saline solutions under septic shock (Paydar et al., 2014; Semler and Rice, 2016; Gokcen et al., 2018). High sodium saline concentrations are associated with suppression in the neutrophil-mediated immune responses but also with an amplification of the macrophages' inflammatory response (Jobin et al., 2021). In addition, the continued infused salts might have altered the sodium ion concentration, a condition that could lead to hypernatremia and metabolic alkalosis. None of those conditions were measured in the present study and unfortunately, previous studies did not report blood pH or gases (Zarrin et al., 2013, 2014, 2017; Swartz et al., 2021b). Metabolic alkalosis predisposes cows to increased blood pH, milk fever, and subclinical hypocalcemia (Goff, 2008). So, it is plausible to suggest all these conditions have influenced the cow's health in our study. To overcome these obstacles, additional approaches will be required to assess the real role of BHB in dairy cows. Current research using ruminal ketogenic precursors is in this direction (Rico et al., 2021; Ruiz-González et al., 2022; Barrientos-Blanco et al., 2023).

Conclusion

Through a randomized controlled trial, we successfully isolated hyperketonemia from confounding events during the transition period. Under this condition, our model to induce hyperketonemia allowed the evaluation of BHB as a causal factor and its metabolic implications. Our findings demonstrated that the negative trajectories of reduced DMI and milk production, associated with BHB, were only reproduced when hyperketonemia was combined with the immune challenge. This finding highlights the need for additional cofactors beyond BHB to exert the impaired DMI and productivity described in the ketosis metabolic disorder. Future studies must focus on assessing the accuracy of the associations

on health attributed to ketosis to further elucidate the real role of the ketone bodies in the dairy cow's metabolism.

Bibliography

Abaker, J.A., T.L. Xu, D. Jin, G.J. Chang, K. Zhang, and X.Z. Shen. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. J Dairy Sci 100:666–678. doi:10.3168/jds.2016-10871.

Abbas, Z., A. Sammad, L. Hu, H. Fang, Q. Xu, and Y. Wang. 2020. Glucose metabolism and dynamics of facilitative glucose transporters (Gluts) under the influence of heat stress in dairy cattle. Metabolites 10:1–19. doi:10.3390/metabo10080312.

Abdallah, A., J. Hewson, D. Francoz, H. Selim, and S. Buczinski. 2016. Systematic Review of the Diagnostic Accuracy of Haptoglobin, Serum Amyloid A, and Fibrinogen versus Clinical Reference Standards for the Diagnosis of Bovine Respiratory Disease. J Vet Intern Med 30:1356–1368. doi:10.1111/JVIM.13975.

Abuajamieh, M., S.K. Kvidera, M.V.S. Fernandez, A. Nayeri, N.C. Upah, E.A. Nolan, S.M. Lei, J.M. DeFrain, H.B. Green, K.M. Schoenberg, W.E. Trout, and L.H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res Vet Sci 109:81–85. doi:10.1016/j.rvsc.2016.09.015.

Abuelo, A., J. Hernández, J.L. Benedito, and C. Castillo. 2016. Association of oxidative status and insulin sensitivity in periparturient dairy cattle: An observational study. J Anim Physiol Anim Nutr (Berl) 100:279–286. doi:10.1111/JPN.12365.

Ali, A., and G. Shook. 1980. An Optimum Transformation for Somatic Cell Concentration in Milk'. Journal fo Dairy Science 63:487–490. doi:10.3168/jds.S0022-0302(80)82959-6.

Allen, M.S. 2020. Review: Control of feed intake by hepatic oxidation in ruminant animals: integration of homeostasis and homeorhesis. doi:10.1017/S1751731119003215.

Alsemgeest, S.P., H.C. Kalsbeek, T. Wensing, J.P. Koeman, A.M. van Ederen, and E. Gruys. 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet Q 16:21–23. doi:10.1080/01652176.1994.9694410.

Ametaj, B.N., B.J. Bradford, G. Bobe, R.A. Nafikov, Y. Lu, J.W. Young, and D.C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can J Anim Sci 85:165–175. doi:10.4141/A04-043.

Anders, H.J., B. Suarez-Alvarez, M. Grigorescu, O. Foresto-Neto, S. Steiger, J. Desai, J.A. Marschner, M. Honarpisheh, C. Shi, J. Jordan, L. Müller, N. Burzlaff, T. Bäuerle, and S.R. Mulay. 2018. The macrophage phenotype and inflammasome component NLRP3 contributes to nephrocalcinosis-related chronic kidney disease independent from IL-1–mediated tissue injury. Kidney Int 93:656–669. doi:10.1016/J.KINT.2017.09.022.

Andrew, S.M., H.F. Tyrrell, C.K. Reynolds, and R.A. Erdman. 1991. Net Energy for Lactation of Calcium Salts of Long-Chain Fatty Acids for Cows Fed Silage-Based Diets. J Dairy Sci 74:2588–2600. doi:10.3168/jds.S0022-0302(91)78437-3.

Annison, E., and W. Bryden. 1998. Perspectives on ruminant nutrition and metabolism I. Metabolism in the Rumen. Nutr Res Rev 11:173` – 198.

Ari, C., C. Murdun, A.P. Koutnik, C.R. Goldhagen, C. Rogers, C. Park, S. Bharwani, D.M. Diamond, M.S. Kindy, D.P. D'Agostino, and Z. Kovács. 2019. Exogenous Ketones Lower Blood Glucose Level in Rested and Exercised Rodent Models. Nutrients 11. doi:10.3390/NU11102330.

Aschenbach, J.R., N.B. Kristensen, S.S. Donkin, H.M. Hammon, and G.B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB Life 62:869–877. doi:10.1002/IUB.400.

Athinarayanan, S.J., R.N. Adams, S.J. Hallberg, A.L. McKenzie, N.H. Bhanpuri, W.W. Campbell, J.S. Volek, S.D. Phinney, and J.P. McCarter. 2019. Long-Term

Effects of a Novel Continuous Remote Care Intervention Including Nutritional Ketosis for the Management of Type 2 Diabetes: A 2-Year Non-randomized Clinical Trial. Front Endocrinol (Lausanne) 10. doi:10.3389/fendo.2019.00348.

Baird, G.D., K.G. Hibbitt, G.D. Hunter, P. Lund, M. Stubbs, and H.A. Krebs. 1968. Biochemical aspects of bovine ketosis. Biochemical Journal 107:683. doi:10.1042/BJ1070683.

Balasse, E.O., and F. Féry. 1989. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 5:247–270. doi:10.1002/DMR.5610050304.

Baldwin, R.L., and M.J. Allison. 1983. Rumen metabolism.. J Anim Sci 57 Suppl 2:461–77.

Baldwin, R.L., and B. Jesse. 1996. Propionate Modulation of Ruminal Ketogenesis Attenuation of the inflammatory phenomena in the transition period of dairy cows View project Gut sensing in calves View project. Article in Journal of Animal Science. doi:10.2527/1996.7471694x.

Baldwin, R.L., R.W. Li, Y. Jia, and C.J. Li. 2018. Transcriptomic Impacts of Rumen Epithelium Induced by Butyrate Infusion in Dairy Cattle in Dry Period. Gene Regul Syst Bio 12. doi:10.1177/1177625018774798.

Barrientos-Blanco, M., A. Celemin-Sarmiento, M. da Silva, N. Bagheri, C. Mercado, N. Galdos, and J. Rico. 2023. The induction of nutritional ketosis in lactating dairy cows using calcium butyrate: effects on lactation performance and health. Page in American Dairy Science Association Conference, Ottawa.

Bauchart, D. 1993. Lipid Absorption and Transport in Ruminants. J Dairy Sci 76:3864–3881. doi:10.3168/JDS.S0022-0302(93)77728-0.

Bauman, D.E., R.E. Brown, and C.L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Arch Biochem Biophys 140:237–244. doi:10.1016/0003-9861(70)90028-7.

Bauman, D.E., and W. Bruce Currie. 1980. Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and

Homeorhesis. J Dairy Sci 63:1514–1529. doi:10.3168/jds.S0022-0302(80)83111-0.

Bauman, D.E., C.J. Peel, W.D. Steinhour, P.J. Reynolds, H.F. Tyrrell, A.C.G. Brown, and G.L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J Nutr 118:1031–1040. doi:10.1093/JN/118.8.1031.

Baumgard, L.H., R.J. Collier, and D.E. Bauman. 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. J Dairy Sci 100:10353–10366. doi:10.3168/JDS.2017-13242.

Beauchemin, K.A., E. Ungerfeld, and R. Gruninger. 2022. Control of Methanogenesis in Dairy Animals. Encyclopedia of Dairy Sciences 65–78. doi:10.1016/B978-0-12-818766-1.00072-6.

Bedford, A., and J. Gong. 2018. Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition 4:151–159. doi:10.1016/j.aninu.2017.08.010.

Belay, T.K., M. Svendsen, Z.M. Kowalski, and T. Ådnøy. 2017. Genetic parameters of blood β-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. J Dairy Sci 100:6298–6311. doi:10.3168/JDS.2016-12458.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation.. J Anim Sci 73:2804–2819. doi:10.2527/1995.7392804x.

Bell, A.W., and D.E. Bauman. 1997. Adaptations of Glucose Metabolism During Pregnancy and Lactation.

Bendridi, N., A. Selmi, A. Balcerczyk, and L. Pirola. 2022. Ketone Bodies as Metabolites and Signalling Molecules at the Crossroad between Inflammation and Epigenetic Control of Cardiometabolic Disorders. Int J Mol Sci 23:14564. doi:10.3390/ijms232314564.

Benedet, A., C.L. Manuelian, A. Zidi, M. Penasa, and M. De Marchi. 2019. Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. Pages 1676–1689 in Animal. Cambridge University Press.

Benson, J.D., and R.S. Emery. 1971. Fatty Acid Esterification by Homogenates of Bovine Liver and Adipose Tissue. J Dairy Sci 54:1034–1040. doi:10.3168/jds.S0022-0302(71)85967-2.

Benzaquen, M.E., C.A. Risco, L.F. Archbald, P. Melendez, M.J. Thatcher, and W.W. Thatcher. 2007. Rectal Temperature, Calving-Related Factors, and the Incidence of Puerperal Metritis in Postpartum Dairy Cows. J Dairy Sci 90:2804–2814. doi:10.3168/JDS.2006-482.

Bergman, E.N. 1971. Hyperketonemia-Ketogenesis and Ketone Body Metabolism. J Dairy Sci 54:936–948. doi:10.3168/jds.S0022-0302(71)85950-7.

Bergman, E.N. 1990. Energy Contributions of Volatile Fatty Acids From the Gastrointestinal Tract in Various Species.

Bergman, E.N., and K. Kon. 1964. Factors affecting acetoacetate production rates by normal and ketotic pregnant sheep. American Journal of Physiology 206:453–457. doi:10.1152/AJPLEGACY.1964.206.2.453.

Bernal-Santos, G., A.M. O'Donnell, J.L. Vicini, G.F. Hartnell, and D.E. Bauman. 2010. Hot topic: Enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. J Dairy Sci 93:32–37. doi:10.3168/jds.2009-2711.

Biden, T.J., and K.W. Taylor. 1983. Effects of ketone bodies on insulin release and islet-cell metabolism in the rat.. Biochemical Journal 212:371. doi:10.1042/BJ2120371.

Bionaz, M., E. Vargas-Bello-Pérez, and S. Busato. 2020. Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. J Anim Sci Biotechnol 11:1–36. doi:10.1186/s40104-020-00512-8.

Block, S.S., W.R. Butler, R.A. Ehrhardt, A.W. Bell, M.E. van Amburgh, and Y.R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance.

Bolon, B., G. Campagnuolo, L. Zhu, D. Duryea, D. Zack, and U. Feige. 2004. Interleukin-1β and tumor necrosis factor-α produce distinct, time-dependent patterns of acute arthritis in the rat knee. Vet Pathol 41:235–243. doi:10.1354/VP.41-3-235/ASSET/IMAGES/LARGE/10.1354_VP.41-3-235-FIG18.JPEG.

Bradford, B., R. Albornoz, and K. Kennedy. 2022. ABST: 1302. Fueling appetite: Nutrient metabolism and the control of feed intake. Pages 119–119 in Ruminant Nutritional Symposium: Integrating the control of energy intake and partitioning into ratio formulation-recognition of the contribution of ADSA fellow Mike Allen.

Bradford, B.J., L.K. Mamedova, J.E. Minton, J.S. Drouillard, and B.J. Johnson. 2009. Daily Injection of Tumor Necrosis Factor-α Increases Hepatic Triglycerides and Alters Transcript Abundance of Metabolic Genes in Lactating Dairy Cattle. J Nutr 139:1451–1456. doi:10.3945/JN.109.108233.

Bradford, B.J., and T.H. Swartz. 2020. Review: Following the smoke signals: Inflammatory signaling in metabolic homeostasis and homeorhesis in dairy cattle. Pages S144–S154 in Animal. Cambridge University Press.

Bradford, B.J., K. Yuan, J.K. Farney, L.K. Mamedova, and A.J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J Dairy Sci 98:6631–6650. doi:10.3168/JDS.2015-9683.

Bradshaw, P.C., W.A. Seeds, A.C. Miller, V.R. Mahajan, and W.M. Curtis. 2020. COVID-19: Proposing a Ketone-Based Metabolic Therapy as a Treatment to Blunt the Cytokine Storm. Oxid Med Cell Longev 2020. doi:10.1155/2020/6401341.

Brigham, K.L., and B. Meyrick. 1986. Endotoxin and lung injury.. Am Rev Respir Dis 133:913–927.

Brown, W.E., and B.J. Bradford. 2021. Invited review: Mechanisms of hypophagia during disease. J Dairy Sci 104:9418–9436. doi:10.3168/jds.2021-20217.

Burgos, S. 2000. Effects of bacterial lipopolysaccharide and dehydration on feeding and metabolism in bovines.

Butler, W.R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 83:211–218. doi:10.1016/S0301-6226(03)00112-X.

Capel, M.B., K.D. Bach, S. Mann, and J.A.A. McArt. 2021. A randomized controlled trial to evaluate propylene glycol alone or in combination with dextrose as a treatment for hyperketonemia in dairy cows. J Dairy Sci 104:2185–2194. doi:10.3168/jds.2020-19111.

Carretta, M.D., Y. Barría, K. Borquez, B. Urra, A. Rivera, P. Alarcón, M.A. Hidalgo, and R.A. Burgos. 2020. β-hydroxybutyrate and hydroxycarboxylic acid receptor 2 agonists activate the AKT, ERK and AMPK pathways, which are involved in bovine neutrophil chemotaxis. Sci Rep 10:12491. doi:10.1038/S41598-020-69500-2.

Chandler, T.L., T.A. Westhoff, A.S. Sipka, T.R. Overton, and S. Mann. 2022. Lipopolysaccharide challenge following intravenous amino acid infusion in postpartum dairy cows: II. Clinical and inflammatory responses. J Dairy Sci 105:4611–4623. doi:10.3168/JDS.2021-21227.

Chapinal, N., M. Carson, T. Duffield, M. Capel, S. Godden, M. Overton, J. Santos, and S. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J Dairy Sci 94:4897–4903. doi:10.3168/jds.2010-4075.

Chapinal, N., M.E. Carson, S.J. LeBlanc, K.E. Leslie, S. Godden, M. Capel, J.E.P. Santos, M.W. Overton, and T.F. Duffield. 2012a. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J Dairy Sci 95:1301–1309. doi:10.3168/JDS.2011-4724.

Chapinal, N., S.J. LeBlanc, M.E. Carson, K.E. Leslie, S. Godden, M. Capel, J.E.P. Santos, M.W. Overton, and T.F. Duffield. 2012b. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J Dairy Sci 95:5676–5682. doi:10.3168/JDS.2011-5132.

Chavez, J.A., T.A. Knotts, L.P. Wang, G. Li, R.T. Dobrowsky, G.L. Florant, and S.A. Summers. 2003. A Role for Ceramide, but Not Diacylglycerol, in the Antagonism of Insulin Signal Transduction by Saturated Fatty Acids *. Journal of Biological Chemistry 278:10297–10303. doi:10.1074/JBC.M212307200.

Choi, S.M., D.F. Tucker, D.N. Gross, R.M. Easton, L.M. DiPilato, A.S. Dean, B.R. Monks, and M.J. Birnbaum. 2010. Insulin Regulates Adipocyte Lipolysis via an Akt-Independent Signaling Pathway. Mol Cell Biol 30:5009. doi:10.1128/MCB.00797-10.

Clark, J., P. Vanegas, M. Panesar, and A. Cope. 2005. What does tumour necrosis factor excess do to the immune system long term?. Annals of the Rheumatic Diseases (ARD) 64:70–76. doi:10.1136/ard.2005.042523.

Contreras, G.A., E. Kabara, J. Brester, L. Neuder, and M. Kiupel. 2015. Macrophage infiltration in the omental and subcutaneous adipose tissues of dairy cows with displaced abomasum. J Dairy Sci 98:6176–6187. doi:10.3168/JDS.2015-9370.

Contreras, G.A., C. Strieder-Barboza, and W. Raphael. 2017. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J Anim Sci Biotechnol 8. doi:10.1186/S40104-017-0174-4.

Coppock, C.E. 1985. Energy Nutrition and Metabolism of the Lactating Dairy Cow. J Dairy Sci 68:3403–3410. doi:10.3168/jds.S0022-0302(85)81253-4.

Cortegiani, A., F. Madotto, C. Gregoretti, G. Bellani, J.G. Laffey, T. Pham, F. Van Haren, A. Giarratano, M. Antonelli, A. Pesenti, and G. Grasselli. 2018. Immunocompromised patients with acute respiratory distress syndrome: secondary analysis of the LUNG SAFE database. Crit Care 22:157. doi:10.1186/s13054-018-2079-9.

Cox, P.J., T. Kirk, T. Ashmore, K. Willerton, R. Evans, A. Smith, A.J. Murray, B. Stubbs, J. West, S.W. McLure, M.T. King, M.S. Dodd, C. Holloway, S. Neubauer, S. Drawer, R.L. Veech, J.L. Griffin, and K. Clarke. 2016. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. Cell Metab 24:256–268. doi:10.1016/j.cmet.2016.07.010.

Cuenoud, B., M. Hartweg, J.P. Godin, E. Croteau, M. Maltais, C.A. Castellano, A.C. Carpentier, and S.C. Cunnane. 2020. Metabolism of Exogenous D-Beta-Hydroxybutyrate, an Energy Substrate Avidly Consumed by the Heart and Kidney. Front Nutr 7. doi:10.3389/fnut.2020.00013.

Dalmas, E. 2019. Innate immune priming of insulin secretion. Curr Opin Immunol 56:44–49. doi:10.1016/J.COI.2018.10.005.

Dämmgen, U., H.-D. Haenel, C. Rösemann, N.J. Hutchings, W. Brade, and P. Lebzien. 2009. Improved national calculation procedures to assess energy requirements, nitrogen and VS excretions of dairy cows in the German emission model GAS-EM.

Dann, H.M., D.E. Morin, G.A. Bollero, M.R. Murphy, and J.K. Drackley. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J Dairy Sci 88:3249–3264. doi:10.3168/jds.S0022-0302(05)73008-3.

Davis, J.D., D. Wirtshafter, K.E. Asin, and D. Brief. 1981. Sustained Intracerebroventricular Infusion of Brain Fuels Reduces Body Weight and Food Intake in Rats. Science (1979) 212:81–83. doi:10.1126/SCIENCE.7193909.

Deemer, S.E., E.P. Plaisance, and C. Martins. 2020. Impact of ketosis on appetite regulation—a review. Nutrition Research 77:1–11. doi:10.1016/J.NUTRES.2020.02.010.

DeFrain, J.M., A.R. Hippen, K.F. Kalscheur, and D.J. Schingoethe. 2004. Feeding lactose increases ruminal butyrate and plasma β- hydroxybutyrate in lactating dairy cows. J Dairy Sci 87:2486–2494. doi:10.3168/jds.S0022-0302(04)73373-1.

Demigné, C., C. Yacoub, C. Rémésy, and P. Fafournoux. 1986. Propionate and butyrate metabolism in rat or sheep hepatocytes. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 875:535–542. doi:10.1016/0005-2760(86)90074-3.

Dinarello, C.A. 1999. Cytokines as Endogenous Pyrogens. J Infect Dis 179:294–304.

Dirksen, G.U., and E. Mayer. 1985. Adaptive Changes of the Ruminal Mucosa and Their Functional and Clinical Significance.

Domingues, D., M. de Beni, and R. Dias Lauritano. 2016. Rumenology. 1st ed. Springer, Cham, Switzerland.

Dong, G., S. Liu, Y. Wu, C. Lei, J. Zhou, and S. Zhang. 2011. Diet-induced bacterial immunogens in the gastrointestinal tract of dairy cows: Impacts on immunity and metabolism. Acta Vet Scand 53:1–7. doi:10.1186/1751-0147-53-48/METRICS.

Dong, G., M. Qiu, C. Ao, J. Zhou, Khas-Erdene, X. Wang, Z. Zhang, and Y. Yang. 2014. Feeding a high-concentrate corn straw diet induced epigenetic alterations in the mammary tissue of dairy cows. PLoS One 9. doi:10.1371/journal.pone.0107659.

Dong, Z., X. Sun, Y. Tang, S. Luo, H. Jia, Q. Xu, Q. Jiang, J.J. Loor, W. Xu, and C. Xu. 2022. β-hydroxybutyrate impairs monocyte function via the ROS-NLR family pyrin domain-containing three inflammasome (NLRP3) pathway in ketotic cows. Front Vet Sci 9:1252. doi:10.3389/FVETS.2022.925900/BIBTEX.

Drackley, J.K. 1999. ADSA foundation scholar award: Biology of dairy cows during the transition period: The final frontier?. J Dairy Sci 82:2259–2273. doi:10.3168/jds.s0022-0302(99)75474-3.

Drackley, J.K., T.R. Overton, and G.N. Douglas. 2001. Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. J Dairy Sci 84:E100–E112. doi:10.3168/jds.s0022-0302(01)70204-4.

Drackley, J.K., J.J. Veenhuizen, M.J. Richard, and J.W. Young. 1991. Metabolic Changes in Blood and Liver of Dairy Cows During Either Feed Restriction or Administration of 1,3-Butanediol. J Dairy Sci 74:4254–4264. doi:10.3168/jds.S0022-0302(91)78620-7.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J Dairy Sci 93:5764–5771. doi:10.3168/JDS.2010-3429.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2012. Risk factors and effects of postpartum anovulation in dairy cows. J Dairy Sci 95:1845–1854. doi:10.3168/jds.2011-4781.

Duffield, T. 2000. Subclinical Ketosis in Lactating Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice 16:231–253. doi:10.1016/S0749-0720(15)30103-1.

Duffield, T., K.D. Lissemore, B.W. McBride, and K.E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci 92:571–580. doi:10.3168/jds.2008-1507.

Eaton, J.W., P. Brandt, J.R. Mahoney, and J.T. Lee. 1982. Haptoglobin: A Natural Bacteriostat. Science (1979) 215:691–693. doi:10.1126/SCIENCE.7036344.

Eckel, E.F., and B.N. Ametaj. 2016. Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. J Dairy Sci 99:5967–5990. doi:10.3168/JDS.2015-10727.

Eckel, E.F., and B.N. Ametaj. 2020. Bacterial Endotoxins and Their Role in Periparturient Diseases of Dairy Cows: Mucosal Vaccine Perspectives. Dairy 2020, Vol. 1, Pages 61-90 1:61–90. doi:10.3390/DAIRY1010006.

Elander, L., L. Engström, M. Hallbeck, and A. Blomqvist. 2007. IL-1β and LPS induce anorexia by distinct mechanisms differentially dependent on microsomal prostaglandin E synthase-1. Am J Physiol Regul Integr Comp Physiol 292:258–267. doi:10.1152/AJPREGU.00511.2006.

Emmanuel, D.G.V., S.M. Dunn, and B.N. Ametaj. 2008. Feeding High Proportions of Barley Grain Stimulates an Inflammatory Response in Dairy Cows. J Dairy Sci 91:606–614. doi:10.3168/JDS.2007-0256.

Engelking, L.E., D.J. Ambrose, and M. Oba. 2022. Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration on serum inflammatory markers and productivity of dairy cows during the calving transition. J Dairy Sci 105:4144–4155. doi:10.3168/jds.2021-21553.

Evans, M., T.S. McClure, A.P. Koutnik, and B. Egan. 2022. Exogenous Ketone Supplements in Athletic Contexts: Past, Present, and Future. Sports Medicine 2022 52:1 52:25–67. doi:10.1007/S40279-022-01756-2.

Falkenhain, K., A. Daraei, S.C. Forbes, and J.P. Little. 2022. Effects of Exogenous Ketone Supplementation on Blood Glucose: A Systematic Review and Meta-analysis. Advances in Nutrition 13:1697–1714. doi:10.1093/advances/nmac036.

Finucane, O.M., C.L. Lyons, A.M. Murphy, C.M. Reynolds, R. Klinger, N.P. Healy, A.A. Cooke, R.C. Coll, L. Mcallan, K.N. Nilaweera, M.E. O'Reilly, A.C. Tierney, M.J. Morine, J.F. Alcala-Diaz, J. Lopez-Miranda, D.P. O'Connor, L.A. O'Neill, F.C. Mcgillicuddy, and H.M. Roche. 2015. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity. Diabetes 64:2116–2128. doi:10.2337/DB14-1098.

Fischer, C., M. Drillich, S. Odau, W. Heuwieser, R. Einspanier, and C. Gabler. 2010. Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the oestrous cycle and elevated in case of subclinical or clinical endometritis. Reprod Fertil Dev 22:818–829. doi:10.1071/RD09120.

Franchi, L., N. Warner, K. Viani, and G. Nuñez. 2009. Function of Nod-like receptors in microbial recognition and host defense. Blackwell Munksgaard • Immunological Reviews 227:106–128.

Froesch, E.R. 1967. The physiology and pharmacology of adipose tissue lipolysis: Its inhibition and implications for the treatment of diabetes. Diabetologia 3:475–487. doi:10.1007/BF01213565/METRICS.

Furukawa, E., Z. Chen, H. Ueshiba, Y. Wu, H. Chiba, Y. Yanagawa, S. Katagiri, M. Nagano, and S.P. Hui. 2021. Postpartum cows showed high oocyte triacylglycerols concurrently with high plasma free fatty acids. Theriogenology 176:174–182. doi:10.1016/J.THERIOGENOLOGY.2021.09.034.

Gabay, C., and I. Kushner. 1999. Acute-Phase Proteins and Other Systemic Responses to Inflammation. https://doi.org/10.1056/NEJM199902113400607 340:448–454. doi:10.1056/NEJM199902113400607.

Garner, J.B., M. Douglas, S.R.O. Williams, W.J. Wales, L.C. Marett, K. DiGiacomo, B.J. Leury, B.J. Hayes, J.B. Garner, M. Douglas, S.R.O. Williams, W.J. Wales, L.C. Marett, K. DiGiacomo, B.J. Leury, and B.J. Hayes. 2017. Responses of dairy

cows to short-term heat stress in controlled-climate chambers. Anim Prod Sci 57:1233–1241. doi:10.1071/AN16472.

Gattorno, M., S. Tassi, S. Carta, L. Delfino, F. Ferlito, M.A. Pelagatti, A. D'Osualdo, A. Buoncompagni, M.G. Alpigiani, M. Alessio, A. Martini, and A. Rubartelli. 2007. Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. Arthritis Rheum 56:3138–3148. doi:10.1002/ART.22842.

Gleerup, K.B., P.H. Andersen, L. Munksgaard, and B. Forkman. 2015. Pain evaluation in dairy cattle. Appl Anim Behav Sci 171:25–32. doi:10.1016/J.APPLANIM.2015.08.023.

Goff, J.P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal 176:50–57. doi:10.1016/j.tvjl.2007.12.020.

Goff, J.P., and R.L. Horst. 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders,. J Dairy Sci 80:1260–1268. doi:10.3168/JDS.S0022-0302(97)76055-7.

Gokcen, E., Y. Cevik, G. Pamukcu Gunaydin, N.O. Dogan, and M. Tekin. 2018. Change in Complete Blood Count Parameters and Vital Signs After Intravenous Infusion of 1 Liter of Normal Saline. Eurasian Journal of Emergency Medicine 17. doi:10.5152/eajem.2018.83007.

Goldhawk, C., N. Chapinal, D.M. Veira, D.M. Weary, and M.A.G. von Keyserlingk. 2009. Prepartum feeding behavior is an early indicator of subclinical ketosis. J Dairy Sci 92:4971–4977. doi:10.3168/jds.2009-2242.

Goldsby, R., T. Kindt, and B. Osborne. 2001. Kuby Immunology. 4th ed. W.H. FREEMAN AND COMPANY, New York.

Gordon, J.L., S.J. Leblanc, and T.F. Duffield. 2013. Ketosis Treatment in Lactating Dairy Cattle. doi:10.1016/j.cvfa.2013.03.001.

Górka, P., Z.M. Kowalski, R. Zabielski, and P. Guilloteau. 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. J Dairy Sci 101:4785–4800. doi:10.3168/jds.2017-14086.

Gozho, G.N., D.O. Krause, and J.C. Plaizier. 2007. Ruminal Lipopolysaccharide Concentration and Inflammatory Response During Grain-Induced Subacute Ruminal Acidosis in Dairy Cows. J Dairy Sci 90:856–866. doi:10.3168/JDS.S0022-0302(07)71569-2.

Griffin, M.E., M.J. Marcucci, G.W. Cline, K. Bell, N. Barucci, D. Lee, L.J. Goodyear, E.W. Kraegen, M.F. White, and G.I. Shulman. 1999. Free Fatty Acid-Induced Insulin Resistance Is Associated With Activation of Protein Kinase C and Alterations in the Insulin Signaling Cascade. 1270 DIABETES 48.

Grinberg, N., S. Elazar, I. Rosenshine, and N.Y. Shpigel. 2008. β-hydroxybutyrate abrogates formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic Escherichia coli. Infect Immun 76:2802–2807. doi:10.1128/IAI.00051-08/ASSET/C36B113B-0391-4750-A0B7-98586ED222E1/ASSETS/GRAPHIC/ZII0060873660004.JPEG.

Grinnell, K.L., H. Chichger, J. Braza, H. Duong, and E.O. Harrington. 2012. Protection against LPS-Induced Pulmonary Edema through the Attenuation of Protein Tyrosine Phosphatase–1B Oxidation. Am J Respir Cell Mol Biol 46:623–632. doi:10.1165/rcmb.2011-0271OC.

Gröhn, Y., L.A. Lindberg, M.L. Bruss, and T.B. Farver. 1983. Fatty infiltration of liver in spontaneously ketotic dairy cows. J Dairy Sci 66:2320–2328. doi:10.3168/JDS.S0022-0302(83)82088-8.

Gross, J., H.A. van Dorland, R.M. Bruckmaier, and F.J. Schwarz. 2011. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. J Dairy Sci 94:1820–1830. doi:10.3168/JDS.2010-3707.

Gross, J.J., L. Grossen-Rösti, S.K. Wall, O. Wellnitz, and R.M. Bruckmaier. 2020. Metabolic status is associated with the recovery of milk somatic cell count and milk secretion after lipopolysaccharide-induced mastitis in dairy cows. J Dairy Sci 103:5604–5615. doi:10.3168/jds.2019-18032.

Grossi, P., G. Bertoni, F.P. Cappelli, and E. Trevisi. 2013. Effects of the precalving administration of omega-3 fatty acids alone or in combination with acetylsalicylic

acid in periparturient dairy cows1. J Anim Sci 91:2657–2666. doi:10.2527/jas.2012-5661.

Gruber, L., W. Knaus, A. Susenbeth, F.J. Schwarz, T. Guggenberger, B. Fischer, H. Spiekers, H. Steingaß, U. Meyer, A. Münger, T. Jilg, and A. Obermaier. 2021. Evaluation of the net energy for lactation system and estimation of the energy requirements of dairy cows based on a comprehensive analysis of feeding trials. Arch Anim Nutr 75:1–17. doi:10.1080/1745039X.2020.1854595.

Grummer, R.R. 1993. Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy Cows. J Dairy Sci 76:3882–3896. doi:10.3168/jds.S0022-0302(93)77729-2.

Gruys, E., M.J.M. Toussaint, T.A. Niewold, and S.J. Koopmans. 2005. Review: Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B 6:1045. doi:10.1631/JZUS.2005.B1045.

Gulhar, R., M.A. Ashraf, and I. Jialal. 2022. Physiology, Acute Phase Reactants. StatPearls.

Guliński, P. 2021. Ketone bodies - causes and effects of their increased presence in cows' body fluids: A review.. Vet World 14:1492–1503. doi:10.14202/vetworld.2021.1492-1503.

Guo, J., G. Chang, K. Zhang, L. Xu, D. Jin, M. Shahid Bilal, and X. Shen. 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. Nanjing.

Haces, M.L., K. Hernández-Fonseca, O.N. Medina-Campos, T. Montiel, J. Pedraza-Chaverri, and L. Massieu. 2008. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. Exp Neurol 211:85–96. doi:10.1016/J.EXPNEUROL.2007.12.029.

Hackmann, T.J., D.K. Ngugi, J.L. Firkins, and J. Tao. 2017. Genomes of rumen bacteria encode atypical pathways for fermenting hexoses to short-chain fatty acids. Environ Microbiol 19:4670–4683. doi:10.1111/1462-2920.13929.

Hall, M.B. 2023. Invited review: Corrected milk: Reconsideration of common equations and milk energy estimates. J Dairy Sci 106:2230–2246. doi:10.3168/jds.2022-22219.

Hammon, D.S., I.M. Evjen, T.R. Dhiman, J.P. Goff, and J.L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol 113:21–29. doi:10.1016/j.vetimm.2006.03.022.

Hammon, H.M., G. Stürmer, F. Schneider, A. Tuchscherer, H. Blum, T. Engelhard, A. Genzel, R. Staufenbiel, and W. Kanitz. 2009. Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. J Dairy Sci 92:1554–1566. doi:10.3168/JDS.2008-1634.

Hansen, V.L., L.S. Faber, A.A. Salehpoor, and R.D. Miller. 2017. A pronounced uterine pro-inflammatory response at parturition is an ancient feature in mammals. Proceedings of the Royal Society B: Biological Sciences 284. doi:10.1098/RSPB.2017.1694.

Hanson, R.W., and F.J. Ballard. 1967. The Relative Significance of Acetate and Glucose as Precursors for Lipid Synthesis in Liver and Adipose Tissue from Ruminants.

Hart, I.C. 1983. Endocrine control of nutrient partition in lactating ruminants. Proceedings of the Nutrition Society 42:181–194. doi:10.1079/pns19830023.

Hartman, A.L., and J.M. Rho. 2014. The New Ketone Alphabet Soup: BHB, HCA, and HDAC. Epilepsy Curr 14:355. doi:10.5698/1535-7597-14.6.355.

Heitmann, R.N., D.J. Dawes, and S.C. Sensenig. 1987. Hepatic Ketogenesis and Peripheral Ketone Body Utilization in the Ruminant. J Nutr 117:1174–1180. doi:10.1093/JN/117.6.1174.

Herrick, K.J., A.R. Hippen, K.F. Kalscheur, D.J. Schingoethe, D.P. Casper, S.C. Moreland, and J.E. van Eys. 2017. Single-dose infusion of sodium butyrate, but not lactose, increases plasma β-hydroxybutyrate and insulin in lactating dairy cows. J Dairy Sci 100:757–768. doi:10.3168/jds.2016-11634.

Herrick, K.J., A.R. Hippen, K.F. Kalscheur, D.J. Schingoethe, S.D. Ranathunga, J.L. Anderson, S.C. Moreland, and J.E. van Eys. 2018. Infusion of butyrate affects plasma glucose, butyrate, and β-hydroxybutyrate but not plasma insulin in lactating dairy cows. J Dairy Sci 101:3524–3536. doi:10.3168/jds.2017-13842.

Hoeben, D., R. Heyneman, and C. Burvenich. 1997. Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. Vet Immunol Immunopathol 58:165–170. doi:10.1016/S0165-2427(97)00031-7.

Høgild, M.L., A.J. Hjelholt, J. Hansen, S.B. Pedersen, N. Møller, J.F.P. Wojtaszewski, M. Johannsen, N. Jessen, and J.O.L. Jørgensen. 2023. Ketone Body Infusion Abrogates Growth Hormone–Induced Lipolysis and Insulin Resistance. J Clin Endocrinol Metab 108:653–664. doi:10.1210/clinem/dgac595.

Holdsworth, D.A., P.J. Cox, T. Kirk, H. Stradling, S.G. Impey, and K. Clarke. 2017. A Ketone Ester Drink Increases Postexercise Muscle Glycogen Synthesis in Humans. Med Sci Sports Exerc 49:1789–1795. doi:10.1249/MSS.0000000000001292.

Horst, E.A., S.K. Kvidera, and L.H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas. J Dairy Sci 104:8380–8410. doi:10.3168/jds.2021-20330.

Horst, E.A., S.K. Kvidera, M.J. Dickson, C.S. McCarthy, E.J. Mayorga, M. Al-Qaisi, H.A. Ramirez, A.F. Keating, and L.H. Baumgard. 2019a. Effects of continuous and increasing lipopolysaccharide infusion on basal and stimulated metabolism in lactating Holstein cows. J Dairy Sci 102:3584–3597. doi:10.3168/JDS.2018-15627.

Horst, E.A., E.J. Mayorga, S. Rodriguez-Jimenez, M.A. Abeyta, B.M. Goetz, S. Carta, M. Al-Qaisi, S.K. Kvidera, and L.H. Baumgard. 2019b. Causes and Metabolic Consequences of Leaky Gut.

Huang, Z., J.B. Massey, and D. Via. 2000. Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis

factor-alpha (TNF-alpha) in human in vitro differentiated macrophages.. Biochem Pharmacol.

Hughes J. 1962. A simplified instrument for obtaining liver biopsies in cattle.. Am J Vet Res 23:1111–3.

Huhtanen, P., H. Miettinen, and M. Ylinen. 1993. Effect of Increasing Ruminal Butyrate on Milk Yield and Blood Constituents in Dairy Cows Fed a Grass Silage-Based Diet. J Dairy Sci 76:1114–1124. doi:10.3168/jds.S0022-0302(93)77440-8.

Hui, S., A.J. Cowan, X. Zeng, L. Yang, T. TeSlaa, X. Li, C. Bartman, Z. Zhang, C. Jang, L. Wang, W. Lu, J. Rojas, J. Baur, and J.D. Rabinowitz. 2020. Quantitative Fluxomics of Circulating Metabolites. Cell Metab 32:676-688.e4. doi:10.1016/J.CMET.2020.07.013.

Imhasly, S., H. Naegeli, S. Baumann, M. von Bergen, A. Luch, H. Jungnickel, S. Potratz, and C. Gerspach. 2014. Metabolomic biomarkers correlating with hepatic lipidosis in dairy cows. BMC Vet Res 10:1–9. doi:10.1186/1746-6148-10-122/FIGURES/5.

Izumi, K., R. Fukumori, S. Oikawa, and M. Oba. 2019. Short communication: Effects of butyrate supplementation on the productivity of lactating dairy cows fed diets differing in starch content. J Dairy Sci 102:11051–11056. doi:10.3168/jds.2019-17113.

Jacobs, A.A.A., J. Dijkstra, J.S. Liesman, M.J. Vandehaar, A.L. Lock, A.M. van Vuuren, W.H. Hendriks, and J. van Baal. 2013. Effects of short- and long-chain fatty acids on the expression of stearoyl-CoA desaturase and other lipogenic genes in bovine mammary epithelial cells. Animal 7:1508–1516. doi:10.1017/S175173111300061X.

Jain, S.K., K. Kannan, G. Lim, J. Matthews-Greek, R. McVie, and J.A. Bocchini. 2003. Elevated blood interleukin-6 levels in hyperketonemic type 1 diabetic patients and secretion by acetoacetate-treated cultured U937 monocytes. Diabetes Care 26:2139–2143. doi:10.2337/DIACARE.26.7.2139.

Jain, S.K.;, and R. Mcvie. 1999. Hyperketonemia can increase lipid peroxidation and lower glutathione. Diabetes 48:1850.

Jenkins, D.J.A., W.M. Hunter, and D. V. Goff. 1970. Ketone Bodies and Evidence for increased Insulin Secretion. Nature 1970 227:5256 227:384–385. doi:10.1038/227384a0.

Jenkins, T.C. 1993. Lipid Metabolism in the Rumen. J Dairy Sci 76:3851–3863. doi:10.3168/JDS.S0022-0302(93)77727-9.

Jesse, B.W., R.S. Emery, and J.W. Thomas. 1986. Control of Bovine Hepatic Fatty Acid Oxidation. J Dairy Sci 69:2290–2297. doi:10.3168/jds.S0022-0302(86)80667-1.

Jobin, K., D.N. Müller, J. Jantsch, and C. Kurts. 2021. Sodium and its manifold impact on our immune system. Trends Immunol 42:469–479. doi:10.1016/j.it.2021.04.002.

Kaneko, N., M. Kurata, T. Yamamoto, S. Morikawa, and J. Masumoto. 2019. The role of interleukin-1 in general pathology. Inflammation and Regeneration 2019 39:1 39:1–16. doi:10.1186/S41232-019-0101-5.

Kashiwaya, Y., M.T. King, and R.L. Veech. 1997. Substrate Signaling by Insulin: A Ketone Bodies Ratio Mimics Insulin Action in Heart. American Journal of Cardiology 80:50A-64A. doi:10.1016/S0002-9149(97)00458-X.

Kayano, M., and T. Kataoka. 2015. Screening for ketosis using multiple logistic regression based on milk yield and composition. J Vet Med Sci 77:1473. doi:10.1292/JVMS.14-0691.

Kelley, N., D. Jeltema, Y. Duan, and Y. He. 2019. Molecular Sciences The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. doi:10.3390/ijms20133328.

Kelly, B., and L.A.J. O'Neill. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 25:771–784. doi:10.1038/CR.2015.68.

Kerner, J., and C.L. Hoppel. 2004. Ketogenesis. Elsevier.

Kesl, S.L., A.M. Poff, N.P. Ward, T.N. Fiorelli, C. Ari, A.J. Van Putten, J.W. Sherwood, P. Arnold, and D.P. D'Agostino. 2016. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in

Sprague-Dawley rats. Nutr Metab (Lond) 13:1–15. doi:10.1186/S12986-016-0069-Y/FIGURES/7.

Khafipour, E., D.O. Krause, and J.C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci 92:1060–1070. doi:10.3168/JDS.2008-1389.

Kim, S.R., S.G. Lee, S.H. Kim, J.H. Kim, E. Choi, W. Cho, J.H. Rim, I. Hwang, C.J. Lee, M. Lee, C.M. Oh, J.Y. Jeon, H.Y. Gee, J.H. Kim, B.W. Lee, E.S. Kang, B.S. Cha, M.S. Lee, J.W. Yu, J.W. Cho, J.S. Kim, and Y. ho Lee. 2020. SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nature Communications 2020 11:1 11:1–11. doi:10.1038/s41467-020-15983-6.

Kováč, G., C. Tóthová, O. Nagy, H. Seidel, and J. Konvičná. 2009. Acute Phase Proteins and their Relation to Energy Metabolites in Dairy Cows during the Pre- and Postpartal Period. Acta Veterinaria Brno 78:441–447. doi:10.2754/AVB200978030441.

Krebs, H.A. 1966. Bovine ketosis. Vet Rec 78:187–192. doi:10.1136/VR.78.6.187.

Krehbiel, C.R., D.L. Harmon, and J.E. Schneider. 1992a. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers.. J Anim Sci 70:904–914. doi:10.2527/1992.703904x.

Krehbiel, C.R., D.L. Harmon, and J.E. Schneider. 1992b. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. J Anim Sci 70:904–914. doi:10.2527/1992.703904x.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: implications for insufficient feed intake of transition dairy cows. Animal 14:s65–s77. doi:10.1017/S1751731119003124.

Kurtz, R., M.F. Anderman, and B.D. Shepard. 2021. GPCRs get fatty: the role of G protein-coupled receptor signaling in the development and progression of nonalcoholic fatty liver disease. American Journal of Physiology-Gastrointestinal and Liver Physiology 320:G304–G318. doi:10.1152/ajpgi.00275.2020.

Kvidera, S.K., E.A. Horst, M. Abuajamieh, E.J. Mayorga, M.V.S. Fernandez, and L.H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J Dairy Sci 100:2360–2374. doi:10.3168/jds.2016-12001.

Kyoung Lee, A., D.H. Kim, E.J. Bang, Y.J. Choi, and H.Y. Chung. 2020. β-Hydroxybutyrate Suppresses Lipid Accumulation in Aged Liver through GPR109A-mediated Signaling. Aging Dis 11:777. doi:10.14336/AD.2019.0926.

Laarman, A.H., A. Kleinberg, M.A. Steele, O. AlZahal, and B.W. McBride. 2015. Changes in the Rumen Papillae During the Periparturient Transition in Holstein Dairy Cows are Accompanied by Changes in Abundance of Proteins Involved in Intracellular pH Regulation, But not SCFA Transport. Am J Anim Vet Sci 10:14–22. doi:10.3844/ajavsp.2015.14.22.

Laffel, L. 1999. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 15:412–426. doi:10.1002/(sici)1520-7560(199911/12)15:6<412::aid-dmrr72>3.0.co;2-8.

Langhans, W., F. Wiesenreiter, and E. Scharrer. 1983. Different effects of subcutaneous D,L-3-hydroxybutyrate and acetoacetate injections on food intake in rats. Physiol Behav 31:483–486. doi:10.1016/0031-9384(83)90070-7.

LeBlanc, S. 2015. Managing Ketosis in the Transition Cow for Health and Reproduction. Pages 94–101 in Dairy Cattle Reproduction Council.

LeBlanc, S.J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. The Veterinary Journal 176:102–114. doi:10.1016/j.tvjl.2007.12.019.

LeBlanc, S.J., K.E. Leslie, and T.F. Duffield. 2005. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. J Dairy Sci 88:159–170. doi:10.3168/JDS.S0022-0302(05)72674-6.

LeBlanc, S.J., K.D. Lissemore, D.F. Kelton, T.F. Duffield, and K.E. Leslie. 2006. Major Advances in Disease Prevention in Dairy Cattle. J Dairy Sci 89:1267–1279. doi:10.3168/JDS.S0022-0302(06)72195-6.

LeBlanc, S.J., T. Osawa, and J. Dubuc. 2011. Reproductive tract defense and disease in postpartum dairy cows. Theriogenology 76:1610–1618. doi:10.1016/J.THERIOGENOLOGY.2011.07.017.

Lebreton, F., E. Berishvili, G. Parnaud, C. Rouget, D. Bosco, T. Berney, and V. Lavallard. 2018. NLRP3 inflammasome is expressed and regulated in human islets. Cell Death & Disease 2018 9:7 9:1–10. doi:10.1038/s41419-018-0764-x.

Lee, A.K., D.H. Kim, E. Bang, Y.J. Choi, and H.Y. Chung. 2020. β-Hydroxybutyrate Suppresses Lipid Accumulation in Aged Liver through GPR109A-mediated Signaling. Aging Dis 11:777. doi:10.14336/AD.2019.0926.

Li, B., Y. Yu, K. Liu, Y. Zhang, Q. Geng, F. Zhang, Y. Li, and J. Qi. 2021. β-Hydroxybutyrate inhibits histone deacetylase 3 to promote claudin-5 generation and attenuate cardiac microvascular hyperpermeability in diabetes. Diabetologia 64:226–239. doi:10.1007/S00125-020-05305-2.

Li, P., X.B. Li, S.X. Fu, C.C. Wu, X.X. Wang, G.J. Yu, M. Long, Z. Wang, and G.W. Liu. 2012a. Alterations of fatty acid β-oxidation capability in the liver of ketotic cows. J Dairy Sci 95:1759–1766. doi:10.3168/JDS.2011-4580.

Li, R.W., S. Wu, R.L. Baldwin, W. Li, and C. Li. 2012b. Perturbation Dynamics of the Rumen Microbiota in Response to Exogenous Butyrate. PLoS One 7:e29392. doi:10.1371/journal.pone.0029392.

Li, Y., H. Ding, X. Wang, L. Liu, D. Huang, R. Zhang, L. Guo, Z. Wang, X. Li, G. Liu, J. Wu, and X. Li. 2016. High levels of acetoacetate and glucose increase expression of cytokines in bovine hepatocytes, through activation of the NF-κB signalling pathway. Journal of Dairy Research 83:51–57. doi:10.1017/S0022029915000680.

Liang, D., L.M. Arnold, C.J. Stowe, R.J. Harmon, and J.M. Bewley. 2017. Estimating US dairy clinical disease costs with a stochastic simulation model. J Dairy Sci 100:1472–1486. doi:10.3168/JDS.2016-11565.

Liang, J.J., I.D.C. Fraser, and C.E. Bryant. 2021. Lipid regulation of NLRP3 inflammasome activity through organelle stress. Trends Immunol 42:807–823. doi:10.1016/J.IT.2021.07.005.

Linn, J. 2006. Feed Efficiency: Its Economic Impact In Lactating Dairy Cows.

Lisuzzo, A., F. Fiore, K. Harvatine, E. Mazzotta, M. Berlanda, N. Spissu, T. Badon, B. Contiero, L. Moscati, and E. Fiore. 2022. Changes in plasma fatty acids profile in hyperketonemic ewes during early lactation: a preliminary study. Sci Rep 12:17017. doi:10.1038/s41598-022-21088-5.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206. doi:10.1007/s11745-004-1348-6.

Loft, A., S.F. Schmidt, G. Caratti, U. Stifel, J. Havelund, R. Sekar, Y. Kwon, A. Sulaj, K.K. Chow, A.J. Alfaro, T. Schwarzmayr, N. Rittig, M. Svart, F.F. Tsokanos, A. Maida, A. Blutke, A. Feuchtinger, N. Møller, M. Blüher, P. Nawroth, J. Szendrödi, N.J. Færgeman, A. Zeigerer, J. Tuckermann, and S. Herzig. 2022. A macrophage-hepatocyte glucocorticoid receptor axis coordinates fasting ketogenesis. Cell Metab 34:473-486.e9. doi:10.1016/J.CMET.2022.01.004.

Lukasova, M., J. Hanson, S. Tunaru, and S. Offermanns. 2011. Nicotinic acid (niacin): new lipid-independent mechanisms of action and therapeutic potentials. Trends Pharmacol Sci 32:700–707. doi:10.1016/J.TIPS.2011.08.002.

Luo, Z.Z., L.H. Shen, J. Jiang, Y.X. Huang, L.P. Bai, S.M. Yu, X.P. Yao, Z.H. Ren, Y.X. Yang, and S.Z. Cao. 2019. Plasma metabolite changes in dairy cows during parturition identified using untargeted metabolomics. J Dairy Sci 102:4639–4650. doi:10.3168/jds.2018-15601.

Lyons, C.L., and H.M. Roche. 2018. Nutritional Modulation of AMPK-Impact upon Metabolic-Inflammation. International Journal of Molecular Sciences 2018, Vol. 19, Page 3092 19:3092. doi:10.3390/IJMS19103092.

Ma, D., A.C. Wang, I. Parikh, S.J. Green, J.D. Hoffman, G. Chlipala, M.P. Murphy, B.S. Sokola, B. Bauer, A.M.S. Hartz, and A.-L. Lin. 2018. Ketogenic diet enhances neurovascular function with altered gut microbiome in young healthy mice. Sci Rep 8:6670. doi:10.1038/s41598-018-25190-5.

MacKellar, M., and D.J. Vigerust. 2016. Role of Haptoglobin in Health and Disease: A Focus on Diabetes. Clin Diabetes 34:148. doi:10.2337/DIACLIN.34.3.148.

Madison, L.L., D. Mebane, R.H. Unger, and A. Lochner. 1964. THE HYPOGLYCEMIC ACTION OF KETONES. II. EVIDENCE FOR A STIMULATORY. J. Clin. Invest. 43:408. doi:10.1172/jci104925.

Maizels, E.Z., N.B. Ruderman, M.N. Goodman, and D. Lau. 1977. Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat.. Biochemical Journal 162:557. doi:10.1042/BJ1620557.

Malik, A., and T.-D. Kanneganti. 2017. Inflammasome activation and assembly at a glance. J Cell Sci 130:3955–3963. doi:10.1242/jcs.207365.

Mann, S., F.A. Leal Yepes, J.J. Wakshlag, E. Behling-Kelly, and J.A.A. McArt. 2018. The effect of different treatments for early-lactation hyperketonemia on liver triglycerides, glycogen, and expression of key metabolic enzymes in dairy cattle. J Dairy Sci 101:1626–1637. doi:10.3168/JDS.2017-13360.

le Marchand-Brustel, Y., P. Gual, T. Grémeaux, T. Gonzalez, R. Barrès, and J.F. Tanti. 2003. Fatty acid-induced insulin resistance: role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling. Biochem Soc Trans 31:1152–1156. doi:10.1042/BST0311152.

Martens, H., I. Rabbani, Z. Shen, F. Stumpff, and C. Deiner. 2012. Changes in rumen absorption processes during transition. Anim Feed Sci Technol 172:95–102. doi:10.1016/J.ANIFEEDSCI.2011.12.011.

Masi, D., M.E. Spoltore, R. Rossetti, M. Watanabe, R. Tozzi, A. Caputi, R. Risi, A. Balena, O. Gandini, S. Mariani, G. Spera, L. Gnessi, and C. Lubrano. 2022. The Influence of Ketone Bodies on Circadian Processes Regarding Appetite, Sleep and Hormone Release: A Systematic Review of the Literature. Nutrients 14. doi:10.3390/NU14071410.

Matsuki, T., R. Horai, K. Sudo, and Y. Iwakura. 2003. IL-1 Plays an Important Role in Lipid Metabolism by Regulating Insulin Levels under Physiological Conditions. J Exp Med 198:877. doi:10.1084/JEM.20030299.

McArt, J.A.A., D. V. Nydam, and G.R. Oetzel. 2012a. Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci 95:5056–5066. doi:10.3168/jds.2012-5443.

McArt, J.A.A., D. V. Nydam, and G.R. Oetzel. 2012b. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J Dairy Sci 95:2505–2512. doi:10.3168/jds.2011-4908.

McArt, J.A.A., D. v. Nydam, G.R. Oetzel, and C.L. Guard. 2014. An economic analysis of hyperketonemia testing and propylene glycol treatment strategies in early lactation dairy cattle. Prev Vet Med 117:170–179. doi:10.1016/J.PREVETMED.2014.06.017.

McArt, J.A.A., D. V. Nydam, G.R. Oetzel, T.R. Overton, and P.A. Ospina. 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Veterinary Journal 198:560–570. doi:10.1016/j.tvjl.2013.08.011.

McArt, J.A.A., D. V. Nydam, P.A. Ospina, and G.R. Oetzel. 2011. A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. J Dairy Sci 94:6011–6020. doi:10.3168/JDS.2011-4463.

McArt, J.A.A., and G.R. Oetzel. 2015. A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. J Dairy Sci 98:7408–7418. doi:10.3168/JDS.2015-9479.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. Nature 2008 454:7203 454:428–435. doi:10.1038/nature07201.

Meng, L., Z. Song, A. Liu, U. Dahmen, X. Yang, and H. Fang. 2021. Effects of Lipopolysaccharide-Binding Protein (LBP) Single Nucleotide Polymorphism (SNP) in Infections, Inflammatory Diseases, Metabolic Disorders and Cancers. Front Immunol 12:2469. doi:10.3389/FIMMU.2021.681810/BIBTEX.

Mensching, A., M. Zschiesche, J. Hummel, C. Grelet, N. Gengler, S. Dänicke, and A.R. Sharifi. 2021. Development of a subacute ruminal acidosis risk score and its prediction using milk mid-infrared spectra in early-lactation cows. J Dairy Sci 104:4615–4634. doi:10.3168/jds.2020-19516.

Meroni, E., N. Papini, F. Criscuoli, M.C. Casiraghi, L. Massaccesi, N. Basilico, and D. Erba. 2018. Metabolic Responses in Endothelial Cells Following Exposure to Ketone Bodies. Nutrients 10. doi:10.3390/NU10020250.

Mezzetti, M., L. Cattaneo, M.M. Passamonti, V. Lopreiato, A. Minuti, and E. Trevisi. 2021. The Transition Period Updated: A Review of the New Insights into the Adaptation of Dairy Cows to the New Lactation. Dairy 2:617–636. doi:10.3390/dairy2040048.

Mielenz, M. 2017. Invited review: nutrient-sensing receptors for free fatty acids and hydroxycarboxylic acids in farm animals. Animal 11:1008–1016. doi:10.1017/S175173111600238X.

Van Miert, A.S.J.P.A.M. 2011. Pro-inflammatory cytokines in a ruminant model: Pathophysiological, pharmacological, and therapeutic aspects. http://dx.doi.org/10.1080/01652176.1995.9694530 17:41–50. doi:10.1080/01652176.1995.9694530.

Mihaylova, M.M., D.S. Vasquez, K. Ravnskjaer, P.D. Denechaud, R.T. Yu, J.G. Alvarez, M. Downes, R.M. Evans, M. Montminy, and R.J. Shaw. 2011. Class IIa Histone Deacetylases are Hormone-activated regulators of FOXO and Mammalian Glucose Homeostasis. Cell 145:607. doi:10.1016/J.CELL.2011.03.043.

Mills, S.E., D.C. Beitz, and J.W. Young. 1986. Characterization of Metabolic Changes during a Protocol for Inducing Lactation Ketosis in Dairy Cows. J Dairy Sci 69:352–361. doi:10.3168/jds.S0022-0302(86)80413-1.

Moallem, U. 2018. Invited review: Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J Dairy Sci 101:8641–8661. doi:10.3168/jds.2018-14772.

Mohammad, S., and C. Thiemermann. 2021. Role of Metabolic Endotoxemia in Systemic Inflammation and Potential Interventions. Front Immunol 11. doi:10.3389/fimmu.2020.594150.

Mohsin, M.A., H. Yu, R. He, P. Wang, L. Gan, Y. Du, Y. Huang, M.B. Abro, S. Sohaib, M. Pierzchala, P. Sobiech, K. Miętkiewska, C.S. Pareek, and B.X. He.

2022. Differentiation of Subclinical Ketosis and Liver Function Test Indices in Adipose Tissues Associated With Hyperketonemia in Postpartum Dairy Cattle. Front Vet Sci 8:1684. doi:10.3389/FVETS.2021.796494/BIBTEX.

Molla, M.D., Y. Akalu, Z. Geto, B. Dagnew, B. Ayelign, and T. Shibabaw. 2020. Role of caspase-1 in the pathogenesis of inflammatory-associated chronic noncommunicable diseases. J Inflamm Res 13:749–764. doi:10.2147/JIR.S277457.

Murray, R.K. (Robert K., D.A. Bender, K.M. Botham, P.J. Kennelly, V.W. Rodwell, and P.A. Weil. 2009. Harper's Illustrated Biochemistry. McGraw-Hill Medical.

Myette-Côté, É., H. Neudorf, H. Rafiei, K. Clarke, and J.P. Little. 2018. Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. J Physiol 596:1385. doi:10.1113/JP275709.

Nagaraja, T.G., and M.M. Chengappa. 1996. Bud Britton Memorial Symposium on Metabolic Disorders of Feedlot Cattle.

National Research Council. 1980. Effect of Environment on Nutrient Requirements of Domestic Animals. National Academies Press.

National Research Council (U.S.). Subcommittee on Dairy Cattle Nutrition. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press.

Newman, J.C., and E. Verdin. 2017. β-Hydroxybutyrate: A Signaling Metabolite. Annu Rev Nutr 37:51. doi:10.1146/ANNUREV-NUTR-071816-064916.

Novak, T.E., S.L. Rodriguez-Zas, B.R. Southey, J.D. Starkey, R.M. Stockler, G.F. Alfaro, and S.J. Moisá. 2019. Jersey steer ruminal papillae histology and nutrigenomics with diet changes. J Anim Physiol Anim Nutr (Berl) 103:1694–1707. doi:10.1111/JPN.13189.

Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 20:651–674. doi:10.1016/J.CVFA.2004.06.006.

Oetzel, G.R. 2013. Understanding the Impact of Subclinical Ketosis. University of Wisconsin: Madison 15–26.

Oh, K.J., H.S. Han, M.J. Kim, and S.H. Koo. 2013. CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. BMB Rep 46:567. doi:10.5483/BMBREP.2013.46.12.248.

Okawa, T., M. Nagai, and K. Hase. 2021. Dietary Intervention Impacts Immune Cell Functions and Dynamics by Inducing Metabolic Rewiring. Front Immunol 11:3756. doi:10.3389/FIMMU.2020.623989/BIBTEX.

Omenetti, S., and T.T. Pizarro. 2015. The Treg/Th17 axis: A dynamic balance regulated by the gut microbiome. Front Immunol 6:639. doi:10.3389/FIMMU.2015.00639/BIBTEX.

Onizawa, Y., T. Katoh, R. Miura, K. Konda, T. Noguchi, H. Iwata, T. Kuwayama, S. Hamano, and K. Shirasuna. 2022a. Acetoacetate is a trigger of NLRP3 inflammasome activation in bovine peripheral blood mononuclear cells. Vet Immunol Immunopathol 244. doi:10.1016/J.VETIMM.2021.110370.

Onizawa, Y., T. Katoh, R. Miura, K. Konda, T. Noguchi, H. Iwata, T. Kuwayama, S. Hamano, and K. Shirasuna. 2022b. Acetoacetate is a trigger of NLRP3 inflammasome activation in bovine peripheral blood mononuclear cells. Vet Immunol Immunopathol 244. doi:10.1016/J.VETIMM.2021.110370.

Osorio, J.S., J. Lohakare, and M. Bionaz. 2016. Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. Physiol Genomics 48:231–256. doi:10.1152/physiolgenomics.00016.2015.

Ospina, P.A., J.A. McArt, T.R. Overton, T. Stokol, and D. V. Nydam. 2013. Using Nonesterified Fatty Acids and β-Hydroxybutyrate Concentrations During the Transition Period for Herd-Level Monitoring of Increased Risk of Disease and Decreased Reproductive and Milking Performance. Veterinary Clinics of North America: Food Animal Practice 29:387–412. doi:10.1016/J.CVFA.2013.04.003.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010a. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J Dairy Sci 93:546–554. doi:10.3168/JDS.2009-2277.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010b. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J Dairy Sci 93:1596–1603. doi:10.3168/JDS.2009-2852.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010c. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J Dairy Sci 93:1596–1603. doi:10.3168/JDS.2009-2852.

Overton, T.R., J.A.A. McArt, and D. V. Nydam. 2017. A 100-Year Review: Metabolic health indicators and management of dairy cattle. J Dairy Sci 100:10398–10417. doi:10.3168/jds.2017-13054.

Pan, A., X.M. Sun, F.Q. Huang, J.F. Liu, Y.Y. Cai, X. Wu, R.N. Alolga, P. Li, B.L. Liu, Q. Liu, and L.W. Qi. 2022a. The mitochondrial β-oxidation enzyme HADHA restrains hepatic glucagon response by promoting β-hydroxybutyrate production. Nat Commun 13. doi:10.1038/S41467-022-28044-X.

Pan, A., X.-M. Sun, F.-Q. Huang, J.-F. Liu, Y.-Y. Cai, X. Wu, R.N. Alolga, P. Li, B.-L. Liu, Q. Liu, and L.-W. Qi. 2022b. The mitochondrial β-oxidation enzyme HADHA restrains hepatic glucagon response by promoting β-hydroxybutyrate production. Nat Commun 13:386. doi:10.1038/s41467-022-28044-x.

Park, S., D.S. Kim, and J.W. Daily. 2011. Central infusion of ketone bodies modulates body weight and hepatic insulin sensitivity by modifying hypothalamic leptin and insulin signaling pathways in type 2 diabetic rats. Brain Res 1401:95–103. doi:10.1016/J.BRAINRES.2011.05.040.

Paydar, S., H. Bazrafkan, N. Golestani, J. Roozbeh, A. Akrami, and A.M. Moradi. 2014. Effects of Intravenous Fluid Therapy on Clinical and Biochemical Parameters of Trauma Patients.. Emerg (Tehran) 2:90–5.

Pérez-Báez, J., C.A. Risco, R.C. Chebel, G.C. Gomes, L.F. Greco, S. Tao, I.M. Thompson, B.C. do Amaral, M.G. Zenobi, N. Martinez, C.R. Staples, G.E. Dahl,

J.A. Hernández, J.E.P. Santos, and K.N. Galvão. 2019. Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis. J Dairy Sci 102:9151–9164. doi:10.3168/jds.2018-15879.

Pérez-Hernández, E.G., B. Delgado-Coello, I. Luna-Reyes, and J. Mas-Oliva. 2021. New insights into lipopolysaccharide inactivation mechanisms in sepsis. Biomedicine & Pharmacotherapy 141:111890. doi:10.1016/J.BIOPHA.2021.111890.

Petersen, M.C., and G.I. Shulman. 2018. Mechanisms of Insulin Action and Insulin Resistance. Physiol Rev 98:2133. doi:10.1152/PHYSREV.00063.2017.

Peterson, S.E., P. Rezamand, J.E. Williams, W. Price, M. Chahine, and M.A. McGuire. 2012. Effects of dietary betaine on milk yield and milk composition of mid-lactation Holstein dairy cows. J Dairy Sci 95:6557–6562. doi:10.3168/JDS.2011-4808.

Piñeiro, J.M., B.T. Menichetti, A.A. Barragan, A.E. Relling, W.P. Weiss, S. Bas, and G.M. Schuenemann. 2019. Associations of postpartum lying time with culling, milk yield, cyclicity, and reproductive performance of lactating dairy cows. J Dairy Sci 102:3362–3375. doi:10.3168/jds.2018-15387.

Poff, A.M., A.P. Koutnik, and B. Egan. 2020. Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. Curr Sports Med Rep 19:251–259. doi:10.1249/JSR.0000000000000732.

Polawska, E., E. Bagnicka, and A. Wierzbicka. 2012. Relations between the oxidative status, mastitis, milk quality and disorders of reproductive functions in dairy cows - a review. Anim Sci Pap Rep 30:297–307.

Popko, K., E. Gorska, A. Stelmaszczyk-Emmel, R. Plywaczewski, A. Stoklosa, D. Gorecka, B. Pyrzak, and U. Demkow. 2010. Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects. Eur J Med Res 15:120–122. doi:10.1186/2047-783X-15-S2-120/FIGURES/3.

Pralle, R.S., J.D. Amdall, R.H. Fourdraine, G.R. Oetzel, and H.M. White. 2021. Hyperketonemia Predictions Provide an On-Farm Management Tool with Epidemiological Insights. Animals (Basel) 11. doi:10.3390/ANI11051291.

Puchalska, P., S.E. Martin, X. Huang, J.E. Lengfeld, B. Daniel, M.J. Graham, X. Han, L. Nagy, G.J. Patti, and P.A. Crawford. 2019. Hepatocyte-Macrophage Acetoacetate Shuttle Protects against Tissue Fibrosis. Cell Metab 29:383-398.e7. doi:10.1016/J.CMET.2018.10.015/ATTACHMENT/C08FA489-8C1E-4811-8CDE-9C6EDD469458/MMC6.XLSX.

Puigserver, P., J. Rhee, J. Donovan, C.J. Walkey, J.C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili, and B.M. Spiegelman. 2003. Insulin-regulated hepatic gluconeogenesis through FOXO1–PGC-1α interaction. Nature 423:550–555. doi:10.1038/nature01667.

Qi, J., L. Gan, J. Fang, J. Zhang, X. Yu, H. Guo, D. Cai, H. Cui, L. Gou, J. Deng, Z. Wang, and Z. Zuo. 2022. Beta-Hydroxybutyrate: A Dual Function Molecular and Immunological Barrier Function Regulator. Front Immunol 0:2714. doi:10.3389/FIMMU.2022.805881.

Rahman, M., S. Muhammad, M.A. Khan, H. Chen, D.A. Ridder, H. Müller-Fielitz, B. Pokorná, T. Vollbrandt, I. Stölting, R. Nadrowitz, J.G. Okun, S. Offermanns, and M. Schwaninger. 2014. The β-hydroxybutyrate receptor HCA2 activates a neuroprotective subset of macrophages. Nature Communications 2014 5:1 5:1–11. doi:10.1038/ncomms4944.

Ramadori, G., and B. Christ. 1999. Cytokines and the hepatic acute-phase response. Semin Liver Dis 19:141–156. doi:10.1055/s-2007-1007106.

Randle, P.J. 1998. Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263–283. doi:10.1002/(SICI)1099-0895(199812)14:4<263::AID-DMR233>3.0.CO;2-C.

Rathbun, F.M., R.S. Pralle, S.J. Bertics, L.E. Armentano, K. Cho, C. Do, K.A. Weigel, and H.M. White. 2017. Relationships between body condition score change, prior mid-lactation phenotypic residual feed intake, and hyperketonemia onset in transition dairy cows. J Dairy Sci 100:3685–3696. doi:10.3168/jds.2016-12085.

Remond, D., I. Ortigues, and J.P. Jouany. 1995. Energy substrates for the rumen epithelium. Nutrition Society 54.

Rico, D.E., A. Celemín-Sarmiento, and J.E. Rico. 2021. The dose responsive induction of acute hyperketonemia by ruminal infusion of calcium butyrate in dairy cows. Pages 142–143 in ADSA annual meeting 2021.

Ringseis, R., D.K. Gessner, and K. Eder. 2015. Molecular insights into the mechanisms of liver-associated diseases in early-lactating dairy cows: hypothetical role of endoplasmic reticulum stress. J Anim Physiol Anim Nutr (Berl) 99:626–645. doi:10.1111/JPN.12263.

Roberts, T., N. Chapinal, S.J. LeBlanc, D.F. Kelton, J. Dubuc, and T.F. Duffield. 2012. Metabolic parameters in transition cows as indicators for early-lactation culling risk. J Dairy Sci 95:3057–3063. doi:10.3168/JDS.2011-4937.

Roche, J.R., A.W. Bell, T.R. Overton, and J.J. Loor. 2013. Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking. Anim Prod Sci 53:1000. doi:10.1071/AN12293.

Rodriguez, Z., E. Shepley, M.I. Endres, G. Cramer, and L.S. Caixeta. 2022. Assessment of milk yield and composition, early reproductive performance, and herd removal in multiparous dairy cattle based on the week of diagnosis of hyperketonemia in early lactation. J Dairy Sci. doi:10.3168/JDS.2021-20836.

Roekenes, J., and C. Martins. 2021. Ketogenic diets and appetite regulation. Curr Opin Clin Nutr Metab Care 24:359–363. doi:10.1097/MCO.0000000000000760.

Roh, J.S., and D.H. Sohn. 2018. Damage-Associated Molecular Patterns in Inflammatory Diseases. Immune Netw 18. doi:10.4110/IN.2018.18.E27.

Rook, J.A.F., C.C. Balch, and V.W. Johnson. 1965. Further observations on the effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Brit. J. Nutr 19:93. doi:10.1079/BJN19650008.

Le Roy, D., F. Di Padova, Y. Adachi, M.P. Glauser, T. Calandra, and D. Heumann. 2001. Critical Role of Lipopolysaccharide-Binding Protein and CD14 in Immune

Responses against Gram-Negative Bacteria. The Journal of Immunology 167:2759–2765. doi:10.4049/JIMMUNOL.167.5.2759.

Ruiz-González, A., M. Ramírez-Mella, D.E. Rico, and J.E. Rico. 2022. The nutritional induction of episodic hyperketonemia in dairy cows: a randomized controlled trial. Page in American Dairy Science Association. Journal of Dairy Science, Kansas City, MO.

Saco, Y., and A. Bassols. 2023. Acute phase proteins in cattle and swine: A review. Vet Clin Pathol 52 Suppl 1:50–63. doi:10.1111/VCP.13220.

Sadri, H., M.H. Ghaffari, and H. Sauerwein. 2023. Invited review: Muscle protein breakdown and its assessment in periparturient dairy cows. J Dairy Sci 106:822–842. doi:10.3168/jds.2022-22068.

Sahoo, S.S., R.C. Patra, P.C. Behera, and D. Swarup. 2009. Oxidative stress indices in the erythrocytes from lactating cows after treatment for subclinical ketosis with antioxidant incorporated in the therapeutic regime. Vet Res Commun 33:281–290. doi:10.1007/S11259-008-9176-1/FIGURES/1.

Santschi, D.E., R. Lacroix, J. Durocher, M. Duplessis, R.K. Moore, and D.M. Lefebvre. 2016. Prevalence of elevated milk β-hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in Dairy Herd Improvement milk samples and association with milk yield and components. J Dairy Sci 99:9263–9270. doi:10.3168/JDS.2016-11128.

Schroedl, W., B. Fuerll, P. Reinhold, M. Krueger, and C. Schuett. 2001. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP).

Seely, C.R., K.D. Bach, D.M. Barbano, and J.A.A. McArt. 2021. Effect of hyperketonemia on the diurnal patterns of energy-related blood metabolites in early-lactation dairy cows. J Dairy Sci 104:818–825. doi:10.3168/jds.2020-18930.

Semler, M.W., and T.W. Rice. 2016. Sepsis Resuscitation: Fluid Choice and Dose.. Clin Chest Med 37:241–50. doi:10.1016/j.ccm.2016.01.007.

Serra, D., N. Casals, G. Asins, T. Royo, C. Ciudad, and F. Hegardt. 1993. Regulation of Mitochondrial 3-Hydroxy-3-methylglutaryl-coenzyme A Synthase Protein by Starvation, Fat Feeding, and Diabetes. Arch Biochem Biophys 307:40–45.

Sharma, D., and T.D. Kanneganti. 2016. The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. J Cell Biol 213:617. doi:10.1083/JCB.201602089.

Shaw, D.M., F. Merien, A. Braakhuis, E. Maunder, and D.K. Dulson. 2019. Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. Sports Medicine 2019 50:4 50:641–656. doi:10.1007/S40279-019-01246-Y.

Shaw, D.M., F. Merien, A. Braakhuis, E. Maunder, and D.K. Dulson. 2020. Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. Sports Med 50:641–656. doi:10.1007/S40279-019-01246-Y.

Shaw, J.C. 1943. Studies on Ketosis in Dairy Cattle. V. The Development of Ketosis. J Dairy Sci 26:1079–1090. doi:10.3168/JDS.S0022-0302(43)92810-3.

Shaw, J.C. 1956. Ketosis in Dairy Cattle. A Review. J Dairy Sci 39:402–434. doi:10.3168/jds.S0022-0302(56)94765-8.

Shen, L., Y. Yang, T. Ou, C.C.C. Key, S.H. Tong, R.C. Sequeira, J.M. Nelson, Y. Nie, Z. Wang, E. Boudyguina, S. v. Shewale, and X. Zhu. 2017. Dietary PUFAs attenuate NLRP3 inflammasome activation via enhancing macrophage autophagy. J Lipid Res 58:1808–1821. doi:10.1194/JLR.M075879.

Shen, T., X. Li, J.J. Loor, Y. Zhu, X. Du, X. Wang, D. Xing, Z. Shi, Z. Fang, X. Li, and G. Liu. 2019. Hepatic nuclear factor kappa B signaling pathway and NLR family pyrin domain containing 3 inflammasome is over-activated in ketotic dairy cows. J Dairy Sci 102:10554–10563. doi:10.3168/JDS.2019-16706.

Shi, J., J. Fan, Q. Su, and Z. Yang. 2019. Cytokines and Abnormal Glucose and Lipid Metabolism. Front Endocrinol (Lausanne) 10:703. doi:10.3389/FENDO.2019.00703/BIBTEX.

Simkins, K.L., J.W. Suttie, and B.R. Baumgardt. 1965. Regulation of Food Intake in Ruminants. 4. Effect of Acetate, Propionate, Butyrate, and Glucose on Voluntary Food Intake in Dairy Cattle. J Dairy Sci 48:1635–1642. doi:10.3168/jds.S0022-0302(65)88541-1.

Sjollema, B., and J. Van Der Zande. 1923. Metabolism in acetonemia of milch cows. Journal of Metabolic Research 525–533.

Song, Y., J.J. Loor, C. Li, Y. Liang, N. Li, X. Shu, Y. Yang, X. Feng, X. Du, Z. Wang, G. Liu, and X. Li. 2021. Enhanced mitochondrial dysfunction and oxidative stress in the mammary gland of cows with clinical ketosis. J Dairy Sci 104:6909–6918. doi:10.3168/jds.2020-19964.

Sordillo, L.M., and V. Mavangira. 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 54:1204–1214. doi:10.1071/AN14503.

Sordillo, L.M., and W. Raphael. 2013. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. Veterinary Clinics of North America: Food Animal Practice 29:267–278. doi:10.1016/J.CVFA.2013.03.002.

Soto-Mota, A., N.G. Norwitz, R.D. Evans, and K. Clarke. 2022. Exogenous d-β-hydroxybutyrate lowers blood glucose in part by decreasing the availability of L-alanine for gluconeogenesis. Endocrinol Diabetes Metab 5. doi:10.1002/EDM2.300.

Steffen, M.R. 1917. The Clinical Diagnosis of Cattle Diseases.

Stienstra, R., L.A.B. Joosten, T. Koenen, B. van Tits, J.A. van Diepen, S.A.A. van den Berg, P.C.N. Rensen, P.J. Voshol, G. Fantuzzi, A. Hijmans, S. Kersten, M. Müller, W.B. van den Berg, N. van Rooijen, M. Wabitsch, B.J. Kullberg, J.W.M. van der Meer, T. Kanneganti, C.J. Tack, and M.G. Netea. 2010. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab 12:593–605. doi:10.1016/J.CMET.2010.11.011.

Stubbs, B.J., P.J. Cox, R.D. Evans, M. Cyranka, K. Clarke, and H. de Wet. 2018. A Ketone Ester Drink Lowers Human Ghrelin and Appetite. Obesity (Silver Spring) 26:269. doi:10.1002/OBY.22051.

Stubbs, B.J., P.J. Cox, R.D. Evans, P. Santer, J.J. Miller, O.K. Faull, S. Magor-Elliott, S. Hiyama, M. Stirling, and K. Clarke. 2017. On the metabolism of exogenous ketones in humans. Front Physiol 8. doi:10.3389/fphys.2017.00848.

Stubbs, B.J., A.P. Koutnik, E.L. Goldberg, V. Upadhyay, P.J. Turnbaugh, E. Verdin, and J.C. Newman. 2020a. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med 1:43–65. doi:10.1016/j.medj.2020.06.008.

Stubbs, B.J., A.P. Koutnik, E.L. Goldberg, V. Upadhyay, P.J. Turnbaugh, E. Verdin, and J.C. Newman. 2020b. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med 1:43–65. doi:10.1016/j.medj.2020.06.008.

Su, G.L., S.M. Goyert, M.H. Fan, A. Aminlari, K.Q. Gong, R.D. Klein, A. Myc, W.H. Alarcon, L. Steinstraesser, D.G. Remick, and S.C. Wang. 2002. Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. Am J Physiol Gastrointest Liver Physiol 283:640–645. doi:10.1152/AJPGI.00253.2001/ASSET/IMAGES/LARGE/H30920964007.JPEG.

Sun, X., Y. Tang, C. Jiang, S. Luo, H. Jia, Q. Xu, C. Zhao, Y. Liang, Z. Cao, G. Shao, J.J. Loor, and C. Xu. 2021. Oxidative stress, NF-κB signaling, NLRP3 inflammasome, and caspase apoptotic pathways are activated in mammary gland of ketotic Holstein cows. J Dairy Sci 104:849–861. doi:10.3168/jds.2020-18788.

Suthar, V., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci 96:2925–2938. doi:10.3168/jds.2012-6035.

Swartz, T.H., B.J. Bradford, and L.K. Mamedova. 2021a. Diverging in vitro inflammatory responses toward Streptococcus uberis in mouse macrophages

either preconditioned or continuously treated with β-hydroxybutyrate. JDS Communications 2:142–147. doi:10.3168/jdsc.2020-0038.

Swartz, T.H., B.J. Bradford, and L.K. Mamedova. 2021b. Connecting Metabolism to Mastitis: Hyperketonemia Impaired Mammary Gland Defenses During a Streptococcus uberis Challenge in Dairy Cattle. Front Immunol 12. doi:10.3389/fimmu.2021.700278.

Taggart, A.K.P., J. Kero, X. Gan, T.Q. Cai, K. Cheng, M. Ippolito, N. Ren, R. Kaplan, K. Wu, T.J. Wu, L. Jin, C. Liaw, R. Chen, J. Richman, D. Connolly, S. Offermanns, S.D. Wright, and M.G. Waters. 2005. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. J Biol Chem 280:26649–26652. doi:10.1074/JBC.C500213200.

Tavares, E., R. Maldonado, M.L. Ojeda, and F.J. Minano. 2005. Circulating inflammatory mediators during start of fever in differential diagnosis of gram-negative and gram-positive infections in leukopenic rats. Clin Diagn Lab Immunol 12:1085–1093. doi:10.1128/CDLI.12.9.1085-1093.2005.

Thangaraju, M., G.A. Cresci, K. Liu, S. Ananth, J.P. Gnanaprakasam, D.D. Browning, J.D. Mellinger, S.B. Smith, G.J. Digby, N.A. Lambert, P.D. Prasad, and V. Ganapathy. 2009. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res 69:2826. doi:10.1158/0008-5472.CAN-08-4466.

Thio, C.L.-P., A.C.-Y. Lai, Y.-T. Ting, P.-Y. Chi, and Y.-J. Chang. 2022. The ketone body β-hydroxybutyrate mitigates ILC2-driven airway inflammation by regulating mast cell function. Cell Rep 40:111437. doi:10.1016/j.celrep.2022.111437.

Tillett, W.S., and T. Francis. 1930. SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS. J Exp Med 52:561. doi:10.1084/JEM.52.4.561.

Tobias, P.S., K. Soldau, and R.J. Ulevitch. 1989. Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. Journal of Biological Chemistry 264:10867–10871. doi:10.1016/s0021-9258(18)81700-8.

Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res Vet Sci 93:695–704. doi:10.1016/J.RVSC.2011.11.008.

Trevisi, E., N. Jahan, G. Bertoni, A. Ferrari, and A. Minuti. 2016. Pro-Inflammatory Cytokine Profile in Dairy Cows: Consequences for New Lactation. https://doi.org/10.4081/ijas.2015.3862 14:285–292. doi:10.4081/IJAS.2015.3862.

Tweedell, R.E., R.K.S. Malireddi, and T.D. Kanneganti. 2020. A comprehensive guide to studying inflammasome activation and cell death. Nat Protoc 15. doi:10.1038/s41596-020-0374-9.

Tyrrell, H.F., and P.W. Moe. 1975. Effect of Intake on Digestive Efficiency. J Dairy Sci 58:1151–1163. doi:10.3168/jds.S0022-0302(75)84694-7.

Udall, H. 1943. The Practice of Veterinary Medicine. 4th ed. Ithaca.

Urrutia, N., R. Bomberger, C. Matamoros, and K.J. Harvatine. 2019. Effect of dietary supplementation of sodium acetate and calcium butyrate on milk fat synthesis in lactating dairy cows. J Dairy Sci 102:5172–5181. doi:10.3168/jds.2018-16024.

Urrutia, N.L., and K.J. Harvatine. 2017. Acetate Dose-Dependently Stimulates Milk Fat Synthesis in Lactating Dairy Cows. J Nutr 763–769. doi:10.3945/jn.116.245001.

Vandanmagsar, B., Y.H. Youm, A. Ravussin, J.E. Galgani, K. Stadler, R.L. Mynatt, E. Ravussin, J.M. Stephens, and V.D. Dixit. 2011. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–189. doi:10.1038/NM.2279.

Vanholder, T., J. Papen, R. Bemers, G. Vertenten, and A.C.B. Berge. 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J Dairy Sci 98:880–888. doi:10.3168/jds.2014-8362.

Vanitallie, T.B., and T.H. Nufert. 2003. Lead Review Article Ketones: Metabolism's Ugly Duckling 327–341. doi:10.131/nr.2003.oct.327-341.

Vels, L., C.M. Røntved, M. Bjerring, and K.L. Ingvartsen. 2008. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. J Dairy Sci 92:922–934. doi:10.3168/jds.2008-1209.

Vercouteren, M.M.A.A., J.H.J. Bittar, P.J. Pinedo, C.A. Risco, J.E.P. Santos, A. Vieira-Neto, and K.N. Galvão. 2015. Factors associated with early cyclicity in postpartum dairy cows. J Dairy Sci 98:229–239. doi:10.3168/JDS.2014-8460.

de Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production Variables and Fertility. J Dairy Sci 83:62–69. doi:10.3168/jds.S0022-0302(00)74856-9.

Wagner, S.A., and D.E. Schimek. 2010. Evaluation of the effect of bolus administration of 50% dextrose solution on measures of electrolyte and energy balance in postpartum dairy cows. Am J Vet Res 71:1074–1080. doi:10.2460/AJVR.71.9.1074.

Wanders, D., and R.L. Judd. 2011. Future of GPR109A agonists in the treatment of dyslipidaemia. Diabetes Obes Metab 13:685–691. doi:10.1111/j.1463-1326.2011.01400.x.

Wankhade, P.R., A. Manimaran, A. Kumaresan, S. Jeyakumar, K.P. Ramesha, V. Sejian, D. Rajendran, and M.R. Varghese. 2017. Metabolic and immunological changes in transition dairy cows: A review. Vet World 10:1367. doi:10.14202/VETWORLD.2017.1367-1377.

Weigand, E., J.W. Young, and A.D. McGilliard. 1972. Extent of Butyrate Metabolism by Bovine Ruminoreticulum Epithelium and the Relationship to Absorption Rate. J Dairy Sci 55:589–597. doi:10.3168/JDS.S0022-0302(72)85541-3.

Weiss, W.P. 2010. Refining the Net Energy System.

Weiss, W.P., and A.W. Tebbe. 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Transl Anim Sci 3:953–961. doi:10.1093/tas/txy119.

White, H., and B. Venkatesh. 2011. Clinical review: Ketones and brain injury. Crit Care 15:219. doi:10.1186/CC10020.

White, H.M. 2015. The Role of TCA Cycle Anaplerosis in Ketosis and Fatty Liver in Periparturient Dairy Cows. Animals (Basel) 5:793. doi:10.3390/ANI5030384.

White, H.M. 2020. ADSA Foundation Scholar Award: Influencing hepatic metabolism: Can nutrient partitioning be modulated to optimize metabolic health in the transition dairy cow?. J Dairy Sci 103:6741–6750. doi:10.3168/jds.2019-18119.

Williamson, D.H., P. Lund, and H.A. Krebs. 1967. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochemical Journal 103:514. doi:10.1042/BJ1030514.

Wilson, J.J., C.R. Youno, and S.B. Smth. 1992. TRIACYLGLYCEROL BIOSYNTHESIS IN BOVINE LIVER AND SUBCUTANEOUS ADIPOSE TISSUE.

Wu, Y., Y. Sun, R. Zhang, T. He, G. Huang, K. Tian, J. Liu, J. Chen, and G. Dong. 2021. Sodium butyrate more effectively mitigates the negative effects of high-concentrate diet in dairy cows than sodium β-hydroxybutyrate via reducing free bacterial cell wall components in rumen fluid and plasma. Toxins (Basel) 13. doi:10.3390/toxins13050352.

Xu, W., J. Vervoort, E. Saccenti, B. Kemp, R.J. van Hoeij, and A.T.M. van Knegsel. 2020. Relationship between energy balance and metabolic profiles in plasma and milk of dairy cows in early lactation. J Dairy Sci 103:4795–4805. doi:10.3168/JDS.2019-17777.

Yamada, T., S.J. Zhang, H. Westerblad, and A. Katz. 2010. β-hydroxybutyrate inhibits insulin-mediated glucose transport in mouse oxidative muscle. Am J Physiol Endocrinol Metab 299:364–373. doi:10.1152/AJPENDO.00142.2010/ASSET/IMAGES/LARGE/ZH100810603400006.JPEG.

Yamanashi, T., M. Iwata, N. Kamiya, K. Tsunetomi, N. Kajitani, N. Wada, T. Iitsuka, T. Yamauchi, A. Miura, S. Pu, Y. Shirayama, K. Watanabe, R.S. Duman, and K. Kaneko. 2017. Beta-hydroxybutyrate, an endogenic NLRP3 inflammasome

inhibitor, attenuates stress-induced behavioral and inflammatory responses. Scientific Reports 2017 7:1 7:1–11. doi:10.1038/s41598-017-08055-1.

Yan, H., and K.M. Ajuwon. 2015. Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells. PLoS One 10:e0145940. doi:10.1371/journal.pone.0145940.

Yan, T., C.S. Mayne, T.W.J. Keady, and R.E. Agnew. 2006. Effects of dairy cow genotype with two planes of nutrition on energy partitioning between milk and body tissue. J Dairy Sci 89:1031–1042. doi:10.3168/jds.S0022-0302(06)72170-1.

Yao, Q., Y. Li, Q. Meng, and Z. Zhou. 2017. The effect of calcium propionate on the ruminal bacterial community composition in finishing bulls. Asian-Australas J Anim Sci 30:495–504. doi:10.5713/ajas.16.0469.

Yokoi, K., N. Mukaida, A. Harada, Y. Watanabe, and K. Matsushima. 1997. Prevention of endotoxemia-induced acute respiratory distress syndrome-like lung injury in rabbits by a monoclonal antibody to IL-8.. Lab Invest 76:375–384.

You, K., H. Gu, Z. Yuan, and X. Xu. 2021. Tumor Necrosis Factor Alpha Signaling and Organogenesis. Front Cell Dev Biol 9:2055. doi:10.3389/FCELL.2021.727075/BIBTEX.

Youm, Y.H., K.Y. Nguyen, R.W. Grant, E.L. Goldberg, M. Bodogai, D. Kim, D. D'Agostino, N. Planavsky, C. Lupfer, T.D. Kanneganti, S. Kang, T.L. Horvath, T.M. Fahmy, P.A. Crawford, A. Biragyn, E. Alnemri, and V.D. Dixit. 2015. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 21:263–269. doi:10.1038/nm.3804.

Young, J.W. Gluconeogenesis in Cattle: Significance and Methodology ~. J Dairy Sci 60:1–15. doi:10.3168/jds.S0022-0302(77)83821-6.

Yuan, K., J.K. Farney, L.K. Mamedova, L.M. Sordillo, and B.J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8. doi:10.1371/JOURNAL.PONE.0080316.

Yurista, S.R., C.R. Chong, J.J. Badimon, D.P. Kelly, R.A. de Boer, and B.D. Westenbrink. 2021. Therapeutic Potential of Ketone Bodies for Patients With Cardiovascular Disease: JACC State-of-the-Art Review. J Am Coll Cardiol 77:1660–1669. doi:10.1016/J.JACC.2020.12.065.

Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J Dairy Sci 96:5656–5669. doi:10.3168/JDS.2012-6142.

Zarrin, M., L. Grossen-Rösti, R.M. Bruckmaier, and J.J. Gross. 2017. Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. J Dairy Sci 100:2323–2333. doi:10.3168/jds.2016-11714.

Zarrin, M., L. De Matteis, M.C.M.B. Vernay, O. Wellnitz, H.A. van Dorland, and R.M. Bruckmaier. 2013. Long-term elevation of β-hydroxybutyrate in dairy cows through infusion: Effects on feed intake, milk production, and metabolism. J Dairy Sci 96:2960–2972. doi:10.3168/jds.2012-6224.

Zarrin, M., O. Wellnitz, H.A. van Dorland, and R.M. Bruckmaier. 2014. Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. J Dairy Sci 97:330–339. doi:10.3168/jds.2013-7222.

Zebeli, Q., and B. Ametaj. 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. J Dairy Sci 92:3800–3809. doi:10.3168/JDS.2009-2178.

Zhang, F., X. Nan, H. Wang, Y. Zhao, Y. Guo, and B. Xiong. 2020. Effects of propylene glycol on negative energy balance of postpartum dairy cows. Animals 10:1–15. doi:10.3390/ani10091526.

Zhang, G., and B.N. Ametaj. 2020. Ketosis an Old Story Under a New Approach. Dairy 2020, Vol. 1, Pages 42-60 1:42–60. doi:10.3390/DAIRY1010005.

Zhang, G., D. Hailemariam, E. Dervishi, S.A. Goldansaz, Q. Deng, S.M. Dunn, and B.N. Ametaj. 2016. Dairy cows affected by ketosis show alterations in innate

immunity and lipid and carbohydrate metabolism during the dry off period and postpartum. Res Vet Sci 107:246–256. doi:10.1016/j.rvsc.2016.06.012.

Zheng, D., T. Liwinski, and E. Elinav. 2020. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. Cell Discovery 2020 6:1 6:1–22. doi:10.1038/s41421-020-0167-x.

Zhu, H., D. Bi, Y. Zhang, C. Kong, J. Du, X. Wu, Q. Wei, and H. Qin. 2022. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. Signal Transduct Target Ther 7:11. doi:10.1038/s41392-021-00831-w.

Abaker, J.A., T.L. Xu, D. Jin, G.J. Chang, K. Zhang, and X.Z. Shen. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. J Dairy Sci 100:666–678. doi:10.3168/jds.2016-10871.

Abbas, Z., A. Sammad, L. Hu, H. Fang, Q. Xu, and Y. Wang. 2020. Glucose metabolism and dynamics of facilitative glucose transporters (Gluts) under the influence of heat stress in dairy cattle. Metabolites 10:1–19. doi:10.3390/metabo10080312.

Abdallah, A., J. Hewson, D. Francoz, H. Selim, and S. Buczinski. 2016. Systematic Review of the Diagnostic Accuracy of Haptoglobin, Serum Amyloid A, and Fibrinogen versus Clinical Reference Standards for the Diagnosis of Bovine Respiratory Disease. J Vet Intern Med 30:1356–1368. doi:10.1111/JVIM.13975.

Abuajamieh, M., S.K. Kvidera, M.V.S. Fernandez, A. Nayeri, N.C. Upah, E.A. Nolan, S.M. Lei, J.M. DeFrain, H.B. Green, K.M. Schoenberg, W.E. Trout, and L.H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res Vet Sci 109:81–85. doi:10.1016/j.rvsc.2016.09.015.

Abuelo, A., J. Hernández, J.L. Benedito, and C. Castillo. 2016. Association of oxidative status and insulin sensitivity in periparturient dairy cattle: An observational study. J Anim Physiol Anim Nutr (Berl) 100:279–286. doi:10.1111/JPN.12365.

Ali, A., and G. Shook. 1980. An Optimum Transformation for Somatic Cell Concentration in Milk'. Journal fo Dairy Science 63:487–490. doi:10.3168/jds.S0022-0302(80)82959-6.

Allen, M.S. 2020. Review: Control of feed intake by hepatic oxidation in ruminant animals: integration of homeostasis and homeorhesis. doi:10.1017/S1751731119003215.

Alsemgeest, S.P., H.C. Kalsbeek, T. Wensing, J.P. Koeman, A.M. van Ederen, and E. Gruys. 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet Q 16:21–23. doi:10.1080/01652176.1994.9694410.

Ametaj, B.N., B.J. Bradford, G. Bobe, R.A. Nafikov, Y. Lu, J.W. Young, and D.C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can J Anim Sci 85:165–175. doi:10.4141/A04-043.

Anders, H.J., B. Suarez-Alvarez, M. Grigorescu, O. Foresto-Neto, S. Steiger, J. Desai, J.A. Marschner, M. Honarpisheh, C. Shi, J. Jordan, L. Müller, N. Burzlaff, T. Bäuerle, and S.R. Mulay. 2018. The macrophage phenotype and inflammasome component NLRP3 contributes to nephrocalcinosis-related chronic kidney disease independent from IL-1–mediated tissue injury. Kidney Int 93:656–669. doi:10.1016/J.KINT.2017.09.022.

Andrew, S.M., H.F. Tyrrell, C.K. Reynolds, and R.A. Erdman. 1991. Net Energy for Lactation of Calcium Salts of Long-Chain Fatty Acids for Cows Fed Silage-Based Diets. J Dairy Sci 74:2588–2600. doi:10.3168/jds.S0022-0302(91)78437-3.

Annison, E., and W. Bryden. 1998. Perspectives on ruminant nutrition and metabolism I. Metabolism in the Rumen. Nutr Res Rev 11:173` – 198.

Ari, C., C. Murdun, A.P. Koutnik, C.R. Goldhagen, C. Rogers, C. Park, S. Bharwani, D.M. Diamond, M.S. Kindy, D.P. D'Agostino, and Z. Kovács. 2019. Exogenous Ketones Lower Blood Glucose Level in Rested and Exercised Rodent Models. Nutrients 11. doi:10.3390/NU11102330.

Aschenbach, J.R., N.B. Kristensen, S.S. Donkin, H.M. Hammon, and G.B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB Life 62:869–877. doi:10.1002/IUB.400.

Athinarayanan, S.J., R.N. Adams, S.J. Hallberg, A.L. McKenzie, N.H. Bhanpuri, W.W. Campbell, J.S. Volek, S.D. Phinney, and J.P. McCarter. 2019. Long-Term Effects of a Novel Continuous Remote Care Intervention Including Nutritional Ketosis for the Management of Type 2 Diabetes: A 2-Year Non-randomized Clinical Trial. Front Endocrinol (Lausanne) 10. doi:10.3389/fendo.2019.00348.

Baird, G.D., K.G. Hibbitt, G.D. Hunter, P. Lund, M. Stubbs, and H.A. Krebs. 1968. Biochemical aspects of bovine ketosis. Biochemical Journal 107:683. doi:10.1042/BJ1070683.

Balasse, E.O., and F. Féry. 1989. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 5:247–270. doi:10.1002/DMR.5610050304.

Baldwin, R.L., and M.J. Allison. 1983. Rumen metabolism.. J Anim Sci 57 Suppl 2:461–77.

Baldwin, R.L., and B. Jesse. 1996. Propionate Modulation of Ruminal Ketogenesis Attenuation of the inflammatory phenomena in the transition period of dairy cows View project Gut sensing in calves View project. Article in Journal of Animal Science. doi:10.2527/1996.7471694x.

Baldwin, R.L., R.W. Li, Y. Jia, and C.J. Li. 2018. Transcriptomic Impacts of Rumen Epithelium Induced by Butyrate Infusion in Dairy Cattle in Dry Period. Gene Regul Syst Bio 12. doi:10.1177/1177625018774798.

Barrientos-Blanco, M., A. Celemin-Sarmiento, M. da Silva, N. Bagheri, C. Mercado, N. Galdos, and J. Rico. 2023. The induction of nutritional ketosis in lactating dairy cows using calcium butyrate: effects on lactation performance and health. Page in American Dairy Science Association Conference, Ottawa.

Bauchart, D. 1993. Lipid Absorption and Transport in Ruminants. J Dairy Sci 76:3864–3881. doi:10.3168/JDS.S0022-0302(93)77728-0.

Bauman, D.E., R.E. Brown, and C.L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Arch Biochem Biophys 140:237–244. doi:10.1016/0003-9861(70)90028-7.

Bauman, D.E., and W. Bruce Currie. 1980. Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. J Dairy Sci 63:1514–1529. doi:10.3168/jds.S0022-0302(80)83111-0.

Bauman, D.E., C.J. Peel, W.D. Steinhour, P.J. Reynolds, H.F. Tyrrell, A.C.G. Brown, and G.L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J Nutr 118:1031–1040. doi:10.1093/JN/118.8.1031.

Baumgard, L.H., R.J. Collier, and D.E. Bauman. 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. J Dairy Sci 100:10353–10366. doi:10.3168/JDS.2017-13242.

Beauchemin, K.A., E. Ungerfeld, and R. Gruninger. 2022. Control of Methanogenesis in Dairy Animals. Encyclopedia of Dairy Sciences 65–78. doi:10.1016/B978-0-12-818766-1.00072-6.

Bedford, A., and J. Gong. 2018. Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition 4:151–159. doi:10.1016/j.aninu.2017.08.010.

Belay, T.K., M. Svendsen, Z.M. Kowalski, and T. Ådnøy. 2017. Genetic parameters of blood β-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. J Dairy Sci 100:6298–6311. doi:10.3168/JDS.2016-12458.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation.. J Anim Sci 73:2804–2819. doi:10.2527/1995.7392804x.

Bell, A.W., and D.E. Bauman. 1997. Adaptations of Glucose Metabolism During Pregnancy and Lactation.

Bendridi, N., A. Selmi, A. Balcerczyk, and L. Pirola. 2022. Ketone Bodies as Metabolites and Signalling Molecules at the Crossroad between Inflammation and Epigenetic Control of Cardiometabolic Disorders. Int J Mol Sci 23:14564. doi:10.3390/ijms232314564.

Benedet, A., C.L. Manuelian, A. Zidi, M. Penasa, and M. De Marchi. 2019. Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. Pages 1676–1689 in Animal. Cambridge University Press.

Benson, J.D., and R.S. Emery. 1971. Fatty Acid Esterification by Homogenates of Bovine Liver and Adipose Tissue. J Dairy Sci 54:1034–1040. doi:10.3168/jds.S0022-0302(71)85967-2.

Benzaquen, M.E., C.A. Risco, L.F. Archbald, P. Melendez, M.J. Thatcher, and W.W. Thatcher. 2007. Rectal Temperature, Calving-Related Factors, and the Incidence of Puerperal Metritis in Postpartum Dairy Cows. J Dairy Sci 90:2804–2814. doi:10.3168/JDS.2006-482.

Bergman, E.N. 1971. Hyperketonemia-Ketogenesis and Ketone Body Metabolism. J Dairy Sci 54:936–948. doi:10.3168/jds.S0022-0302(71)85950-7.

Bergman, E.N. 1990. Energy Contributions of Volatile Fatty Acids From the Gastrointestinal Tract in Various Species.

Bergman, E.N., and K. Kon. 1964. Factors affecting acetoacetate production rates by normal and ketotic pregnant sheep. American Journal of Physiology 206:453–457. doi:10.1152/AJPLEGACY.1964.206.2.453.

Bernal-Santos, G., A.M. O'Donnell, J.L. Vicini, G.F. Hartnell, and D.E. Bauman. 2010. Hot topic: Enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. J Dairy Sci 93:32–37. doi:10.3168/jds.2009-2711.

Biden, T.J., and K.W. Taylor. 1983. Effects of ketone bodies on insulin release and islet-cell metabolism in the rat.. Biochemical Journal 212:371. doi:10.1042/BJ2120371.

Bionaz, M., E. Vargas-Bello-Pérez, and S. Busato. 2020. Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. J Anim Sci Biotechnol 11:1–36. doi:10.1186/s40104-020-00512-8.

Block, S.S., W.R. Butler, R.A. Ehrhardt, A.W. Bell, M.E. van Amburgh, and Y.R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance.

Bolon, B., G. Campagnuolo, L. Zhu, D. Duryea, D. Zack, and U. Feige. 2004. Interleukin-1β and tumor necrosis factor-α produce distinct, time-dependent patterns of acute arthritis in the rat knee. Vet Pathol 41:235–243. doi:10.1354/VP.41-3-235/ASSET/IMAGES/LARGE/10.1354_VP.41-3-235-FIG18.JPEG.

Bradford, B., R. Albornoz, and K. Kennedy. 2022. ABST: 1302. Fueling appetite: Nutrient metabolism and the control of feed intake. Pages 119–119 in Ruminant Nutritional Symposium: Integrating the control of energy intake and partitioning into ratio formulation-recognition of the contribution of ADSA fellow Mike Allen.

Bradford, B.J., L.K. Mamedova, J.E. Minton, J.S. Drouillard, and B.J. Johnson. 2009. Daily Injection of Tumor Necrosis Factor-α Increases Hepatic Triglycerides and Alters Transcript Abundance of Metabolic Genes in Lactating Dairy Cattle. J Nutr 139:1451–1456. doi:10.3945/JN.109.108233.

Bradford, B.J., and T.H. Swartz. 2020. Review: Following the smoke signals: Inflammatory signaling in metabolic homeostasis and homeorhesis in dairy cattle. Pages S144–S154 in Animal. Cambridge University Press.

Bradford, B.J., K. Yuan, J.K. Farney, L.K. Mamedova, and A.J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J Dairy Sci 98:6631–6650. doi:10.3168/JDS.2015-9683.

Bradshaw, P.C., W.A. Seeds, A.C. Miller, V.R. Mahajan, and W.M. Curtis. 2020. COVID-19: Proposing a Ketone-Based Metabolic Therapy as a Treatment to Blunt the Cytokine Storm. Oxid Med Cell Longev 2020. doi:10.1155/2020/6401341.

Brigham, K.L., and B. Meyrick. 1986. Endotoxin and lung injury.. Am Rev Respir Dis 133:913–927.

Brown, W.E., and B.J. Bradford. 2021. Invited review: Mechanisms of hypophagia during disease. J Dairy Sci 104:9418–9436. doi:10.3168/jds.2021-20217.

Burgos, S. 2000. Effects of bacterial lipopolysaccharide and dehydration on feeding and metabolism in bovines.

Butler, W.R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 83:211–218. doi:10.1016/S0301-6226(03)00112-X.

Capel, M.B., K.D. Bach, S. Mann, and J.A.A. McArt. 2021. A randomized controlled trial to evaluate propylene glycol alone or in combination with dextrose as a treatment for hyperketonemia in dairy cows. J Dairy Sci 104:2185–2194. doi:10.3168/jds.2020-19111.

Carretta, M.D., Y. Barría, K. Borquez, B. Urra, A. Rivera, P. Alarcón, M.A. Hidalgo, and R.A. Burgos. 2020. β-hydroxybutyrate and hydroxycarboxylic acid receptor 2 agonists activate the AKT, ERK and AMPK pathways, which are involved in bovine neutrophil chemotaxis. Sci Rep 10:12491. doi:10.1038/S41598-020-69500-2.

Chandler, T.L., T.A. Westhoff, A.S. Sipka, T.R. Overton, and S. Mann. 2022. Lipopolysaccharide challenge following intravenous amino acid infusion in postpartum dairy cows: II. Clinical and inflammatory responses. J Dairy Sci 105:4611–4623. doi:10.3168/JDS.2021-21227.

Chapinal, N., M. Carson, T. Duffield, M. Capel, S. Godden, M. Overton, J. Santos, and S. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J Dairy Sci 94:4897–4903. doi:10.3168/jds.2010-4075.

Chapinal, N., M.E. Carson, S.J. LeBlanc, K.E. Leslie, S. Godden, M. Capel, J.E.P. Santos, M.W. Overton, and T.F. Duffield. 2012a. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J Dairy Sci 95:1301–1309. doi:10.3168/JDS.2011-4724.

Chapinal, N., S.J. LeBlanc, M.E. Carson, K.E. Leslie, S. Godden, M. Capel, J.E.P. Santos, M.W. Overton, and T.F. Duffield. 2012b. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J Dairy Sci 95:5676–5682. doi:10.3168/JDS.2011-5132.

Chavez, J.A., T.A. Knotts, L.P. Wang, G. Li, R.T. Dobrowsky, G.L. Florant, and S.A. Summers. 2003. A Role for Ceramide, but Not Diacylglycerol, in the Antagonism of Insulin Signal Transduction by Saturated Fatty Acids *. Journal of Biological Chemistry 278:10297–10303. doi:10.1074/JBC.M212307200.

Choi, S.M., D.F. Tucker, D.N. Gross, R.M. Easton, L.M. DiPilato, A.S. Dean, B.R. Monks, and M.J. Birnbaum. 2010. Insulin Regulates Adipocyte Lipolysis via an Akt-Independent Signaling Pathway. Mol Cell Biol 30:5009. doi:10.1128/MCB.00797-10.

Clark, J., P. Vanegas, M. Panesar, and A. Cope. 2005. What does tumour necrosis factor excess do to the immune system long term?. Annals of the Rheumatic Diseases (ARD) 64:70–76. doi:10.1136/ard.2005.042523.

Contreras, G.A., E. Kabara, J. Brester, L. Neuder, and M. Kiupel. 2015. Macrophage infiltration in the omental and subcutaneous adipose tissues of dairy cows with displaced abomasum. J Dairy Sci 98:6176–6187. doi:10.3168/JDS.2015-9370.

Contreras, G.A., C. Strieder-Barboza, and W. Raphael. 2017. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J Anim Sci Biotechnol 8. doi:10.1186/S40104-017-0174-4.

Coppock, C.E. 1985. Energy Nutrition and Metabolism of the Lactating Dairy Cow. J Dairy Sci 68:3403–3410. doi:10.3168/jds.S0022-0302(85)81253-4.

Cortegiani, A., F. Madotto, C. Gregoretti, G. Bellani, J.G. Laffey, T. Pham, F. Van Haren, A. Giarratano, M. Antonelli, A. Pesenti, and G. Grasselli. 2018. Immunocompromised patients with acute respiratory distress syndrome: secondary analysis of the LUNG SAFE database. Crit Care 22:157. doi:10.1186/s13054-018-2079-9.

Cox, P.J., T. Kirk, T. Ashmore, K. Willerton, R. Evans, A. Smith, A.J. Murray, B. Stubbs, J. West, S.W. McLure, M.T. King, M.S. Dodd, C. Holloway, S. Neubauer, S. Drawer, R.L. Veech, J.L. Griffin, and K. Clarke. 2016. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. Cell Metab 24:256–268. doi:10.1016/j.cmet.2016.07.010.

Cuenoud, B., M. Hartweg, J.P. Godin, E. Croteau, M. Maltais, C.A. Castellano, A.C. Carpentier, and S.C. Cunnane. 2020. Metabolism of Exogenous D-Beta-Hydroxybutyrate, an Energy Substrate Avidly Consumed by the Heart and Kidney. Front Nutr 7. doi:10.3389/fnut.2020.00013.

Dalmas, E. 2019. Innate immune priming of insulin secretion. Curr Opin Immunol 56:44–49. doi:10.1016/J.COI.2018.10.005.

Dämmgen, U., H.-D. Haenel, C. Rösemann, N.J. Hutchings, W. Brade, and P. Lebzien. 2009. Improved national calculation procedures to assess energy requirements, nitrogen and VS excretions of dairy cows in the German emission model GAS-EM.

Dann, H.M., D.E. Morin, G.A. Bollero, M.R. Murphy, and J.K. Drackley. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J Dairy Sci 88:3249–3264. doi:10.3168/jds.S0022-0302(05)73008-3.

Davis, J.D., D. Wirtshafter, K.E. Asin, and D. Brief. 1981. Sustained Intracerebroventricular Infusion of Brain Fuels Reduces Body Weight and Food Intake in Rats. Science (1979) 212:81–83. doi:10.1126/SCIENCE.7193909.

Deemer, S.E., E.P. Plaisance, and C. Martins. 2020. Impact of ketosis on appetite regulation—a review. Nutrition Research 77:1–11. doi:10.1016/J.NUTRES.2020.02.010.

DeFrain, J.M., A.R. Hippen, K.F. Kalscheur, and D.J. Schingoethe. 2004. Feeding lactose increases ruminal butyrate and plasma β- hydroxybutyrate in lactating dairy cows. J Dairy Sci 87:2486–2494. doi:10.3168/jds.S0022-0302(04)73373-1.

Demigné, C., C. Yacoub, C. Rémésy, and P. Fafournoux. 1986. Propionate and butyrate metabolism in rat or sheep hepatocytes. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 875:535–542. doi:10.1016/0005-2760(86)90074-3.

Dinarello, C.A. 1999. Cytokines as Endogenous Pyrogens. J Infect Dis 179:294–304.

Dirksen, G.U., and E. Mayer. 1985. Adaptive Changes of the Ruminal Mucosa and Their Functional and Clinical Significance.

Domingues, D., M. de Beni, and R. Dias Lauritano. 2016. Rumenology. 1st ed. Springer, Cham, Switzerland.

Dong, G., S. Liu, Y. Wu, C. Lei, J. Zhou, and S. Zhang. 2011. Diet-induced bacterial immunogens in the gastrointestinal tract of dairy cows: Impacts on immunity and metabolism. Acta Vet Scand 53:1–7. doi:10.1186/1751-0147-53-48/METRICS.

Dong, G., M. Qiu, C. Ao, J. Zhou, Khas-Erdene, X. Wang, Z. Zhang, and Y. Yang. 2014. Feeding a high-concentrate corn straw diet induced epigenetic alterations in the mammary tissue of dairy cows. PLoS One 9. doi:10.1371/journal.pone.0107659.

Dong, Z., X. Sun, Y. Tang, S. Luo, H. Jia, Q. Xu, Q. Jiang, J.J. Loor, W. Xu, and C. Xu. 2022. β-hydroxybutyrate impairs monocyte function via the ROS-NLR family pyrin

domain-containing three inflammasome (NLRP3) pathway in ketotic cows. Front Vet Sci 9:1252. doi:10.3389/FVETS.2022.925900/BIBTEX.

Drackley, J.K. 1999. ADSA foundation scholar award: Biology of dairy cows during the transition period: The final frontier?. J Dairy Sci 82:2259–2273. doi:10.3168/jds.s0022-0302(99)75474-3.

Drackley, J.K., T.R. Overton, and G.N. Douglas. 2001. Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. J Dairy Sci 84:E100–E112. doi:10.3168/jds.s0022-0302(01)70204-4.

Drackley, J.K., J.J. Veenhuizen, M.J. Richard, and J.W. Young. 1991. Metabolic Changes in Blood and Liver of Dairy Cows During Either Feed Restriction or Administration of 1,3-Butanediol. J Dairy Sci 74:4254–4264. doi:10.3168/jds.S0022-0302(91)78620-7.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J Dairy Sci 93:5764–5771. doi:10.3168/JDS.2010-3429.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2012. Risk factors and effects of postpartum anovulation in dairy cows. J Dairy Sci 95:1845–1854. doi:10.3168/jds.2011-4781.

Duffield, T. 2000. Subclinical Ketosis in Lactating Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice 16:231–253. doi:10.1016/S0749-0720(15)30103-1.

Duffield, T., K.D. Lissemore, B.W. McBride, and K.E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci 92:571–580. doi:10.3168/jds.2008-1507.

Eaton, J.W., P. Brandt, J.R. Mahoney, and J.T. Lee. 1982. Haptoglobin: A Natural Bacteriostat. Science (1979) 215:691–693. doi:10.1126/SCIENCE.7036344.

Eckel, E.F., and B.N. Ametaj. 2016. Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. J Dairy Sci 99:5967–5990. doi:10.3168/JDS.2015-10727.

Eckel, E.F., and B.N. Ametaj. 2020. Bacterial Endotoxins and Their Role in Periparturient Diseases of Dairy Cows: Mucosal Vaccine Perspectives. Dairy 2020, Vol. 1, Pages 61-90 1:61–90. doi:10.3390/DAIRY1010006.

Elander, L., L. Engström, M. Hallbeck, and A. Blomqvist. 2007. IL-1β and LPS induce anorexia by distinct mechanisms differentially dependent on microsomal prostaglandin E synthase-1. Am J Physiol Regul Integr Comp Physiol 292:258–267. doi:10.1152/AJPREGU.00511.2006.

Emmanuel, D.G.V., S.M. Dunn, and B.N. Ametaj. 2008. Feeding High Proportions of Barley Grain Stimulates an Inflammatory Response in Dairy Cows. J Dairy Sci 91:606–614. doi:10.3168/JDS.2007-0256.

Engelking, L.E., D.J. Ambrose, and M. Oba. 2022. Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration on serum inflammatory markers and productivity of dairy cows during the calving transition. J Dairy Sci 105:4144–4155. doi:10.3168/jds.2021-21553.

Evans, M., T.S. McClure, A.P. Koutnik, and B. Egan. 2022. Exogenous Ketone Supplements in Athletic Contexts: Past, Present, and Future. Sports Medicine 2022 52:1 52:25–67. doi:10.1007/S40279-022-01756-2.

Falkenhain, K., A. Daraei, S.C. Forbes, and J.P. Little. 2022. Effects of Exogenous Ketone Supplementation on Blood Glucose: A Systematic Review and Meta-analysis. Advances in Nutrition 13:1697–1714. doi:10.1093/advances/nmac036.

Finucane, O.M., C.L. Lyons, A.M. Murphy, C.M. Reynolds, R. Klinger, N.P. Healy, A.A. Cooke, R.C. Coll, L. Mcallan, K.N. Nilaweera, M.E. O'Reilly, A.C. Tierney, M.J. Morine, J.F. Alcala-Diaz, J. Lopez-Miranda, D.P. O'Connor, L.A. O'Neill, F.C. Mcgillicuddy, and H.M. Roche. 2015. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity. Diabetes 64:2116–2128. doi:10.2337/DB14-1098.

Fischer, C., M. Drillich, S. Odau, W. Heuwieser, R. Einspanier, and C. Gabler. 2010. Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the oestrous cycle and elevated in case of subclinical or clinical endometritis. Reprod Fertil Dev 22:818–829. doi:10.1071/RD09120.

Franchi, L., N. Warner, K. Viani, and G. Nuñez. 2009. Function of Nod-like receptors in microbial recognition and host defense. Blackwell Munksgaard • Immunological Reviews 227:106–128.

Froesch, E.R. 1967. The physiology and pharmacology of adipose tissue lipolysis: Its inhibition and implications for the treatment of diabetes. Diabetologia 3:475–487. doi:10.1007/BF01213565/METRICS.

Furukawa, E., Z. Chen, H. Ueshiba, Y. Wu, H. Chiba, Y. Yanagawa, S. Katagiri, M. Nagano, and S.P. Hui. 2021. Postpartum cows showed high oocyte triacylglycerols concurrently with high plasma free fatty acids. Theriogenology 176:174–182. doi:10.1016/J.THERIOGENOLOGY.2021.09.034.

Gabay, C., and I. Kushner. 1999. Acute-Phase Proteins and Other Systemic Responses to Inflammation. https://doi.org/10.1056/NEJM199902113400607 340:448–454. doi:10.1056/NEJM199902113400607.

Garner, J.B., M. Douglas, S.R.O. Williams, W.J. Wales, L.C. Marett, K. DiGiacomo, B.J. Leury, B.J. Hayes, J.B. Garner, M. Douglas, S.R.O. Williams, W.J. Wales, L.C. Marett, K. DiGiacomo, B.J. Leury, and B.J. Hayes. 2017. Responses of dairy cows to short-term heat stress in controlled-climate chambers. Anim Prod Sci 57:1233–1241. doi:10.1071/AN16472.

Gattorno, M., S. Tassi, S. Carta, L. Delfino, F. Ferlito, M.A. Pelagatti, A. D'Osualdo, A. Buoncompagni, M.G. Alpigiani, M. Alessio, A. Martini, and A. Rubartelli. 2007. Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. Arthritis Rheum 56:3138–3148. doi:10.1002/ART.22842.

Gleerup, K.B., P.H. Andersen, L. Munksgaard, and B. Forkman. 2015. Pain evaluation in dairy cattle. Appl Anim Behav Sci 171:25–32. doi:10.1016/J.APPLANIM.2015.08.023.

Goff, J.P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal 176:50–57. doi:10.1016/j.tvjl.2007.12.020.

Goff, J.P., and R.L. Horst. 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders,. J Dairy Sci 80:1260–1268. doi:10.3168/JDS.S0022-0302(97)76055-7.

Gokcen, E., Y. Cevik, G. Pamukcu Gunaydin, N.O. Dogan, and M. Tekin. 2018. Change in Complete Blood Count Parameters and Vital Signs After Intravenous Infusion of 1 Liter of Normal Saline. Eurasian Journal of Emergency Medicine 17. doi:10.5152/eajem.2018.83007.

Goldhawk, C., N. Chapinal, D.M. Veira, D.M. Weary, and M.A.G. von Keyserlingk. 2009. Prepartum feeding behavior is an early indicator of subclinical ketosis. J Dairy Sci 92:4971–4977. doi:10.3168/jds.2009-2242.

Goldsby, R., T. Kindt, and B. Osborne. 2001. Kuby Immunology. 4th ed. W.H. FREEMAN AND COMPANY, New York.

Gordon, J.L., S.J. Leblanc, and T.F. Duffield. 2013. Ketosis Treatment in Lactating Dairy Cattle. doi:10.1016/j.cvfa.2013.03.001.

Górka, P., Z.M. Kowalski, R. Zabielski, and P. Guilloteau. 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. J Dairy Sci 101:4785–4800. doi:10.3168/jds.2017-14086.

Gozho, G.N., D.O. Krause, and J.C. Plaizier. 2007. Ruminal Lipopolysaccharide Concentration and Inflammatory Response During Grain-Induced Subacute Ruminal Acidosis in Dairy Cows. J Dairy Sci 90:856–866. doi:10.3168/JDS.S0022-0302(07)71569-2.

Griffin, M.E., M.J. Marcucci, G.W. Cline, K. Bell, N. Barucci, D. Lee, L.J. Goodyear, E.W. Kraegen, M.F. White, and G.I. Shulman. 1999. Free Fatty Acid-Induced Insulin Resistance Is Associated With Activation of Protein Kinase C and Alterations in the Insulin Signaling Cascade. 1270 DIABETES 48.

Grinberg, N., S. Elazar, I. Rosenshine, and N.Y. Shpigel. 2008. β-hydroxybutyrate abrogates formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic Escherichia coli. Infect Immun 76:2802–2807. doi:10.1128/IAI.00051-08/ASSET/C36B113B-0391-4750-A0B7-98586ED222E1/ASSETS/GRAPHIC/ZII0060873660004.JPEG.

Grinnell, K.L., H. Chichger, J. Braza, H. Duong, and E.O. Harrington. 2012. Protection against LPS-Induced Pulmonary Edema through the Attenuation of Protein Tyrosine Phosphatase–1B Oxidation. Am J Respir Cell Mol Biol 46:623–632. doi:10.1165/rcmb.2011-0271OC.

Gröhn, Y., L.A. Lindberg, M.L. Bruss, and T.B. Farver. 1983. Fatty infiltration of liver in spontaneously ketotic dairy cows. J Dairy Sci 66:2320–2328. doi:10.3168/JDS.S0022-0302(83)82088-8.

Gross, J., H.A. van Dorland, R.M. Bruckmaier, and F.J. Schwarz. 2011. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. J Dairy Sci 94:1820–1830. doi:10.3168/JDS.2010-3707.

Gross, J.J., L. Grossen-Rösti, S.K. Wall, O. Wellnitz, and R.M. Bruckmaier. 2020. Metabolic status is associated with the recovery of milk somatic cell count and milk secretion after lipopolysaccharide-induced mastitis in dairy cows. J Dairy Sci 103:5604–5615. doi:10.3168/jds.2019-18032.

Grossi, P., G. Bertoni, F.P. Cappelli, and E. Trevisi. 2013. Effects of the precalving administration of omega-3 fatty acids alone or in combination with acetylsalicylic acid in periparturient dairy cows1. J Anim Sci 91:2657–2666. doi:10.2527/jas.2012-5661.

Gruber, L., W. Knaus, A. Susenbeth, F.J. Schwarz, T. Guggenberger, B. Fischer, H. Spiekers, H. Steingaß, U. Meyer, A. Münger, T. Jilg, and A. Obermaier. 2021. Evaluation of the net energy for lactation system and estimation of the energy requirements of dairy cows based on a comprehensive analysis of feeding trials. Arch Anim Nutr 75:1–17. doi:10.1080/1745039X.2020.1854595.

Grummer, R.R. 1993. Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy Cows. J Dairy Sci 76:3882–3896. doi:10.3168/jds.S0022-0302(93)77729-2.

Gruys, E., M.J.M. Toussaint, T.A. Niewold, and S.J. Koopmans. 2005. Review: Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B 6:1045. doi:10.1631/JZUS.2005.B1045.

Gulhar, R., M.A. Ashraf, and I. Jialal. 2022. Physiology, Acute Phase Reactants. StatPearls.

Guliński, P. 2021. Ketone bodies - causes and effects of their increased presence in cows' body fluids: A review.. Vet World 14:1492–1503. doi:10.14202/vetworld.2021.1492-1503.

Guo, J., G. Chang, K. Zhang, L. Xu, D. Jin, M. Shahid Bilal, and X. Shen. 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. Nanjing.

Haces, M.L., K. Hernández-Fonseca, O.N. Medina-Campos, T. Montiel, J. Pedraza-Chaverri, and L. Massieu. 2008. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. Exp Neurol 211:85–96. doi:10.1016/J.EXPNEUROL.2007.12.029.

Hackmann, T.J., D.K. Ngugi, J.L. Firkins, and J. Tao. 2017. Genomes of rumen bacteria encode atypical pathways for fermenting hexoses to short-chain fatty acids. Environ Microbiol 19:4670–4683. doi:10.1111/1462-2920.13929.

Hall, M.B. 2023. Invited review: Corrected milk: Reconsideration of common equations and milk energy estimates. J Dairy Sci 106:2230–2246. doi:10.3168/jds.2022-22219.

Hammon, D.S., I.M. Evjen, T.R. Dhiman, J.P. Goff, and J.L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol 113:21–29. doi:10.1016/j.vetimm.2006.03.022.

Hammon, H.M., G. Stürmer, F. Schneider, A. Tuchscherer, H. Blum, T. Engelhard, A. Genzel, R. Staufenbiel, and W. Kanitz. 2009. Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. J Dairy Sci 92:1554–1566. doi:10.3168/JDS.2008-1634.

Hansen, V.L., L.S. Faber, A.A. Salehpoor, and R.D. Miller. 2017. A pronounced uterine pro-inflammatory response at parturition is an ancient feature in mammals. Proceedings of the Royal Society B: Biological Sciences 284. doi:10.1098/RSPB.2017.1694.

Hanson, R.W., and F.J. Ballard. 1967. The Relative Significance of Acetate and Glucose as Precursors for Lipid Synthesis in Liver and Adipose Tissue from Ruminants.

Hart, I.C. 1983. Endocrine control of nutrient partition in lactating ruminants. Proceedings of the Nutrition Society 42:181–194. doi:10.1079/pns19830023.

Hartman, A.L., and J.M. Rho. 2014. The New Ketone Alphabet Soup: BHB, HCA, and HDAC. Epilepsy Curr 14:355. doi:10.5698/1535-7597-14.6.355.

Heitmann, R.N., D.J. Dawes, and S.C. Sensenig. 1987. Hepatic Ketogenesis and Peripheral Ketone Body Utilization in the Ruminant. J Nutr 117:1174–1180. doi:10.1093/JN/117.6.1174.

Herrick, K.J., A.R. Hippen, K.F. Kalscheur, D.J. Schingoethe, D.P. Casper, S.C. Moreland, and J.E. van Eys. 2017. Single-dose infusion of sodium butyrate, but not lactose, increases plasma β-hydroxybutyrate and insulin in lactating dairy cows. J Dairy Sci 100:757–768. doi:10.3168/jds.2016-11634.

Herrick, K.J., A.R. Hippen, K.F. Kalscheur, D.J. Schingoethe, S.D. Ranathunga, J.L. Anderson, S.C. Moreland, and J.E. van Eys. 2018. Infusion of butyrate affects plasma glucose, butyrate, and β-hydroxybutyrate but not plasma insulin in lactating dairy cows. J Dairy Sci 101:3524–3536. doi:10.3168/jds.2017-13842.

Hoeben, D., R. Heyneman, and C. Burvenich. 1997. Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. Vet Immunol Immunopathol 58:165–170. doi:10.1016/S0165-2427(97)00031-7.

Høgild, M.L., A.J. Hjelholt, J. Hansen, S.B. Pedersen, N. Møller, J.F.P. Wojtaszewski, M. Johannsen, N. Jessen, and J.O.L. Jørgensen. 2023. Ketone Body Infusion Abrogates Growth Hormone–Induced Lipolysis and Insulin Resistance. J Clin Endocrinol Metab 108:653–664. doi:10.1210/clinem/dgac595.

Holdsworth, D.A., P.J. Cox, T. Kirk, H. Stradling, S.G. Impey, and K. Clarke. 2017. A Ketone Ester Drink Increases Postexercise Muscle Glycogen Synthesis in Humans. Med Sci Sports Exerc 49:1789–1795. doi:10.1249/MSS.0000000000001292.

Horst, E.A., S.K. Kvidera, and L.H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas. J Dairy Sci 104:8380–8410. doi:10.3168/jds.2021-20330.

Horst, E.A., S.K. Kvidera, M.J. Dickson, C.S. McCarthy, E.J. Mayorga, M. Al-Qaisi, H.A. Ramirez, A.F. Keating, and L.H. Baumgard. 2019a. Effects of continuous and increasing lipopolysaccharide infusion on basal and stimulated metabolism in lactating Holstein cows. J Dairy Sci 102:3584–3597. doi:10.3168/JDS.2018-15627.

Horst, E.A., E.J. Mayorga, S. Rodriguez-Jimenez, M.A. Abeyta, B.M. Goetz, S. Carta, M. Al-Qaisi, S.K. Kvidera, and L.H. Baumgard. 2019b. Causes and Metabolic Consequences of Leaky Gut.

Huang, Z., J.B. Massey, and D. Via. 2000. Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in human in vitro differentiated macrophages.. Biochem Pharmacol.

Hughes J. 1962. A simplified instrument for obtaining liver biopsies in cattle.. Am J Vet Res 23:1111–3.

Huhtanen, P., H. Miettinen, and M. Ylinen. 1993. Effect of Increasing Ruminal Butyrate on Milk Yield and Blood Constituents in Dairy Cows Fed a Grass Silage-Based Diet. J Dairy Sci 76:1114–1124. doi:10.3168/jds.S0022-0302(93)77440-8.

Hui, S., A.J. Cowan, X. Zeng, L. Yang, T. TeSlaa, X. Li, C. Bartman, Z. Zhang, C. Jang, L. Wang, W. Lu, J. Rojas, J. Baur, and J.D. Rabinowitz. 2020. Quantitative Fluxomics of Circulating Metabolites. Cell Metab 32:676-688.e4. doi:10.1016/J.CMET.2020.07.013.

Imhasly, S., H. Naegeli, S. Baumann, M. von Bergen, A. Luch, H. Jungnickel, S. Potratz, and C. Gerspach. 2014. Metabolomic biomarkers correlating with hepatic lipidosis in dairy cows. BMC Vet Res 10:1–9. doi:10.1186/1746-6148-10-122/FIGURES/5.

Izumi, K., R. Fukumori, S. Oikawa, and M. Oba. 2019. Short communication: Effects of butyrate supplementation on the productivity of lactating dairy cows fed diets differing in starch content. J Dairy Sci 102:11051–11056. doi:10.3168/jds.2019-17113.

Jacobs, A.A.A., J. Dijkstra, J.S. Liesman, M.J. Vandehaar, A.L. Lock, A.M. van Vuuren, W.H. Hendriks, and J. van Baal. 2013. Effects of short- and long-chain fatty acids on the expression of stearoyl-CoA desaturase and other lipogenic genes in bovine mammary epithelial cells. Animal 7:1508–1516. doi:10.1017/S175173111300061X.

Jain, S.K., K. Kannan, G. Lim, J. Matthews-Greek, R. McVie, and J.A. Bocchini. 2003. Elevated blood interleukin-6 levels in hyperketonemic type 1 diabetic patients and secretion by acetoacetate-treated cultured U937 monocytes. Diabetes Care 26:2139–2143. doi:10.2337/DIACARE.26.7.2139.

Jain, S.K.;, and R. Mcvie. 1999. Hyperketonemia can increase lipid peroxidation and lower glutathione. Diabetes 48:1850.

Jenkins, D.J.A., W.M. Hunter, and D. V. Goff. 1970. Ketone Bodies and Evidence for increased Insulin Secretion. Nature 1970 227:5256 227:384–385. doi:10.1038/227384a0.

Jenkins, T.C. 1993. Lipid Metabolism in the Rumen. J Dairy Sci 76:3851–3863. doi:10.3168/JDS.S0022-0302(93)77727-9.

Jesse, B.W., R.S. Emery, and J.W. Thomas. 1986. Control of Bovine Hepatic Fatty Acid Oxidation. J Dairy Sci 69:2290–2297. doi:10.3168/jds.S0022-0302(86)80667-1.

Jobin, K., D.N. Müller, J. Jantsch, and C. Kurts. 2021. Sodium and its manifold impact on our immune system. Trends Immunol 42:469–479. doi:10.1016/j.it.2021.04.002.

Kaneko, N., M. Kurata, T. Yamamoto, S. Morikawa, and J. Masumoto. 2019. The role of interleukin-1 in general pathology. Inflammation and Regeneration 2019 39:1 39:1–16. doi:10.1186/S41232-019-0101-5.

Kashiwaya, Y., M.T. King, and R.L. Veech. 1997. Substrate Signaling by Insulin: A Ketone Bodies Ratio Mimics Insulin Action in Heart. American Journal of Cardiology 80:50A-64A. doi:10.1016/S0002-9149(97)00458-X.

Kayano, M., and T. Kataoka. 2015. Screening for ketosis using multiple logistic regression based on milk yield and composition. J Vet Med Sci 77:1473. doi:10.1292/JVMS.14-0691.

Kelley, N., D. Jeltema, Y. Duan, and Y. He. 2019. Molecular Sciences The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. doi:10.3390/ijms20133328.

Kelly, B., and L.A.J. O'Neill. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 25:771–784. doi:10.1038/CR.2015.68.

Kerner, J., and C.L. Hoppel. 2004. Ketogenesis. Elsevier.

Kesl, S.L., A.M. Poff, N.P. Ward, T.N. Fiorelli, C. Ari, A.J. Van Putten, J.W. Sherwood, P. Arnold, and D.P. D'Agostino. 2016. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague-Dawley rats. Nutr Metab (Lond) 13:1–15. doi:10.1186/S12986-016-0069-Y/FIGURES/7.

Khafipour, E., D.O. Krause, and J.C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci 92:1060–1070. doi:10.3168/JDS.2008-1389.

Kim, S.R., S.G. Lee, S.H. Kim, J.H. Kim, E. Choi, W. Cho, J.H. Rim, I. Hwang, C.J. Lee, M. Lee, C.M. Oh, J.Y. Jeon, H.Y. Gee, J.H. Kim, B.W. Lee, E.S. Kang, B.S. Cha, M.S. Lee, J.W. Yu, J.W. Cho, J.S. Kim, and Y. ho Lee. 2020. SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nature Communications 2020 11:1 11:1–11. doi:10.1038/s41467-020-15983-6.

Kováč, G., C. Tóthová, O. Nagy, H. Seidel, and J. Konvičná. 2009. Acute Phase Proteins and their Relation to Energy Metabolites in Dairy Cows during the Pre- and Postpartal Period. Acta Veterinaria Brno 78:441–447. doi:10.2754/AVB200978030441.

Krebs, H.A. 1966. Bovine ketosis. Vet Rec 78:187–192. doi:10.1136/VR.78.6.187.

Krehbiel, C.R., D.L. Harmon, and J.E. Schneider. 1992a. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers.. J Anim Sci 70:904–914. doi:10.2527/1992.703904x.

Krehbiel, C.R., D.L. Harmon, and J.E. Schneider. 1992b. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. J Anim Sci 70:904–914. doi:10.2527/1992.703904x.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: implications for insufficient feed intake of transition dairy cows. Animal 14:s65–s77. doi:10.1017/S1751731119003124.

Kurtz, R., M.F. Anderman, and B.D. Shepard. 2021. GPCRs get fatty: the role of G protein-coupled receptor signaling in the development and progression of nonalcoholic fatty liver disease. American Journal of Physiology-Gastrointestinal and Liver Physiology 320:G304–G318. doi:10.1152/ajpgi.00275.2020.

Kvidera, S.K., E.A. Horst, M. Abuajamieh, E.J. Mayorga, M.V.S. Fernandez, and L.H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J Dairy Sci 100:2360–2374. doi:10.3168/jds.2016-12001.

Kyoung Lee, A., D.H. Kim, E.J. Bang, Y.J. Choi, and H.Y. Chung. 2020. β-Hydroxybutyrate Suppresses Lipid Accumulation in Aged Liver through GPR109A-mediated Signaling. Aging Dis 11:777. doi:10.14336/AD.2019.0926.

Laarman, A.H., A. Kleinberg, M.A. Steele, O. AlZahal, and B.W. McBride. 2015. Changes in the Rumen Papillae During the Periparturient Transition in Holstein Dairy Cows are Accompanied by Changes in Abundance of Proteins Involved in Intracellular pH Regulation, But not SCFA Transport. Am J Anim Vet Sci 10:14–22. doi:10.3844/ajavsp.2015.14.22.

Laffel, L. 1999. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 15:412–426. doi:10.1002/(sici)1520-7560(199911/12)15:6<412::aid-dmrr72>3.0.co;2-8.

Langhans, W., F. Wiesenreiter, and E. Scharrer. 1983. Different effects of subcutaneous D,L-3-hydroxybutyrate and acetoacetate injections on food intake in rats. Physiol Behav 31:483–486. doi:10.1016/0031-9384(83)90070-7.

LeBlanc, S. 2015. Managing Ketosis in the Transition Cow for Health and Reproduction. Pages 94–101 in Dairy Cattle Reproduction Council.

LeBlanc, S.J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. The Veterinary Journal 176:102–114. doi:10.1016/j.tvjl.2007.12.019.

LeBlanc, S.J., K.E. Leslie, and T.F. Duffield. 2005. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. J Dairy Sci 88:159–170. doi:10.3168/JDS.S0022-0302(05)72674-6.

LeBlanc, S.J., K.D. Lissemore, D.F. Kelton, T.F. Duffield, and K.E. Leslie. 2006. Major Advances in Disease Prevention in Dairy Cattle. J Dairy Sci 89:1267–1279. doi:10.3168/JDS.S0022-0302(06)72195-6.

LeBlanc, S.J., T. Osawa, and J. Dubuc. 2011. Reproductive tract defense and disease in postpartum dairy cows. Theriogenology 76:1610–1618. doi:10.1016/J.THERIOGENOLOGY.2011.07.017.

Lebreton, F., E. Berishvili, G. Parnaud, C. Rouget, D. Bosco, T. Berney, and V. Lavallard. 2018. NLRP3 inflammasome is expressed and regulated in human islets. Cell Death & Disease 2018 9:7 9:1–10. doi:10.1038/s41419-018-0764-x.

Lee, A.K., D.H. Kim, E. Bang, Y.J. Choi, and H.Y. Chung. 2020. β-Hydroxybutyrate Suppresses Lipid Accumulation in Aged Liver through GPR109A-mediated Signaling. Aging Dis 11:777. doi:10.14336/AD.2019.0926.

Li, B., Y. Yu, K. Liu, Y. Zhang, Q. Geng, F. Zhang, Y. Li, and J. Qi. 2021. β-Hydroxybutyrate inhibits histone deacetylase 3 to promote claudin-5 generation and attenuate cardiac microvascular hyperpermeability in diabetes. Diabetologia 64:226–239. doi:10.1007/S00125-020-05305-2.

Li, P., X.B. Li, S.X. Fu, C.C. Wu, X.X. Wang, G.J. Yu, M. Long, Z. Wang, and G.W. Liu. 2012a. Alterations of fatty acid β-oxidation capability in the liver of ketotic cows. J Dairy Sci 95:1759–1766. doi:10.3168/JDS.2011-4580.

Li, R.W., S. Wu, R.L. Baldwin, W. Li, and C. Li. 2012b. Perturbation Dynamics of the Rumen Microbiota in Response to Exogenous Butyrate. PLoS One 7:e29392. doi:10.1371/journal.pone.0029392.

Li, Y., H. Ding, X. Wang, L. Liu, D. Huang, R. Zhang, L. Guo, Z. Wang, X. Li, G. Liu, J. Wu, and X. Li. 2016. High levels of acetoacetate and glucose increase expression of cytokines in bovine hepatocytes, through activation of the NF-κB signalling pathway. Journal of Dairy Research 83:51–57. doi:10.1017/S0022029915000680.

Liang, D., L.M. Arnold, C.J. Stowe, R.J. Harmon, and J.M. Bewley. 2017. Estimating US dairy clinical disease costs with a stochastic simulation model. J Dairy Sci 100:1472–1486. doi:10.3168/JDS.2016-11565.

Liang, J.J., I.D.C. Fraser, and C.E. Bryant. 2021. Lipid regulation of NLRP3 inflammasome activity through organelle stress. Trends Immunol 42:807–823. doi:10.1016/J.IT.2021.07.005.

Linn, J. 2006. Feed Efficiency: Its Economic Impact In Lactating Dairy Cows.

Lisuzzo, A., F. Fiore, K. Harvatine, E. Mazzotta, M. Berlanda, N. Spissu, T. Badon, B. Contiero, L. Moscati, and E. Fiore. 2022. Changes in plasma fatty acids profile in

hyperketonemic ewes during early lactation: a preliminary study. Sci Rep 12:17017. doi:10.1038/s41598-022-21088-5.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206. doi:10.1007/s11745-004-1348-6.

Loft, A., S.F. Schmidt, G. Caratti, U. Stifel, J. Havelund, R. Sekar, Y. Kwon, A. Sulaj, K.K. Chow, A.J. Alfaro, T. Schwarzmayr, N. Rittig, M. Svart, F.F. Tsokanos, A. Maida, A. Blutke, A. Feuchtinger, N. Møller, M. Blüher, P. Nawroth, J. Szendrödi, N.J. Færgeman, A. Zeigerer, J. Tuckermann, and S. Herzig. 2022. A macrophage-hepatocyte glucocorticoid receptor axis coordinates fasting ketogenesis. Cell Metab 34:473-486.e9. doi:10.1016/J.CMET.2022.01.004.

Lukasova, M., J. Hanson, S. Tunaru, and S. Offermanns. 2011. Nicotinic acid (niacin): new lipid-independent mechanisms of action and therapeutic potentials. Trends Pharmacol Sci 32:700–707. doi:10.1016/J.TIPS.2011.08.002.

Luo, Z.Z., L.H. Shen, J. Jiang, Y.X. Huang, L.P. Bai, S.M. Yu, X.P. Yao, Z.H. Ren, Y.X. Yang, and S.Z. Cao. 2019. Plasma metabolite changes in dairy cows during parturition identified using untargeted metabolomics. J Dairy Sci 102:4639–4650. doi:10.3168/jds.2018-15601.

Lyons, C.L., and H.M. Roche. 2018. Nutritional Modulation of AMPK-Impact upon Metabolic-Inflammation. International Journal of Molecular Sciences 2018, Vol. 19, Page 3092 19:3092. doi:10.3390/IJMS19103092.

Ma, D., A.C. Wang, I. Parikh, S.J. Green, J.D. Hoffman, G. Chlipala, M.P. Murphy, B.S. Sokola, B. Bauer, A.M.S. Hartz, and A.-L. Lin. 2018. Ketogenic diet enhances neurovascular function with altered gut microbiome in young healthy mice. Sci Rep 8:6670. doi:10.1038/s41598-018-25190-5.

MacKellar, M., and D.J. Vigerust. 2016. Role of Haptoglobin in Health and Disease: A Focus on Diabetes. Clin Diabetes 34:148. doi:10.2337/DIACLIN.34.3.148.

Madison, L.L., D. Mebane, R.H. Unger, and A. Lochner. 1964. THE HYPOGLYCEMIC ACTION OF KETONES. II. EVIDENCE FOR A STIMULATORY. J. Clin. Invest. 43:408. doi:10.1172/jci104925.

Maizels, E.Z., N.B. Ruderman, M.N. Goodman, and D. Lau. 1977. Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat.. Biochemical Journal 162:557. doi:10.1042/BJ1620557.

Malik, A., and T.-D. Kanneganti. 2017. Inflammasome activation and assembly at a glance. J Cell Sci 130:3955–3963. doi:10.1242/jcs.207365.

Mann, S., F.A. Leal Yepes, J.J. Wakshlag, E. Behling-Kelly, and J.A.A. McArt. 2018. The effect of different treatments for early-lactation hyperketonemia on liver triglycerides, glycogen, and expression of key metabolic enzymes in dairy cattle. J Dairy Sci 101:1626–1637. doi:10.3168/JDS.2017-13360.

le Marchand-Brustel, Y., P. Gual, T. Grémeaux, T. Gonzalez, R. Barrès, and J.F. Tanti. 2003. Fatty acid-induced insulin resistance: role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling. Biochem Soc Trans 31:1152–1156. doi:10.1042/BST0311152.

Martens, H., I. Rabbani, Z. Shen, F. Stumpff, and C. Deiner. 2012. Changes in rumen absorption processes during transition. Anim Feed Sci Technol 172:95–102. doi:10.1016/J.ANIFEEDSCI.2011.12.011.

Masi, D., M.E. Spoltore, R. Rossetti, M. Watanabe, R. Tozzi, A. Caputi, R. Risi, A. Balena, O. Gandini, S. Mariani, G. Spera, L. Gnessi, and C. Lubrano. 2022. The Influence of Ketone Bodies on Circadian Processes Regarding Appetite, Sleep and Hormone Release: A Systematic Review of the Literature. Nutrients 14. doi:10.3390/NU14071410.

Matsuki, T., R. Horai, K. Sudo, and Y. Iwakura. 2003. IL-1 Plays an Important Role in Lipid Metabolism by Regulating Insulin Levels under Physiological Conditions. J Exp Med 198:877. doi:10.1084/JEM.20030299.

McArt, J.A.A., D. V. Nydam, and G.R. Oetzel. 2012a. Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci 95:5056–5066. doi:10.3168/jds.2012-5443.

McArt, J.A.A., D. V. Nydam, and G.R. Oetzel. 2012b. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J Dairy Sci 95:2505–2512. doi:10.3168/jds.2011-4908.

McArt, J.A.A., D. v. Nydam, G.R. Oetzel, and C.L. Guard. 2014. An economic analysis of hyperketonemia testing and propylene glycol treatment strategies in early lactation dairy cattle. Prev Vet Med 117:170–179. doi:10.1016/J.PREVETMED.2014.06.017.

McArt, J.A.A., D. V. Nydam, G.R. Oetzel, T.R. Overton, and P.A. Ospina. 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Veterinary Journal 198:560–570. doi:10.1016/j.tvjl.2013.08.011.

McArt, J.A.A., D. V. Nydam, P.A. Ospina, and G.R. Oetzel. 2011. A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. J Dairy Sci 94:6011–6020. doi:10.3168/JDS.2011-4463.

McArt, J.A.A., and G.R. Oetzel. 2015. A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. J Dairy Sci 98:7408–7418. doi:10.3168/JDS.2015-9479.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. Nature 2008 454:7203 454:428–435. doi:10.1038/nature07201.

Meng, L., Z. Song, A. Liu, U. Dahmen, X. Yang, and H. Fang. 2021. Effects of Lipopolysaccharide-Binding Protein (LBP) Single Nucleotide Polymorphism (SNP) in Infections, Inflammatory Diseases, Metabolic Disorders and Cancers. Front Immunol 12:2469. doi:10.3389/FIMMU.2021.681810/BIBTEX.

Mensching, A., M. Zschiesche, J. Hummel, C. Grelet, N. Gengler, S. Dänicke, and A.R. Sharifi. 2021. Development of a subacute ruminal acidosis risk score and its prediction using milk mid-infrared spectra in early-lactation cows. J Dairy Sci 104:4615–4634. doi:10.3168/jds.2020-19516.

Meroni, E., N. Papini, F. Criscuoli, M.C. Casiraghi, L. Massaccesi, N. Basilico, and D. Erba. 2018. Metabolic Responses in Endothelial Cells Following Exposure to Ketone Bodies. Nutrients 10. doi:10.3390/NU10020250.

Mezzetti, M., L. Cattaneo, M.M. Passamonti, V. Lopreiato, A. Minuti, and E. Trevisi. 2021. The Transition Period Updated: A Review of the New Insights into the Adaptation of Dairy Cows to the New Lactation. Dairy 2:617–636. doi:10.3390/dairy2040048.

Van Miert, A.S.J.P.A.M. 2011. Pro-inflammatory cytokines in a ruminant model: Pathophysiological, pharmacological, and therapeutic aspects. http://dx.doi.org/10.1080/01652176.1995.9694530 17:41–50. doi:10.1080/01652176.1995.9694530.

Mihaylova, M.M., D.S. Vasquez, K. Ravnskjaer, P.D. Denechaud, R.T. Yu, J.G. Alvarez, M. Downes, R.M. Evans, M. Montminy, and R.J. Shaw. 2011. Class IIa Histone Deacetylases are Hormone-activated regulators of FOXO and Mammalian Glucose Homeostasis. Cell 145:607. doi:10.1016/J.CELL.2011.03.043.

Mills, S.E., D.C. Beitz, and J.W. Young. 1986. Characterization of Metabolic Changes during a Protocol for Inducing Lactation Ketosis in Dairy Cows. J Dairy Sci 69:352–361. doi:10.3168/jds.S0022-0302(86)80413-1.

Moallem, U. 2018. Invited review: Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J Dairy Sci 101:8641–8661. doi:10.3168/jds.2018-14772.

Mohammad, S., and C. Thiemermann. 2021. Role of Metabolic Endotoxemia in Systemic Inflammation and Potential Interventions. Front Immunol 11. doi:10.3389/fimmu.2020.594150.

Mohsin, M.A., H. Yu, R. He, P. Wang, L. Gan, Y. Du, Y. Huang, M.B. Abro, S. Sohaib, M. Pierzchala, P. Sobiech, K. Miętkiewska, C.S. Pareek, and B.X. He. 2022. Differentiation of Subclinical Ketosis and Liver Function Test Indices in Adipose Tissues Associated With Hyperketonemia in Postpartum Dairy Cattle. Front Vet Sci 8:1684. doi:10.3389/FVETS.2021.796494/BIBTEX.

Molla, M.D., Y. Akalu, Z. Geto, B. Dagnew, B. Ayelign, and T. Shibabaw. 2020. Role of caspase-1 in the pathogenesis of inflammatory-associated chronic noncommunicable diseases. J Inflamm Res 13:749–764. doi:10.2147/JIR.S277457.

Murray, R.K. (Robert K., D.A. Bender, K.M. Botham, P.J. Kennelly, V.W. Rodwell, and P.A. Weil. 2009. Harper's Illustrated Biochemistry. McGraw-Hill Medical.

Myette-Côté, É., H. Neudorf, H. Rafiei, K. Clarke, and J.P. Little. 2018. Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. J Physiol 596:1385. doi:10.1113/JP275709.

Nagaraja, T.G., and M.M. Chengappa. 1996. Bud Britton Memorial Symposium on Metabolic Disorders of Feedlot Cattle.

National Research Council. 1980. Effect of Environment on Nutrient Requirements of Domestic Animals. National Academies Press.

National Research Council (U.S.). Subcommittee on Dairy Cattle Nutrition. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press.

Newman, J.C., and E. Verdin. 2017. β-Hydroxybutyrate: A Signaling Metabolite. Annu Rev Nutr 37:51. doi:10.1146/ANNUREV-NUTR-071816-064916.

Novak, T.E., S.L. Rodriguez-Zas, B.R. Southey, J.D. Starkey, R.M. Stockler, G.F. Alfaro, and S.J. Moisá. 2019. Jersey steer ruminal papillae histology and nutrigenomics with diet changes. J Anim Physiol Anim Nutr (Berl) 103:1694–1707. doi:10.1111/JPN.13189.

Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 20:651–674. doi:10.1016/J.CVFA.2004.06.006.

Oetzel, G.R. 2013. Understanding the Impact of Subclinical Ketosis. University of Wisconsin: Madison 15–26.

Oh, K.J., H.S. Han, M.J. Kim, and S.H. Koo. 2013. CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. BMB Rep 46:567. doi:10.5483/BMBREP.2013.46.12.248.

Okawa, T., M. Nagai, and K. Hase. 2021. Dietary Intervention Impacts Immune Cell Functions and Dynamics by Inducing Metabolic Rewiring. Front Immunol 11:3756. doi:10.3389/FIMMU.2020.623989/BIBTEX.

Omenetti, S., and T.T. Pizarro. 2015. The Treg/Th17 axis: A dynamic balance regulated by the gut microbiome. Front Immunol 6:639. doi:10.3389/FIMMU.2015.00639/BIBTEX.

Onizawa, Y., T. Katoh, R. Miura, K. Konda, T. Noguchi, H. Iwata, T. Kuwayama, S. Hamano, and K. Shirasuna. 2022a. Acetoacetate is a trigger of NLRP3 inflammasome activation in bovine peripheral blood mononuclear cells. Vet Immunol Immunopathol 244. doi:10.1016/J.VETIMM.2021.110370.

Onizawa, Y., T. Katoh, R. Miura, K. Konda, T. Noguchi, H. Iwata, T. Kuwayama, S. Hamano, and K. Shirasuna. 2022b. Acetoacetate is a trigger of NLRP3 inflammasome activation in bovine peripheral blood mononuclear cells. Vet Immunol Immunopathol 244. doi:10.1016/J.VETIMM.2021.110370.

Osorio, J.S., J. Lohakare, and M. Bionaz. 2016. Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. Physiol Genomics 48:231–256. doi:10.1152/physiolgenomics.00016.2015.

Ospina, P.A., J.A. McArt, T.R. Overton, T. Stokol, and D. V. Nydam. 2013. Using Nonesterified Fatty Acids and β-Hydroxybutyrate Concentrations During the Transition Period for Herd-Level Monitoring of Increased Risk of Disease and Decreased Reproductive and Milking Performance. Veterinary Clinics of North America: Food Animal Practice 29:387–412. doi:10.1016/J.CVFA.2013.04.003.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010a. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J Dairy Sci 93:546–554. doi:10.3168/JDS.2009-2277.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010b. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J Dairy Sci 93:1596–1603. doi:10.3168/JDS.2009-2852.

Ospina, P.A., D. V. Nydam, T. Stokol, and T.R. Overton. 2010c. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J Dairy Sci 93:1596–1603. doi:10.3168/JDS.2009-2852.

Overton, T.R., J.A.A. McArt, and D. V. Nydam. 2017. A 100-Year Review: Metabolic health indicators and management of dairy cattle. J Dairy Sci 100:10398–10417. doi:10.3168/jds.2017-13054.

Pan, A., X.M. Sun, F.Q. Huang, J.F. Liu, Y.Y. Cai, X. Wu, R.N. Alolga, P. Li, B.L. Liu, Q. Liu, and L.W. Qi. 2022a. The mitochondrial β-oxidation enzyme HADHA restrains

hepatic glucagon response by promoting β-hydroxybutyrate production. Nat Commun 13. doi:10.1038/S41467-022-28044-X.

Pan, A., X.-M. Sun, F.-Q. Huang, J.-F. Liu, Y.-Y. Cai, X. Wu, R.N. Alolga, P. Li, B.-L. Liu, Q. Liu, and L.-W. Qi. 2022b. The mitochondrial β-oxidation enzyme HADHA restrains hepatic glucagon response by promoting β-hydroxybutyrate production. Nat Commun 13:386. doi:10.1038/s41467-022-28044-x.

Park, S., D.S. Kim, and J.W. Daily. 2011. Central infusion of ketone bodies modulates body weight and hepatic insulin sensitivity by modifying hypothalamic leptin and insulin signaling pathways in type 2 diabetic rats. Brain Res 1401:95–103. doi:10.1016/J.BRAINRES.2011.05.040.

Paydar, S., H. Bazrafkan, N. Golestani, J. Roozbeh, A. Akrami, and A.M. Moradi. 2014. Effects of Intravenous Fluid Therapy on Clinical and Biochemical Parameters of Trauma Patients.. Emerg (Tehran) 2:90–5.

Pérez-Báez, J., C.A. Risco, R.C. Chebel, G.C. Gomes, L.F. Greco, S. Tao, I.M. Thompson, B.C. do Amaral, M.G. Zenobi, N. Martinez, C.R. Staples, G.E. Dahl, J.A. Hernández, J.E.P. Santos, and K.N. Galvão. 2019. Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis. J Dairy Sci 102:9151–9164. doi:10.3168/jds.2018-15879.

Pérez-Hernández, E.G., B. Delgado-Coello, I. Luna-Reyes, and J. Mas-Oliva. 2021. New insights into lipopolysaccharide inactivation mechanisms in sepsis. Biomedicine & Pharmacotherapy 141:111890. doi:10.1016/J.BIOPHA.2021.111890.

Petersen, M.C., and G.I. Shulman. 2018. Mechanisms of Insulin Action and Insulin Resistance. Physiol Rev 98:2133. doi:10.1152/PHYSREV.00063.2017.

Peterson, S.E., P. Rezamand, J.E. Williams, W. Price, M. Chahine, and M.A. McGuire. 2012. Effects of dietary betaine on milk yield and milk composition of mid-lactation Holstein dairy cows. J Dairy Sci 95:6557–6562. doi:10.3168/JDS.2011-4808.

Piñeiro, J.M., B.T. Menichetti, A.A. Barragan, A.E. Relling, W.P. Weiss, S. Bas, and G.M. Schuenemann. 2019. Associations of postpartum lying time with culling, milk yield, cyclicity, and reproductive performance of lactating dairy cows. J Dairy Sci 102:3362–3375. doi:10.3168/jds.2018-15387.

Poff, A.M., A.P. Koutnik, and B. Egan. 2020. Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. Curr Sports Med Rep 19:251–259. doi:10.1249/JSR.0000000000000732.

Polawska, E., E. Bagnicka, and A. Wierzbicka. 2012. Relations between the oxidative status, mastitis, milk quality and disorders of reproductive functions in dairy cows - a review. Anim Sci Pap Rep 30:297–307.

Popko, K., E. Gorska, A. Stelmaszczyk-Emmel, R. Plywaczewski, A. Stoklosa, D. Gorecka, B. Pyrzak, and U. Demkow. 2010. Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects. Eur J Med Res 15:120–122. doi:10.1186/2047-783X-15-S2-120/FIGURES/3.

Pralle, R.S., J.D. Amdall, R.H. Fourdraine, G.R. Oetzel, and H.M. White. 2021. Hyperketonemia Predictions Provide an On-Farm Management Tool with Epidemiological Insights. Animals (Basel) 11. doi:10.3390/ANI11051291.

Puchalska, P., S.E. Martin, X. Huang, J.E. Lengfeld, B. Daniel, M.J. Graham, X. Han, L. Nagy, G.J. Patti, and P.A. Crawford. 2019. Hepatocyte-Macrophage Acetoacetate Shuttle Protects against Tissue Fibrosis. Cell Metab 29:383-398.e7. doi:10.1016/J.CMET.2018.10.015/ATTACHMENT/C08FA489-8C1E-4811-8CDE-9C6EDD469458/MMC6.XLSX.

Puigserver, P., J. Rhee, J. Donovan, C.J. Walkey, J.C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili, and B.M. Spiegelman. 2003. Insulin-regulated hepatic gluconeogenesis through FOXO1–PGC-1α interaction. Nature 423:550–555. doi:10.1038/nature01667.

Qi, J., L. Gan, J. Fang, J. Zhang, X. Yu, H. Guo, D. Cai, H. Cui, L. Gou, J. Deng, Z. Wang, and Z. Zuo. 2022. Beta-Hydroxybutyrate: A Dual Function Molecular and Immunological Barrier Function Regulator. Front Immunol 0:2714. doi:10.3389/FIMMU.2022.805881.

Rahman, M., S. Muhammad, M.A. Khan, H. Chen, D.A. Ridder, H. Müller-Fielitz, B. Pokorná, T. Vollbrandt, I. Stölting, R. Nadrowitz, J.G. Okun, S. Offermanns, and M. Schwaninger. 2014. The β-hydroxybutyrate receptor HCA2 activates a neuroprotective

subset of macrophages. Nature Communications 2014 5:1 5:1–11. doi:10.1038/ncomms4944.

Ramadori, G., and B. Christ. 1999. Cytokines and the hepatic acute-phase response. Semin Liver Dis 19:141–156. doi:10.1055/s-2007-1007106.

Randle, P.J. 1998. Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263–283. doi:10.1002/(SICI)1099-0895(199812)14:4<263::AID-DMR233>3.0.CO;2-C.

Rathbun, F.M., R.S. Pralle, S.J. Bertics, L.E. Armentano, K. Cho, C. Do, K.A. Weigel, and H.M. White. 2017. Relationships between body condition score change, prior mid-lactation phenotypic residual feed intake, and hyperketonemia onset in transition dairy cows. J Dairy Sci 100:3685–3696. doi:10.3168/jds.2016-12085.

Remond, D., I. Ortigues, and J.P. Jouany. 1995. Energy substrates for the rumen epithelium. Nutrition Society 54.

Rico, D.E., A. Celemín-Sarmiento, and J.E. Rico. 2021. The dose responsive induction of acute hyperketonemia by ruminal infusion of calcium butyrate in dairy cows. Pages 142–143 in ADSA annual meeting 2021.

Ringseis, R., D.K. Gessner, and K. Eder. 2015. Molecular insights into the mechanisms of liver-associated diseases in early-lactating dairy cows: hypothetical role of endoplasmic reticulum stress. J Anim Physiol Anim Nutr (Berl) 99:626–645. doi:10.1111/JPN.12263.

Roberts, T., N. Chapinal, S.J. LeBlanc, D.F. Kelton, J. Dubuc, and T.F. Duffield. 2012. Metabolic parameters in transition cows as indicators for early-lactation culling risk. J Dairy Sci 95:3057–3063. doi:10.3168/JDS.2011-4937.

Roche, J.R., A.W. Bell, T.R. Overton, and J.J. Loor. 2013. Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking. Anim Prod Sci 53:1000. doi:10.1071/AN12293.

Rodriguez, Z., E. Shepley, M.I. Endres, G. Cramer, and L.S. Caixeta. 2022. Assessment of milk yield and composition, early reproductive performance, and herd removal in multiparous dairy cattle based on the week of diagnosis of hyperketonemia in early lactation. J Dairy Sci. doi:10.3168/JDS.2021-20836.

Roekenes, J., and C. Martins. 2021. Ketogenic diets and appetite regulation. Curr Opin Clin Nutr Metab Care 24:359–363. doi:10.1097/MCO.0000000000000760.

Roh, J.S., and D.H. Sohn. 2018. Damage-Associated Molecular Patterns in Inflammatory Diseases. Immune Netw 18. doi:10.4110/IN.2018.18.E27.

Rook, J.A.F., C.C. Balch, and V.W. Johnson. 1965. Further observations on the effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Brit. J. Nutr 19:93. doi:10.1079/BJN19650008.

Le Roy, D., F. Di Padova, Y. Adachi, M.P. Glauser, T. Calandra, and D. Heumann. 2001. Critical Role of Lipopolysaccharide-Binding Protein and CD14 in Immune Responses against Gram-Negative Bacteria. The Journal of Immunology 167:2759–2765. doi:10.4049/JIMMUNOL.167.5.2759.

Ruiz-González, A., M. Ramírez-Mella, D.E. Rico, and J.E. Rico. 2022. The nutritional induction of episodic hyperketonemia in dairy cows: a randomized controlled trial. Page in American Dairy Science Association. Journal of Dairy Science, Kansas City, MO.

Saco, Y., and A. Bassols. 2023. Acute phase proteins in cattle and swine: A review. Vet Clin Pathol 52 Suppl 1:50–63. doi:10.1111/VCP.13220.

Sadri, H., M.H. Ghaffari, and H. Sauerwein. 2023. Invited review: Muscle protein breakdown and its assessment in periparturient dairy cows. J Dairy Sci 106:822–842. doi:10.3168/jds.2022-22068.

Sahoo, S.S., R.C. Patra, P.C. Behera, and D. Swarup. 2009. Oxidative stress indices in the erythrocytes from lactating cows after treatment for subclinical ketosis with antioxidant incorporated in the therapeutic regime. Vet Res Commun 33:281–290. doi:10.1007/S11259-008-9176-1/FIGURES/1.

Santschi, D.E., R. Lacroix, J. Durocher, M. Duplessis, R.K. Moore, and D.M. Lefebvre. 2016. Prevalence of elevated milk β-hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in Dairy Herd Improvement milk samples and association with milk yield and components. J Dairy Sci 99:9263–9270. doi:10.3168/JDS.2016-11128.

Schroedl, W., B. Fuerll, P. Reinhold, M. Krueger, and C. Schuett. 2001. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP).

Seely, C.R., K.D. Bach, D.M. Barbano, and J.A.A. McArt. 2021. Effect of hyperketonemia on the diurnal patterns of energy-related blood metabolites in early-lactation dairy cows. J Dairy Sci 104:818–825. doi:10.3168/jds.2020-18930.

Semler, M.W., and T.W. Rice. 2016. Sepsis Resuscitation: Fluid Choice and Dose.. Clin Chest Med 37:241–50. doi:10.1016/j.ccm.2016.01.007.

Serra, D., N. Casals, G. Asins, T. Royo, C. Ciudad, and F. Hegardt. 1993. Regulation of Mitochondrial 3-Hydroxy-3-methylglutaryl-coenzyme A Synthase Protein by Starvation, Fat Feeding, and Diabetes. Arch Biochem Biophys 307:40–45.

Sharma, D., and T.D. Kanneganti. 2016. The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. J Cell Biol 213:617. doi:10.1083/JCB.201602089.

Shaw, D.M., F. Merien, A. Braakhuis, E. Maunder, and D.K. Dulson. 2019. Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. Sports Medicine 2019 50:4 50:641–656. doi:10.1007/S40279-019-01246-Y.

Shaw, D.M., F. Merien, A. Braakhuis, E. Maunder, and D.K. Dulson. 2020. Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. Sports Med 50:641–656. doi:10.1007/S40279-019-01246-Y.

Shaw, J.C. 1943. Studies on Ketosis in Dairy Cattle. V. The Development of Ketosis. J Dairy Sci 26:1079–1090. doi:10.3168/JDS.S0022-0302(43)92810-3.

Shaw, J.C. 1956. Ketosis in Dairy Cattle. A Review. J Dairy Sci 39:402–434. doi:10.3168/jds.S0022-0302(56)94765-8.

Shen, L., Y. Yang, T. Ou, C.C.C. Key, S.H. Tong, R.C. Sequeira, J.M. Nelson, Y. Nie, Z. Wang, E. Boudyguina, S. v. Shewale, and X. Zhu. 2017. Dietary PUFAs attenuate NLRP3 inflammasome activation via enhancing macrophage autophagy. J Lipid Res 58:1808–1821. doi:10.1194/JLR.M075879.

Shen, T., X. Li, J.J. Loor, Y. Zhu, X. Du, X. Wang, D. Xing, Z. Shi, Z. Fang, X. Li, and G. Liu. 2019. Hepatic nuclear factor kappa B signaling pathway and NLR family pyrin domain containing 3 inflammasome is over-activated in ketotic dairy cows. J Dairy Sci 102:10554–10563. doi:10.3168/JDS.2019-16706.

Shi, J., J. Fan, Q. Su, and Z. Yang. 2019. Cytokines and Abnormal Glucose and Lipid Metabolism. Front Endocrinol (Lausanne) 10:703. doi:10.3389/FENDO.2019.00703/BIBTEX.

Simkins, K.L., J.W. Suttie, and B.R. Baumgardt. 1965. Regulation of Food Intake in Ruminants. 4. Effect of Acetate, Propionate, Butyrate, and Glucose on Voluntary Food Intake in Dairy Cattle. J Dairy Sci 48:1635–1642. doi:10.3168/jds.S0022-0302(65)88541-1.

Sjollema, B., and J. Van Der Zande. 1923. Metabolism in acetonemia of milch cows. Journal of Metabolic Research 525–533.

Song, Y., J.J. Loor, C. Li, Y. Liang, N. Li, X. Shu, Y. Yang, X. Feng, X. Du, Z. Wang, G. Liu, and X. Li. 2021. Enhanced mitochondrial dysfunction and oxidative stress in the mammary gland of cows with clinical ketosis. J Dairy Sci 104:6909–6918. doi:10.3168/jds.2020-19964.

Sordillo, L.M., and V. Mavangira. 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 54:1204–1214. doi:10.1071/AN14503.

Sordillo, L.M., and W. Raphael. 2013. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. Veterinary Clinics of North America: Food Animal Practice 29:267–278. doi:10.1016/J.CVFA.2013.03.002.

Soto-Mota, A., N.G. Norwitz, R.D. Evans, and K. Clarke. 2022. Exogenous d-β-hydroxybutyrate lowers blood glucose in part by decreasing the availability of L-alanine for gluconeogenesis. Endocrinol Diabetes Metab 5. doi:10.1002/EDM2.300.

Steffen, M.R. 1917. The Clinical Diagnosis of Cattle Diseases.

Stienstra, R., L.A.B. Joosten, T. Koenen, B. van Tits, J.A. van Diepen, S.A.A. van den Berg, P.C.N. Rensen, P.J. Voshol, G. Fantuzzi, A. Hijmans, S. Kersten, M. Müller, W.B. van den Berg, N. van Rooijen, M. Wabitsch, B.J. Kullberg, J.W.M. van der Meer, T.

Kanneganti, C.J. Tack, and M.G. Netea. 2010. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab 12:593–605. doi:10.1016/J.CMET.2010.11.011.

Stubbs, B.J., P.J. Cox, R.D. Evans, M. Cyranka, K. Clarke, and H. de Wet. 2018. A Ketone Ester Drink Lowers Human Ghrelin and Appetite. Obesity (Silver Spring) 26:269. doi:10.1002/OBY.22051.

Stubbs, B.J., P.J. Cox, R.D. Evans, P. Santer, J.J. Miller, O.K. Faull, S. Magor-Elliott, S. Hiyama, M. Stirling, and K. Clarke. 2017. On the metabolism of exogenous ketones in humans. Front Physiol 8. doi:10.3389/fphys.2017.00848.

Stubbs, B.J., A.P. Koutnik, E.L. Goldberg, V. Upadhyay, P.J. Turnbaugh, E. Verdin, and J.C. Newman. 2020a. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med 1:43–65. doi:10.1016/j.medj.2020.06.008.

Stubbs, B.J., A.P. Koutnik, E.L. Goldberg, V. Upadhyay, P.J. Turnbaugh, E. Verdin, and J.C. Newman. 2020b. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med 1:43–65. doi:10.1016/j.medj.2020.06.008.

Su, G.L., S.M. Goyert, M.H. Fan, A. Aminlari, K.Q. Gong, R.D. Klein, A. Myc, W.H. Alarcon, L. Steinstraesser, D.G. Remick, and S.C. Wang. 2002. Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. Am J Physiol Gastrointest Liver Physiol 283:640–645. doi:10.1152/AJPGI.00253.2001/ASSET/IMAGES/LARGE/H30920964007.JPEG.

Sun, X., Y. Tang, C. Jiang, S. Luo, H. Jia, Q. Xu, C. Zhao, Y. Liang, Z. Cao, G. Shao, J.J. Loor, and C. Xu. 2021. Oxidative stress, NF-κB signaling, NLRP3 inflammasome, and caspase apoptotic pathways are activated in mammary gland of ketotic Holstein cows. J Dairy Sci 104:849–861. doi:10.3168/jds.2020-18788.

Suthar, V., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci 96:2925–2938. doi:10.3168/jds.2012-6035.

Swartz, T.H., B.J. Bradford, and L.K. Mamedova. 2021a. Diverging in vitro inflammatory responses toward Streptococcus uberis in mouse macrophages either preconditioned or continuously treated with β-hydroxybutyrate. JDS Communications 2:142–147. doi:10.3168/jdsc.2020-0038.

Swartz, T.H., B.J. Bradford, and L.K. Mamedova. 2021b. Connecting Metabolism to Mastitis: Hyperketonemia Impaired Mammary Gland Defenses During a Streptococcus uberis Challenge in Dairy Cattle. Front Immunol 12. doi:10.3389/fimmu.2021.700278.

Taggart, A.K.P., J. Kero, X. Gan, T.Q. Cai, K. Cheng, M. Ippolito, N. Ren, R. Kaplan, K. Wu, T.J. Wu, L. Jin, C. Liaw, R. Chen, J. Richman, D. Connolly, S. Offermanns, S.D. Wright, and M.G. Waters. 2005. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. J Biol Chem 280:26649–26652. doi:10.1074/JBC.C500213200.

Tavares, E., R. Maldonado, M.L. Ojeda, and F.J. Minano. 2005. Circulating inflammatory mediators during start of fever in differential diagnosis of gram-negative and gram-positive infections in leukopenic rats. Clin Diagn Lab Immunol 12:1085–1093. doi:10.1128/CDLI.12.9.1085-1093.2005.

Thangaraju, M., G.A. Cresci, K. Liu, S. Ananth, J.P. Gnanaprakasam, D.D. Browning, J.D. Mellinger, S.B. Smith, G.J. Digby, N.A. Lambert, P.D. Prasad, and V. Ganapathy. 2009. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res 69:2826. doi:10.1158/0008-5472.CAN-08-4466.

Thio, C.L.-P., A.C.-Y. Lai, Y.-T. Ting, P.-Y. Chi, and Y.-J. Chang. 2022. The ketone body β-hydroxybutyrate mitigates ILC2-driven airway inflammation by regulating mast cell function. Cell Rep 40:111437. doi:10.1016/j.celrep.2022.111437.

Tillett, W.S., and T. Francis. 1930. SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS. J Exp Med 52:561. doi:10.1084/JEM.52.4.561.

Tobias, P.S., K. Soldau, and R.J. Ulevitch. 1989. Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. Journal of Biological Chemistry 264:10867–10871. doi:10.1016/s0021-9258(18)81700-8.

Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res Vet Sci 93:695–704. doi:10.1016/J.RVSC.2011.11.008.

Trevisi, E., N. Jahan, G. Bertoni, A. Ferrari, and A. Minuti. 2016. Pro-Inflammatory Cytokine Profile in Dairy Cows: Consequences for New Lactation. https://doi.org/10.4081/ijas.2015.3862 14:285–292. doi:10.4081/IJAS.2015.3862.

Tweedell, R.E., R.K.S. Malireddi, and T.D. Kanneganti. 2020. A comprehensive guide to studying inflammasome activation and cell death. Nat Protoc 15. doi:10.1038/s41596-020-0374-9.

Tyrrell, H.F., and P.W. Moe. 1975. Effect of Intake on Digestive Efficiency. J Dairy Sci 58:1151–1163. doi:10.3168/jds.S0022-0302(75)84694-7.

Udall, H. 1943. The Practice of Veterinary Medicine. 4th ed. Ithaca.

Urrutia, N., R. Bomberger, C. Matamoros, and K.J. Harvatine. 2019. Effect of dietary supplementation of sodium acetate and calcium butyrate on milk fat synthesis in lactating dairy cows. J Dairy Sci 102:5172–5181. doi:10.3168/jds.2018-16024.

Urrutia, N.L., and K.J. Harvatine. 2017. Acetate Dose-Dependently Stimulates Milk Fat Synthesis in Lactating Dairy Cows. J Nutr 763–769. doi:10.3945/jn.116.245001.

Vandanmagsar, B., Y.H. Youm, A. Ravussin, J.E. Galgani, K. Stadler, R.L. Mynatt, E. Ravussin, J.M. Stephens, and V.D. Dixit. 2011. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–189. doi:10.1038/NM.2279.

Vanholder, T., J. Papen, R. Bemers, G. Vertenten, and A.C.B. Berge. 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J Dairy Sci 98:880–888. doi:10.3168/jds.2014-8362.

Vanitallie, T.B., and T.H. Nufert. 2003. Lead Review Article Ketones: Metabolism's Ugly Duckling 327–341. doi:10.131/nr.2003.oct.327-341.

Vels, L., C.M. Røntved, M. Bjerring, and K.L. Ingvartsen. 2008. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. J Dairy Sci 92:922–934. doi:10.3168/jds.2008-1209.

Vercouteren, M.M.A.A., J.H.J. Bittar, P.J. Pinedo, C.A. Risco, J.E.P. Santos, A. Vieira-Neto, and K.N. Galvão. 2015. Factors associated with early cyclicity in postpartum dairy cows. J Dairy Sci 98:229–239. doi:10.3168/JDS.2014-8460.

de Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production Variables and Fertility. J Dairy Sci 83:62–69. doi:10.3168/jds.S0022-0302(00)74856-9.

Wagner, S.A., and D.E. Schimek. 2010. Evaluation of the effect of bolus administration of 50% dextrose solution on measures of electrolyte and energy balance in postpartum dairy cows. Am J Vet Res 71:1074–1080. doi:10.2460/AJVR.71.9.1074.

Wanders, D., and R.L. Judd. 2011. Future of GPR109A agonists in the treatment of dyslipidaemia. Diabetes Obes Metab 13:685–691. doi:10.1111/j.1463-1326.2011.01400.x.

Wankhade, P.R., A. Manimaran, A. Kumaresan, S. Jeyakumar, K.P. Ramesha, V. Sejian, D. Rajendran, and M.R. Varghese. 2017. Metabolic and immunological changes in transition dairy cows: A review. Vet World 10:1367. doi:10.14202/VETWORLD.2017.1367-1377.

Weigand, E., J.W. Young, and A.D. McGilliard. 1972. Extent of Butyrate Metabolism by Bovine Ruminoreticulum Epithelium and the Relationship to Absorption Rate. J Dairy Sci 55:589–597. doi:10.3168/JDS.S0022-0302(72)85541-3.

Weiss, W.P. 2010. Refining the Net Energy System.

Weiss, W.P., and A.W. Tebbe. 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Transl Anim Sci 3:953–961. doi:10.1093/tas/txy119.

White, H., and B. Venkatesh. 2011. Clinical review: Ketones and brain injury. Crit Care 15:219. doi:10.1186/CC10020.

White, H.M. 2015. The Role of TCA Cycle Anaplerosis in Ketosis and Fatty Liver in Periparturient Dairy Cows. Animals (Basel) 5:793. doi:10.3390/ANI5030384.

White, H.M. 2020. ADSA Foundation Scholar Award: Influencing hepatic metabolism: Can nutrient partitioning be modulated to optimize metabolic health in the transition dairy cow?. J Dairy Sci 103:6741–6750. doi:10.3168/jds.2019-18119.

Williamson, D.H., P. Lund, and H.A. Krebs. 1967. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochemical Journal 103:514. doi:10.1042/BJ1030514.

Wilson, J.J., C.R. Youno, and S.B. Smth. 1992. TRIACYLGLYCEROL BIOSYNTHESIS IN BOVINE LIVER AND SUBCUTANEOUS ADIPOSE TISSUE.

Wu, Y., Y. Sun, R. Zhang, T. He, G. Huang, K. Tian, J. Liu, J. Chen, and G. Dong. 2021. Sodium butyrate more effectively mitigates the negative effects of high-concentrate diet in dairy cows than sodium β-hydroxybutyrate via reducing free bacterial cell wall components in rumen fluid and plasma. Toxins (Basel) 13. doi:10.3390/toxins13050352.

Xu, W., J. Vervoort, E. Saccenti, B. Kemp, R.J. van Hoeij, and A.T.M. van Knegsel. 2020. Relationship between energy balance and metabolic profiles in plasma and milk of dairy cows in early lactation. J Dairy Sci 103:4795–4805. doi:10.3168/JDS.2019-17777.

Yamada, T., S.J. Zhang, H. Westerblad, and A. Katz. 2010. β-hydroxybutyrate inhibits insulin-mediated glucose transport in mouse oxidative muscle. Am J Physiol Endocrinol Metab 299:364–373. doi:10.1152/AJPENDO.00142.2010/ASSET/IMAGES/LARGE/ZH10081060340006.JPEG.

Yamanashi, T., M. Iwata, N. Kamiya, K. Tsunetomi, N. Kajitani, N. Wada, T. Iitsuka, T. Yamauchi, A. Miura, S. Pu, Y. Shirayama, K. Watanabe, R.S. Duman, and K. Kaneko. 2017. Beta-hydroxybutyrate, an endogenic NLRP3 inflammasome inhibitor, attenuates stress-induced behavioral and inflammatory responses. Scientific Reports 2017 7:1 7:1–11. doi:10.1038/s41598-017-08055-1.

Yan, T., C.S. Mayne, T.W.J. Keady, and R.E. Agnew. 2006. Effects of dairy cow genotype with two planes of nutrition on energy partitioning between milk and body tissue. J Dairy Sci 89:1031–1042. doi:10.3168/jds.S0022-0302(06)72170-1.

Yao, Q., Y. Li, Q. Meng, and Z. Zhou. 2017. The effect of calcium propionate on the ruminal bacterial community composition in finishing bulls. Asian-Australas J Anim Sci 30:495–504. doi:10.5713/ajas.16.0469.

Yokoi, K., N. Mukaida, A. Harada, Y. Watanabe, and K. Matsushima. 1997. Prevention of endotoxemia-induced acute respiratory distress syndrome-like lung injury in rabbits by a monoclonal antibody to IL-8.. Lab Invest 76:375–384.

You, K., H. Gu, Z. Yuan, and X. Xu. 2021. Tumor Necrosis Factor Alpha Signaling and Organogenesis. Front Cell Dev Biol 9:2055. doi:10.3389/FCELL.2021.727075/BIBTEX.

Youm, Y.H., K.Y. Nguyen, R.W. Grant, E.L. Goldberg, M. Bodogai, D. Kim, D. D'Agostino, N. Planavsky, C. Lupfer, T.D. Kanneganti, S. Kang, T.L. Horvath, T.M. Fahmy, P.A. Crawford, A. Biragyn, E. Alnemri, and V.D. Dixit. 2015. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 21:263–269. doi:10.1038/nm.3804.

Young, J.W. Gluconeogenesis in Cattle: Significance and Methodology ~. J Dairy Sci 60:1–15. doi:10.3168/jds.S0022-0302(77)83821-6.

Yuan, K., J.K. Farney, L.K. Mamedova, L.M. Sordillo, and B.J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8. doi:10.1371/JOURNAL.PONE.0080316.

Yurista, S.R., C.R. Chong, J.J. Badimon, D.P. Kelly, R.A. de Boer, and B.D. Westenbrink. 2021. Therapeutic Potential of Ketone Bodies for Patients With Cardiovascular Disease: JACC State-of-the-Art Review. J Am Coll Cardiol 77:1660–1669. doi:10.1016/J.JACC.2020.12.065.

Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific

insulin resistance occurs in cows prone to high weight loss. J Dairy Sci 96:5656–5669. doi:10.3168/JDS.2012-6142.

Zarrin, M., L. Grossen-Rösti, R.M. Bruckmaier, and J.J. Gross. 2017. Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. J Dairy Sci 100:2323–2333. doi:10.3168/jds.2016-11714.

Zarrin, M., L. De Matteis, M.C.M.B. Vernay, O. Wellnitz, H.A. van Dorland, and R.M. Bruckmaier. 2013. Long-term elevation of β-hydroxybutyrate in dairy cows through infusion: Effects on feed intake, milk production, and metabolism. J Dairy Sci 96:2960–2972. doi:10.3168/jds.2012-6224.

Zarrin, M., O. Wellnitz, H.A. van Dorland, and R.M. Bruckmaier. 2014. Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. J Dairy Sci 97:330–339. doi:10.3168/jds.2013-7222.

Zebeli, Q., and B. Ametaj. 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. J Dairy Sci 92:3800–3809. doi:10.3168/JDS.2009-2178.

Zhang, F., X. Nan, H. Wang, Y. Zhao, Y. Guo, and B. Xiong. 2020. Effects of propylene glycol on negative energy balance of postpartum dairy cows. Animals 10:1–15. doi:10.3390/ani10091526.

Zhang, G., and B.N. Ametaj. 2020. Ketosis an Old Story Under a New Approach. Dairy 2020, Vol. 1, Pages 42-60 1:42–60. doi:10.3390/DAIRY1010005.

Zhang, G., D. Hailemariam, E. Dervishi, S.A. Goldansaz, Q. Deng, S.M. Dunn, and B.N. Ametaj. 2016. Dairy cows affected by ketosis show alterations in innate immunity and lipid and carbohydrate metabolism during the dry off period and postpartum. Res Vet Sci 107:246–256. doi:10.1016/j.rvsc.2016.06.012.

Zheng, D., T. Liwinski, and E. Elinav. 2020. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. Cell Discovery 2020 6:1 6:1–22. doi:10.1038/s41421-020-0167-x.

Zhu, H., D. Bi, Y. Zhang, C. Kong, J. Du, X. Wu, Q. Wei, and H. Qin. 2022. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical

implementations. Signal Transduct Target Ther 7:11. doi:10.1038/s41392-021-00831-w.