Ketosis Diagnosis/Monitoring in Dairy Cows

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Contributor: João Simões

Ketosis is a major issue in high-producing cows, easily reaching a prevalence of 20% during early postpartum when the negative energy balance is well established.

Keywords: beta-hydroxybutyrate ; diagnosis ; metabolic diseases ; negative energy balance

1. Introduction

Ketosis is one of the most harmful and damaging metabolic diseases in early lactating dairy cows characterized by high concentrations of circulating ketone bodies which frequently imply productive and reproductive losses, and even death or early culling [1][2]. All these problems represent financial losses that are worsened with the inherent treatment associated with them. In most cases, the treatment of other concomitant diseases, that appear as a cause or an effect of ketosis, further aggravates the financial problem [3].

Knowing that ketosis appears because of an energetic unbalance, it is crucial to invest in prevention, monitoring the most sensible and debile known moments of the productive life of a cow. Even knowing that laboratory tests are more accurate, cowside tests won enlarged utilization over the last few years as they offer real-time results with good accuracy and are simple to perform. Monitorization of milk parameters has been widely studied as a diagnostic method because of its non-invasive sample collection and because these values can be presented on the monthly milk controls. Moreover, they can be used to assess related metabolic diseases such we reported recently [4]. Assessment of body condition and monitoring the thickness of the dorsal fat layer, ruminatory activity, and control of the known risk factors are the most commonly used methods for monitoring ketosis. Understanding that information is the best weapon to success, this review aimed to highlight the main aspects of the impact and monitoring of hyperketonemia in high-producing dairy cow farms.

Considering a global ketosis prevalence higher than 20% [5], and with estimated costs per case of ketosis that can reach more than €250 [3] in high producing dairy cows, it is imperative to review and update all the available knowledge about ketosis, with particular emphasis on prevention and diagnostic strategies.

2. Energy Metabolism in Ruminants

The ruminants' diet contains considerable amounts of structural carbohydrates (cellulose, hemicelluloses, and pectin) and reserve carbohydrates (starch and other water-soluble carbohydrates). Of all these carbohydrates, approximately 90% are degraded in fermentation processes performed by the reticulorumen commensal microbiota; all carbohydrates, except indissoluble fiber—lignin—are a target of the action of ruminal microorganisms. Only about 10%, corresponding to water-soluble starch or carbohydrates, are digested and absorbed in the small intestine [6].

The degradation of dietary carbohydrates in the rumen occurs in two stages: The first of which involves the digestion of complex carbohydrates into simple sugars, fundamentally through the action of extracellular microbial enzymes; and the second, through the intracellular metabolization of these sugars by microorganisms. This microbial fermentation process results in short-chain volatile fatty acids (VFA)—acetic, propionic, and butyric acid (the most abundant)—as well as in the production of methane and carbon dioxide, which are being eructed [6].

Acetic acid, or acetate, is the main product of the fermentative digestion of carbohydrates in ruminants and is the VFA found in more significant amounts in the peripheral circulation. This can then be used as an energy source, through the Krebs cycle, in various tissues, such as adipose tissue [7]. Still, its main destination is the synthesis of milk fat; several studies prove that dietary supplementation with acetate increases the butter content of milk as it increases lipogenesis in the mammary gland mainly by stimulating the "de novo" synthesis pathways [8][9][10].

Propionic acid, or propionate, after being produced, passes passively through the ruminal wall (only a small part is converted into lactate upon absorption by the ruminal wall), and from there, the majority is transported to the liver to participate in gluconeogenesis, originating glucose that can be used as an energy source [7]. Propionate represents 90 to 95% of the contribution of VFA to gluconeogenesis [11].

Butyric acid, or butyrate, is converted to BHB, during the absorption process by the rumen and omasum walls, which can then be used as an energy source, through the Krebs cycle [7][12], or as a precursor of milk fat synthesized “de novo” in the mammary gland [13].

To supply the needs of carbohydrates in full, the organism, mainly the liver, dedicates itself to the endogenous production of the 90% of glucose missing, which are not directly supplied by food [14]. Two important processes, gluconeogenesis, and glycogenolysis intervene in the metabolism of carbohydrate synthesis.

Gluconeogenesis has propionate as its main precursor, followed by isobutyrate and valerate [11]; to a lesser extent, this route also uses acetate, lactate, butyrate, non-esterified free fatty acids (NEFA), glycerol (mostly resulting from lipolysis of adipose tissue) [7] and amino acids (especially from Alanine) [15].

As mentioned above, energy can also come from triglycerides (TG) and NEFA in the diet or TG reserves in the body; however, the proportion of lipids in the ruminant diet is generally low. Even so, TG are subjected to the action of bacterial lipases, giving rise to NEFA and glycerol, absorbed by the ruminal wall. On the other hand, long-chain free fatty acids are not directly absorbed by the rumen wall, and therefore, in the small intestine, they are hydrogenated/saturated and hydrolyzed or esterified [6]. These are then transported in chylomicrons to the liver; the TG are hydrolyzed, as described above [7]. In both situations, glycerol enters the glycolysis pathway (in the form of phosphate dihydroxyacetone) for later energy production. The maximum contribution of glycerol to glucose production is observed in the postpartum period, reinforcing its role as an indicator of TG mobilization in adipose tissue. Interestingly, the enzyme responsible for the transformation of glycerol into phosphate-dihydroxyacetone has, until now, only been found in the liver and mammary gland, and therefore, it is expected that only these two tissues metabolize glycerol [11].

Nevertheless, the greatest contribution of TG to energy production comes from the β-oxidation of NEFA (progressive shortening of two carbons in the carbon chain), which culminates in the production of acetyl coenzyme A (Acetyl-CoA) that is sent to the Krebs cycle for obtaining energy [7].

When in more demanding phases, the organism is forced to choose alternative substrates to maintain its essential functions, amino acids can be catabolized to obtain energy. This phenomenon generally occurs in the liver. Again, the final product is acetyl-CoA which can be sent to the Krebs cycle and converted there into energy. One of the by-products of this process is ammonia, a very toxic compound. Fortunately, much of the ammonia not used by the body to synthesize amino acids is efficiently excreted in the form of urea (in mammals). In ruminants, specifically, part of the urea is recycled via saliva or directly through the rumen wall, depending on the nitrogen levels in the body [7].

3. Pathophysiology of Ketosis and Inclusion in the Negative Energy Balance (NEB)

Peripartum is a critical moment for dairy cows. It is marked by profound nutritional, endocrine, metabolic, immune, and reproductive changes [16][17]. Between the 3rd precalving week and the 3rd postcalving week, the transition period [18] occurs, during which the cow experiences a stage of energy deficit [19] that affects health and metabolism, production, and reproduction of the remaining lactation of a cow [20].

It is important to reflect that the last stage of pregnancy can be almost entirely dedicated to the growth of the fetus, so the energy needs to increase [21]. These needs are aggravated by the demand for nutrients for developing the mammary gland that begins to appear at this stage; all these requirements are then aggravated/impaired by the reduction in dry matter intake that occurs at the end of pregnancy [22], the latter mainly due to the compression that the pregnant uterus exerts on the rumen.

Compared to prepartum, at the beginning of lactation, milk production requires an expected energy supply in the diet 30 to 50% higher [23]. These requirements are mirrored by the plasma glucose concentration that decreases after calving, mainly in high producing cows, reflecting the priority energy supply for milk production in the mammary gland; mainly to produce lactose (glucose + galactose), the main osmotic regulator for the mammary secretion of water, thus determining the total volume of produced milk. Effectively, about 85% of the glucose produced at the beginning of lactation is destined for the mammary gland [24].

However, this increased contribution does not occur because, on the contrary, and besides, there is a more pronounced decrease in dry matter intake 24 h before delivery, which only returns to the values prior to this fall 24 h after delivery [25] for the emergence of the sensation of hunger in response to the increased nutritional demand [22]; even so, values equivalent to those of predelivery intake are insufficient to supply these 30 to 50% more energy needed for milk production [23].

This discrepancy between the nutritional demand and the available provisions contributes to the installation of NEB [19].

Given the increased energy requirements for milk production (which peak is usually reached by four weeks) and considering that the maximum intake capacity is only recovered 7 to 8 weeks after delivery, the expected adaptive response (an adaptation of the metabolism of anabolic to catabolic [26]) is generally insufficient to meet these same needs, especially in cows with high productive performance [27].

Being glucose an essential nutrient and inherently associated with the maintenance of the normal vital functions of most tissues and with lactogenesis [27][19], given the insufficient available quantity of it, obtained by gluconeogenesis, in periods of NEB, the body urgently needs to mobilize alternative energy sources—fat reserves in the form of TG; their lipolysis, in adipocytes, results in glycerol and NEFA that are released into circulation and sent to the liver [28].

High concentrations of these circulating fatty acids can impair the insulin signaling pathway, decreasing its sensitivity [29], which in turn exacerbates the mobilization of fat reserves and the entry into circulation of NEFA, thus creating a vicious cycle [30]. As noted by De Koster and Opsomer [31], most studies on the subject confirm that dairy cows go through a phase of insulin resistance between the end of pregnancy and the beginning of lactation; these homeoresis mechanisms are a physiological, adaptive, and transitory phenomenon that aims to prioritize the supply of glucose to the pregnant uterus and the mammary gland over other tissues. Thus, the correct development and survival of the offspring are always the priority.

In the liver, glycerol is used for gluconeogenesis, and NEFA are converted to acetyl-CoA that can have various destinations, such as oxidation to carbon dioxide, oxidation to ketone bodies, hepatic storage in the form of TG, or incorporation into very-low-density lipoproteins, which will be exported as fuel for other tissues. In a demanding phase, such as the beginning of lactation, the number of reserves mobilized is much higher than normal, leading to the production of a large amount of acetyl-CoA. If the Krebs cycle is unable to metabolize the excess acetyl-CoA, this is transformed (oxidized) into ketone bodies [17][32]—acetone, acetoacetate, and BHB [28]—or stored in the liver as TG, which can lead to the fatty liver with negative repercussions on hepatic metabolism [32]. As a result, an increased amount of ketone bodies enters the circulation, which, in a short time, will also occur in milk and urine [22].

4. Classification and Forms of Ketosis

4.1. Primary and Secondary Ketosis

Ketosis can be classified according to its origin and pathophysiology.

Type I ketosis, also known as primary or described as spontaneous ketosis, corresponds to hyperketonemia in the period between 3 and 6 weeks postpartum, close to the peak of lactation when milk production exceeds the amount of glucose available. In this very demanding phase, glucose precursors coming from the diet (mostly propionate) or muscle protein are insufficient, resulting in a state of chronic hypoglycemia that triggers hypoinsulinemia. In return, lipolysis and ketogenesis pathways are activated [28]. These animals generally do not develop severe hepatic steatosis since, given the high energy requirements, the gluconeogenesis pathway is stimulated to the maximum, and most NEFA are converted into ketone bodies and very little into TG, with no significant accumulation of lipids in the liver [19]. Upon diagnosis, these animals reveal hyperketonemia and hypoglycemia [28].

In contrast, type II, mainly related to secondary ketosis (to other pathologies), occurs at the beginning of lactation, before or at birth, because of the excessive mobilization of adipose tissue [28]. This mobilization results in large amounts of NEFA, which if the gluconeogenesis and ketogenesis pathway are not stimulated to their maximum, are re-esterified in TG. Due to the limited capacity of ruminants to produce low-density lipoproteins in sufficient quantities to export all these TG to other tissues, a large part of them accumulates in the liver, resulting in hepatic steatosis. The high circulation of ketone bodies (although lower than in type I ketosis) [19] cause the body to be resistant to insulin, impairing the use of glucose and starting a vicious cycle. Exaggerated body condition score (BCS) and overfeeding during the dry season are critical risk factors for developing this type of ketosis. At the time of diagnosis, these animals present hyperketonemia accompanied by hyperglycemia [28].

4.2. Subclinical and Clinical Manifestation of Hyperketonemia

Hyperketonemia can develop as subclinical ketosis or clinical ketosis [27]. Subclinical ketosis corresponds to hyperketonemia without manifestation of evident clinical signs—the cow maintains the usual appetite without reducing the dry matter intake [28]—and it is verified, according to several authors, at BHB values in the blood (serum) from 1.2 mmol to 1.4 mmol [33], due to the energy demand for dairy production. Clinical ketosis, as the designation suggests, applies to cases of hyperketonemia accompanied by the manifestation of clinical signs and hypoglycemia [34], usually evident at blood BHB concentrations ≥3.0 mmol/L [35].

High concentrations of BHB are not guaranteed to correspond to the depletion of the animal's health status [36]; some animals show clinical signs for slight increases in plasma BHB concentration [37] however, cases without clinical signs associated with BHB concentrations above 3.0 mmol/L are also documented [38].

The high circulating concentrations of TG, NEFA, and ketone bodies in the blood, which characterize ketosis, generate inappetence. BHB, in particular, has the effect of reducing the signaling of hypothalamic cells responsible for stimulating appetite [39]. Consequently, there is a decrease in food intake and rumen filling/volume followed by anorexia, aggravating the already existing condition [40]. Generally, if they have a choice, the affected animals decrease their intake of concentrate and opt for forages [41].

When associating anorexia with NEB and the excessive mobilization of lipid reserves (which are directly related to the pathophysiology of ketosis), it is expected to observe decreased milk production and loss of BC in affected cows [40].

The feces of the affected animals generally have a drier consistency than those of other animals in the same lactation phase (reminding horse feces), and fur may have a dull, dry, and erect aspect [41].

If the nervous form develops, the cow may lick itself or inanimate objects persistently, exhibit erratic aggressive behavior, present abnormal head posture, and even suffer from blindness. The mechanism that leads to the emergence of nerve ketosis is not yet fully understood [41]. Despite the lack of understanding, Foster [42] suggested that of the three more likely causes for ketosis (hypoglycemia, hyperketonemia, and isopropyl alcohol), increased serum concentrations of isopropyl alcohol are associated with the appearance of nervous signals. This fact was confirmed by another study (Adler et al., 1955 cited by Foster [42], p. 258), where it was demonstrated that the injection of isopropyl alcohol causes clinical signs similar to those of animals with nervous ketosis. Isopropyl alcohol can be produced in the rumen from acetoacetate (and through the rumen wall enter the bloodstream) or in the brain from BHB [42]. Contrary to this, fasting cows which have even lower levels of glucose and similar levels of blood ketones to cows with ketosis, do not show the same signs of nerve ketosis, so hypoglycemia and hyperketonemia do not seem to be the most prevalent factors in triggering nervous ketosis [42].

More often than not, ketotic animals are more apathetic and less active, and some may even manifest ataxia or even the inability to get up. Signs that usually result from the installed hypoglycemia [41].

The fruity odor of ketone bodies in breath and/or milk, mainly caused by ketone [40][41], is another sign which, when present, is not always easy to detect.

According to Dar et al. [43], productive breakdown and selective food intake are the signs most consistently found in cattle with ketosis (**Table 1**).

Table 1. Relative frequency of the different clinical signs that can be manifested by a cow with ketosis (adapted from [43]).

Clinical Sign	Number of Animals that Showed the Sign	Percentage from the Affected Animals (%)
Nervous signs	1	4
Reluctance to movement	1	4
Constipation	4	14

Clinical Sign	Number of Animals that Showed the Sign	Percentage from the Affected Animals (%)
Acetone odor on breath or milk	5	18
Dry and fewer feces	6	21
Complete anorexia	7	25
Prostration	10	36
Selective food intake	21	75
Abrupt drop in productivity	28	100

Still, all these signs are nonspecific, and some are often not very evident, and therefore, difficult to detect, thereby increasing the risk of obtaining an erroneous diagnosis and the difficulty of correctly distinguishing clinical from subclinical ketosis [27].

Parameters, such as temperature, pulse, and breathing pattern, only deviate from normal if there are other concomitant nosological conditions [41].

5. Laboratory Diagnosis and Methods of Monitoring Ketosis

Deviations from metabolic homeostasis are reflected in changes in body fluids, such as blood, urine, milk, and saliva [5]. Of these, the evaluation of some serum metabolites has been a key point in diagnosing various pathologies, particularly metabolic diseases [38]. Thus, the most common diagnostic method used in diagnosing ketosis has been the analysis of some of these fluids in suspect cows [23].

Concentrations of ketone bodies have been used for diagnosing ketosis in dairy cows for many years [28]. Due to its stability in the blood, BHB has been the ketone body most used in the laboratory diagnosis of ketosis [35], which is considered the “golden standard” method.

The hypothesis of using milk to measure BHB concentration has been increasingly investigated since the analysis and recording of parameters evaluated in milk is already a routine, non-invasive procedure that facilitates monitoring at the herd level. Moreover, unlike blood samples, milk samples reflect the animal’s metabolic state for a period of time and not just at the time of harvest [27]. However, the accuracy of the BHB concentration prediction equations in milk has not been sufficiently high to predict the exact BHB concentration. Even so, it has proved useful for monitoring and signaling cows with high concentrations of BHB [44][45].

It is important to emphasize that any instrument and method intended to be used by veterinarians or producers to detect pathologies must be non-invasive, simple to use, and low cost [46].

6. Control and Prevention

In addition to the methods of monitoring ketosis described in the previous point, it is important to identify some procedures and a set of modifiable and non-modifiable risk factors that have an impact on the control and prevention of ketosis.

6.1. Ruminatory Activity

Mann et al. [47] clarified that, compared to those that receive diets that provide energy well above maintenance needs, cows fed in the dry period with diets with restricted/controlled energy, have a lower risk of being affected by ketosis in the postpartum period, without prejudice to milk production. These animals manifested a less deep NEB and episodes of ketosis to a lesser extent and number.

The study by Kaufman et al. [48], suggested that the monitoring of ruminatory activity during the peripartum, for example, with the use of rumination collars, may contribute to the individual and timely identification of pathologies of the initial stage of lactation. In this study, they found that multiparous cows that had reduced rumination time (by about 25 ± 12.8 min/day less than healthy cows between the 2nd week before delivery and the 4th week after giving birth) in the week immediately before giving birth were more likely to be affected by ketosis. Moreover, those who manifested this reduction (approximately 44 ± 15.6 min/day less than healthy cows between the 2nd week before delivery and the 4th week after delivery) in the week immediately after delivery would be more probably affected, not only by ketosis, but also by another pathology of early lactation. In the same year, Schirmann et al. [49] testified that cows with postpartum ketosis (BHB ≥ 1.2 mmol/L) spent 14% less time ruminating during precalving (considered in the study, 10 days before calving) than healthy cows.

6.2. Assessment of Body Condition and Monitoring the Thickness of the Dorsal Fat Layer

Monitoring the BCS of cows in the precalving period had proved to be a useful tool in managing the health of the herd [50] [51], because Busato et al. [52] found that high scores in the assessment of BCS (>3.25) before parturition were associated with a high loss of body mass in the first postpartum weeks since these animals experienced higher rates of fat mobilization; this phenomenon has been proven by the increased concentrations of circulating NEFA and BHB. Gillund et al. [50] and Roche et al. [51] verified the same phenomenon. Still, Busato et al. [52] found that the metabolic state is optimal in animals of BCS = 3.25 in case they do not lose much body condition in the postpartum period (ΔBCS between prepartum and eight weeks postpartum ≤0.75).

For this monitoring, producers can use the Edmonson BCS classification table (scale 1—emaciated to 5—severely over-conditioned, with increases of 0.25 point) (Edmonson et al. [53]) based on which Gillund et al. [50] recommended a BCS score of <3.5 at delivery to prevent massive fat mobilization. If each animal is evaluated and this evaluation recorded at least once, the producer can, from then on, regularly check changes in the BCS [23].

On the other hand, the method of measuring the thickness of the subcutaneous fat layer on the back using ultrasound necessarily involves the intervention of a veterinarian, which generally makes it more laborious; however, the results obtained are more objective and precise, and therefore, its implementation is recommended to collect measurements/results comparable to those obtained by assessing body condition within the same herd [23]. The method involves measuring the thickness of the subcutaneous fat layer on the back, accumulated between the skin and the deep trunk fascia, which presents itself as a white line hyperechogenic to ultrasounds, after discounting 5–6 mm for the dermis. The sacrum region is the place of choice for this measurement because it is where the largest reserve of adipose tissue on the back is gathered; for said measurement, a horizontal line should be imagined between the ischial tuberosity and the coxal tuberosity and join the fourth to the fifth caudal part of that line, vertically, to the junction of the sacrum with the first caudal vertebra. Due to the high correlation between the amount of dorsal fat and the body fat content, we can assess the body condition of each animal from the first (Staufenbiel, 1992 cited by Schröder and Staufenbiel [54] (pp. 5–6); [23]).

Although these two methods seem practical and simple, the tendency to increase the size of herds discourages producers from regularly monitoring these variables, given the time and workforce required [23].

6.3. Risk Factors of Ketosis

To prevent the emergence of ketosis, the study of risk factors and the most appropriate nutritional management has been the subject of discussion in various parts of the globe [55][56]. The most prevalent and consistently referred risk factors are increased parity (number of lactations), high concentrations of NEFA in the predelivery period, and elevated body condition [5]. Vanholder et al. [56] also add the birthing season, duration of the dry period, duration of the previous lactation, and liters of colostrum produced as risk factors for developing hyperketonemia.

References

1. McArt, J.A.A.; Nydam, D.V.; Overton, M.W. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. J. Dairy Sci. 2015, 98, 2043–2054.
2. Mostert, P.F.; Bokkers, E.A.M.; van Middelaar, C.E.; Hogeveen, H.; de Boer, I.J.M. Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. Animal 2018, 12, 145–154.
3. Raboisson, D.; Mounié, M.; Khenifar, E.; Maigné, E. The economic impact of subclinical ketosis at the farm level: Tackling the challenge of over-estimation due to multiple interactions. Prev. Vet. Med. 2015, 122, 417–425.

4. Lei, M.A.C.; Simões, J. Milk beta-hydroxybutyrate and fat to protein ratio patterns during the first five months of lactation in Holstein dairy cows presenting treated left displaced abomasum and other post-partum diseases. Animals 2021, 11, 816.
5. Overton, T.R.; McArt, J.A.A.; Nydam, D.V. A 100-year review: Metabolic health indicators and management of dairy cattle. J. Dairy Sci. 2017, 100, 10398–10417.
6. McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D.; Morgan, C.A.; Sinclair, L.A.; Wilkinson, R.G. Microbial digestion in ruminants and other herbivores. In Animal Nutrition, 7th ed.; Prentice Hall/Pearson: Harlow, UK, 2011; pp. 171–186.
7. McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D.; Morgan, C.A.; Sinclair, L.A.; Wilkinson, R.G. Energy metabolism. In Animal Nutrition, 7th ed.; Prentice Hall/Pearson: Harlow, UK, 2011; pp. 192–234.
8. Urrutia, N.; Harvatine, K.J. Acetate dose-dependently stimulates milk fat synthesis in lactating dairy cows. J. Nutr. 2017, 147, 763–769.
9. Urrutia, N.; Harvatine, K.J. Effect of conjugated linoleic acid and acetate on milk fat synthesis and adipose lipogenesis in lactating dairy cows. J. Dairy Sci. 2017, 100, 5792–5804.
10. Urrutia, N.; Bomberger, R.; Matamoros, C.; Harvatine, K.J. Effect of dietary supplementation of sodium acetate and calcium butyrate on milk fat synthesis in lactating dairy cows. J. Dairy Sci. 2019, 102, 5172–5181.
11. Larsen, M.; Kristensen, N.B. Precursors for liver gluconeogenesis in periparturient dairy cows. Animal 2013, 7, 1640–1650.
12. Månsson, H.L. Fatty acids in bovine milk fat fatty acids in bovine milk fat. J. Food Nutr. Res. 2008, 52, 1–3.
13. Zhang, M.; Zhang, S.; Hui, Q.; Lei, L.; Du, X.; Gao, W.; Zhang, R.; Liu, G.; Li, X.; Li, X. B-Hydroxybutyrate facilitates fatty acids synthesis mediated by sterol regulatory element-binding protein1 in bovine mammary epithelial cells. Cell. Physiol. Biochem. 2015, 37, 2115–2124.
14. Drackley, J.K.; Overton, T.R.; Douglas, G.N. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 2001, 84, E100–E112.
15. Larsen, M.; Kristensen, N.B. Effect of abomasal glucose infusion on splanchnic amino acid metabolism in periparturient dairy cows. J. Dairy Sci. 2009, 92, 3306–3318.
16. Klevenhusen, F.; Humer, E.; Metzler-Zebeli, B.; Podstatzky-Lichtenstein, L.; Wittek, T.; Zebeli, Q. Metabolic profile and inflammatory responses in dairy cows with left displaced abomasum kept under small-scaled farm conditions. Animals 2015, 5, 1021–1033.
17. White, H.M. The role of TCA cycle anaplerosis in ketosis and fatty liver in periparturient dairy cows. Animals 2015, 5, 793–802.
18. Drackley, J.K. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 1999, 82, 2259–2273.
19. Herdt, T.H. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. N. Am. Small Anim. Pract. 2000, 16, 215–230.
20. Bisinotto, R.S.; Greco, L.F.; Ribeiro, E.S.; Martinez, N.; Lima, F.S.; Staples, C.R.; Thatcher, W.W.; Santos, J.E.P. Influences of nutrition and metabolism on fertility of dairy cows. Anim. Reprod. 2012, 9, 260–272.
21. Bell, A.W. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 1995, 73, 2804–2819.
22. Ingvartsen, K.L. Feeding-and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim. Feed Sci. Technol. 2006, 126, 175–213.
23. Gruber, S.; Mansfeld, R. Herd health monitoring in dairy farms-discover metabolic diseases. An overview. Tierarztl Prax Ausg G Grosstiere Nutztiere 2019, 47, 246–255.
24. Van Knegsel, A.T.M.; Hammon, H.M.; Bernabucci, U.; Bertoni, G.; Bruckmaier, R.M.; Goselink, R.M.A.; Gross, J.J.; Kuhla, B.; Metges, C.C.; Parmentier, H.K.; et al. Metabolic adaptation during early lactation: Key to cow health, longevity and a sustainable dairy production chain. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 2014, 9, 1–15.
25. Schirmann, K.; Chapinal, N.; Weary, D.M.; Vickers, L.; von Keyserlingk, M.A.G. Short communication: Rumination and feeding behavior before and after calving in dairy cows. J. Dairy Sci. 2013, 96, 7088–7092.
26. Belić, B.; Cincović, M.; Lakić, I.; Đoković, R.; Petrović, M.; Ježek, J.; Starič, J. Metabolic status of dairy cows grouped by anabolic and catabolic indicators of metabolic stress in early lactation. Acta Sci. Vet. 2018, 46, 1–9.
27. Benedet, A.; Manuelian, C.L.; Zidi, A.; Penasa, M.; De Marchi, M. Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. Animal 2019, 13, 1676–1689.

28. Zhang, G.; Ametaj, B.N. Ketosis an old story under a new approach. Dairy 2020, 1, 5.

29. Gao, W.; Du, X.; Lei, L.; Wang, H.; Zhang, M.; Wang, Z.; Li, X.; Liu, G.; Li, X. NEFA-induced ROS impaired insulin signalling through the JNK and p38MAPK pathways in non-alcoholic steatohepatitis. J. Cell. Mol. Med. 2018, 22, 3408–3422.

30. De Koster, J.; Hostens, M.; Van Eetvelde, M.; Hermans, K.; Moerman, S.; Bogaert, H.; Depreester, E.; Van den Broeck, W.; Opsomer, G. Insulin response of the glucose and fatty acid metabolism in dry dairy cows across a range of body condition scores. J. Dairy Sci. 2015, 98, 4580–4592.

31. De Koster, J.D.; Opsomer, G. Insulin resistance in dairy cows. Vet. Clin. N. Am. Food Anim. Pract. 2013, 29, 299–322.

32. Furken, C.; Nakao, T.; Hoedemaker, M. Energy balance in transition cows and its association with health, reproduction and milk production. Tierarztl Prax Ausg G Grosstiere Nutztiere 2015, 43, 341–349.

33. Brunner, N.; Groeger, S.; Raposo, J.C.; Bruckmaier, R.M.; Gross, J.J. Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe. Transl. Anim. Sci. 2018, 3, 84–92.

34. Gordon, J.L.; Leblanc, S.J.; Duffield, T.F. Ketosis treatment in lactating dairy cattle. Vet. Clin. N. Am. Food Anim. Pract. 2013, 29, 433–445.

35. Oetzel, G.R. Herd-Level Ketosis-Diagnosis and Risk Factors. In Proceedings of the Preconference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting, American Association of Bovine Practitioners 40th Annual Conference, Vancouver, BC, Canada, 19 September 2007; pp. 67–91.

36. Gross, J.J.; Bruckmaier, R.M. Review: Metabolic challenges in lactating dairy cows and their assessment via established and novel indicators in milk. Animal 2019, 13, s75–s81.

37. Bruckmaier, R.M.; Gross, J.J. Lactational challenges in transition dairy cows. Anim. Prod. Sci. 2017, 57, 1471–1481.

38. Oetzel, G.R. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. N. Am. Food Anim. Pract. 2004, 20, 651–674.

39. Derno, M.; Nürnberg, G.; Schön, P.; Schwarm, A.; Röntgen, M.; Hammon, H.M.; Metges, C.C.; Bruckmaier, R.M.; Kuhla, B. Short-term feed intake is regulated by macronutrient oxidation in lactating Holstein cows. J. Dairy Sci. 2013, 96, 971–980.

40. Berge, A.C.; Vertenten, G. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. J. Dairy Sci. 2014, 97, 2145–2154.

41. Mcart, J.A.A.; Peek, S.F.; Divers, T.J. Metabolic Diseases. In Rebhun's Diseases of Dairy Cattle, 3rd ed.; Peek, S.F., Divers, T.J., Eds.; Elsevier, Inc.: St. Louis, MO, USA, 2018; pp. 713–722.

42. Foster, L.A. Clinical Ketosis. Vet. Clin. N. Am. Food Anim. Pract. 1988, 4, 253–267.

43. Dar, A.M.; Malik, H.U.; Beigh, S.A.; Hussain, S.A.; Nabi, S.U.; Dar, A.A.; Dar, P.A.; Bhat, A.M. Clinico-biochemical alternation in bovine ketosis. J. Entomol. Zool. Stud. 2018, 6, 1146–1150.

44. Grelet, C.; Bastin, C.; Gelé, M.; Davière, J.B.; Johan, M.; Werner, A.; Reding, R.; Fernandez Pierna, J.A.; Colinet, F.G.; Dardenne, P.; et al. Development of Fourier transform mid-infrared calibrations to predict acetone, β-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci. 2016, 99, 4816–4825.

45. Renaud, D.L.; Kelton, D.F.; Duffield, T.F. Short communication: Validation of a test-day milk test for β-hydroxybutyrate for identifying cows with hyperketonemia. J. Dairy Sci. 2019, 102, 1589–1593.

46. King, M.T.M.; Duffield, T.F.; DeVries, T.J. Short communication: Assessing the accuracy of inline milk fat-to-protein ratio data as an indicator of hyperketonemia in dairy cows in herds with automated milking systems. J. Dairy Sci. 2019, 102, 8417–8422.

47. Mann, S.; Leal Yepes, F.A.; Overton, T.R.; Wakshlag, J.J.; Lock, A.L.; Ryan, C.M.; Nydam, D.V. Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. J. Dairy Sci. 2015, 98, 3366–3382.

48. Kaufman, E.I.; Leblanc, S.J.; Mcbride, B.W.; Duffield, T.F.; Devries, T.J. Association of rumination time with subclinical ketosis in transition dairy cows. J. Dairy Sci. 2016, 99, 5604–5618.

49. Schirmann, K.; Weary, D.M.; Heuwieser, W.; Chapinal, N.; Cerri, R.L.A.; von Keyserlingk, M.A.G. Short communication: Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. J. Dairy Sci. 2016, 99, 9917–9924.

50. Gillund, P.; Reksen, O.; Gröhn, Y.T.; Karlberg, K. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J. Dairy Sci. 2001, 84, 1390–1396.

51. Roche, J.R.; Kay, J.K.; Friggens, N.C.; Loor, J.J.; Berry, D.P. Assessing and managing body condition score for the prevention of metabolic disease in dairy cows. Vet. Clin. N. Am. Small Anim. Pract. 2013, 29, 323–336.
52. Busato, A.; Faissler, D.; Küpfer, U.; Blum, J.W. Body condition scores in dairy cows: Associations with metabolic and endocrine changes in healthy dairy cows. J. Vet. Med. A Physiol. Pathol. Clin. Med. 2002, 49, 455–460.
53. Edmonson, A.J.; Lean, I.J.; Weaver, L.D.; Farver, T.; Webster, G. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 1989, 72, 68–78.
54. Schröder, U.J.; Staufenbiel, R. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J. Dairy Sci. 2006, 89, 1–14.
55. McArt, J.A.A.; Nydam, D.V.; Oetzel, G.R. Dry period and parturient predictors of early lactation hyperketonemia in dairy. J. Dairy Sci. 2013, 96, 198–209.
56. Vanholder, T.; Papen, J.; Bemers, R.; Vertenten, G.; Berge, A.C.B. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J. Dairy Sci. 2015, 98, 880–888.
