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Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance

A. Benedet[1], C. L. Manuelian[1], A. Zidi[2], M. Penasa[1] and M. De Marchi[1†]

[1]*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy;*
[2]*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

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Hyperketonemia (HYK) is one of the most frequent and costly metabolic disorders in high-producing dairy cows and its diagnosis is based on β-hydroxybutyrate (BHB) concentration in blood. In the last 10 years, the number of papers that have dealt with the impact of elevated BHB levels in dairy cattle has increased. Therefore, this paper reviewed the recent literature on BHB concentration in blood and milk, and its relationships with dairy cow health and performance, and farm profitability. Most studies applied the threshold of 1.2 mmol/l of BHB concentration in blood to indicate HYK; several authors considered BHB concentrations between 1.2 and 2.9 mmol/l as subclinical ketosis, and values ⩾ 3.0 mmol/l as clinical ketosis. Results on HYK frequency (prevalence and incidence) and cow performance varied according to parity and days in milk, being greater in multiparous than in primiparous cows, and in the first 2 weeks of lactation than in later stages. Hyperketonemia has been associated with greater milk fat content, fat-to-protein ratio and energy-corrected milk, and lower protein and urea nitrogen in milk. The relationships with milk yield and somatic cell count are still controversial. In general, HYK impairs health of dairy cows by increasing the risk of the onset of other early lactation diseases, and it negatively affects reproductive performance. The economic cost of HYK is mainly due to impaired reproductive performance and milk loss. From a genetic point of view, results from the literature suggested the feasibility of selecting cows with low susceptibility to HYK. The present review highlights that milk is the most promising matrix to identify HYK, because it is easy to sample and allows a complete screening of the herd through BHB concentration predicted using mid-IR spectroscopy during routine milk recording. Further research is needed to validate accurate and convenient methods to discriminate between cows in risk of HYK and healthy animals in field conditions and to support farmers to achieve an early detection and minimise the economic losses.

Keywords: cattle, health, hyperketonemia, ketone body, milk production

Implications

Hyperketonemia (HYK) is a complex metabolic status described by high levels of ketone bodies in blood, the most common being β-hydroxybutyrate (BHB). In general, HYK increases the risk of health problems during early lactation, with consequent negative effects on herd profitability. This review shows controversial results in the relationship between BHB and milk production and composition, which could be related to the use of a single cut-off to discriminate between healthy and hyperketonemic animals. More research is still needed to identify and validate trustworthy methods to discriminate between cows affected by HYK and healthy animals in field conditions.

† E-mail: massimo.demarchi@unipd.it

Introduction

Ketosis is one of the most detrimental metabolic diseases in dairy cows. It occurs in early lactation when animals experience a negative energy balance (NEB), defined as the lack of trade-off between energy intake (input) and demands for increased milk production (output) (Herdt, 2000). During this period, cows use alternative energy sources to supply the decrease of available glucose that comes from gluconeogenesis; glucose is a fundamental nutrient strictly linked to the maintenance of normal functions in most of body tissues and lactogenesis. The inability of cows to cope with NEB and glucose drop due to an inadequate metabolic adaptation leads to an excessive mobilisation of adipose reserves, releasing abnormal concentrations of non-esterified fatty acids (NEFA) and ketone bodies (acetone, acetoacetate and BHB) in the blood. Thus, biochemical analyses are

fundamental to help with the diagnosis of this complex disease. Elevated concentrations of ketone bodies in blood, defined as HYK, negatively affect immune function, health and milk production (McArt *et al*., 2013). The BHB is the most common ketone body used to diagnose HYK (Oetzel, 2004) because it is the predominant and more stable circulating ketone body in cow fluids (Duffield *et al*., 2009). However, BHB measurements vary for several reasons such as diurnal variation in body fluids (Nielsen *et al*., 2003), and methods of sampling and analysis (Krogh *et al*., 2011; Bach *et al*., 2016).

Hyperketonemia can result in subclinical ketosis (SCK) or clinical ketosis (CK). Some signs described in literature in animals suffering from CK are ketone smell in breath, reduced activity and appetite, excessive loss of body condition, weakness and apparent blindness (Berge and Vertenten, 2014), but those signs are unspecific and/or difficult to be detected; this increases the error of diagnosis and makes arduous to precisely discriminate between CK and SCK. Both CK and SCK affect milk production, reproduction performance and health of dairy cattle (McArt *et al*., 2013; Raboisson *et al*., 2014), and thus, they are responsible of increased culling rate (Seifi *et al*., 2011) and costs at herd level (Liang *et al*., 2017; Mostert *et al*., 2018). Given this background, the reinforcement of farmers' awareness of HYK effects, the search for new opportunities of genetic improvement (Pryce *et al*., 2016) and the implementation of herd management strategies against HYK have recently gained more and more importance in the scientific community and dairy sector. The aim of this review is to summarise results on the associations of BHB concentration in blood and milk with cow health, milk production and composition, and reproductive performance, and to describe its genetic aspects and economic implications in dairy cattle.

Criteria and methodology of the review

Papers included in the present review were retrieved from Scopus (www.scopus.com) and ISI Web of Science (www.webofknowledge.com) databases for the period January 2007 to January 2018. The keywords used in the literature search were: ketosis, dairy cows, cattle, bovine, beta-hydroxybutyrate, β-hydroxybutyrate, BHB, milk beta-hydroxybutyrate, milk β-hydroxybutyrate, milk BHB, milk composition, milk yield, milk production, genetic, genomic, economic, cost, hyperketonemia, subclinical ketosis, performance, health, reproduction, fertility, ketone body and metabolic disease. Even if ketosis is a complex disease, only papers that focussed on the effects of blood and milk BHB concentrations on milk production, milk composition, reproduction and health performance, and on economic and genetic aspects of this ketone body and HYK or ketosis were considered. The determination of the most accurate blood BHB cut-off to diagnose HYK was not an aim of this review because it has been previously discussed in McArt *et al*. (2013).

In the reviewed papers ketosis and HYK have been often used as synonyms (McArt *et al*., 2012; Vanholder *et al*., 2015; Rutherford *et al*., 2016), and thus, we decided to use the term HYK because we consider it more adequate when investigating BHB concentration in blood or milk. The terms SCK and CK have been used when referring to studies that made clear reference to SCK and CK in the text. The unification of terminology such as HYK, CK and SCK, or frequency defined as prevalence or incidence, was sometimes very problematic and made difficult the comparison among studies. Data quality when analysing health events differed among the reviewed studies because some of them were based on voluntary declaration from farmers (Ospina *et al*., 2010a; Koeck *et al*., 2014; Parker Gaddis *et al*., 2018) and others were from veterinarians (Suthar *et al*., 2013). Moreover, although efforts have been made to standardise the clinical diagnosis, the participation of several observers leads to an unquantifiable error of diagnosis, involving a difficult interpretation of the results.

Studies on BHB effects on cow production have increased since 2012 (Figure 1), with an increasing incidence of papers dealing with genetic parameters and economic aspects. The increment of BHB studies underlines that metabolic disorders have been assuming more and more relevance in dairy industry and scientific community. Moreover, the increase of papers on genetic and economic aspects of HYK is likely related to the more recent availability of large data including BHB concentration as a routinely recorded trait in some production systems, which is necessary, for example, to estimate its genetic parameters.

Table 1 reports some information retrieved from the reviewed studies on HYK in dairy cattle, namely the country where the study was conducted, number of herds and cows, cow breed and parity, and observation period. Papers ($n = 4$) that dealt with economic aspects were not included in Table 1 because they were based on literature values and data simulations. The papers mainly dealt with Holstein, as it is the most popular and productive cosmopolitan dairy cattle breed, and only few studies focussed on other breeds such as Jersey, Brown Swiss, Norwegian Red and local populations. The studies were conducted in 20 countries, with United States and Canada being the most represented with 15 and 10 papers, respectively (Table 1). As only few studies dealt

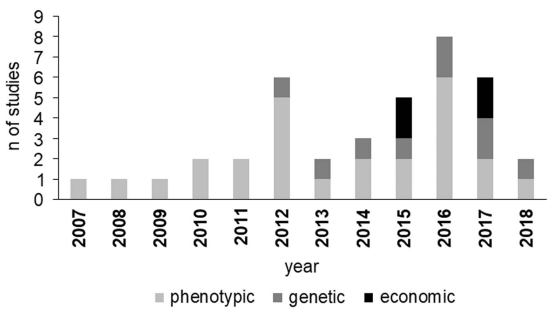

Figure 1 Number of reviewed studies in dairy cattle per area of interest and year of publication. Phenotypic area includes milk production and composition, reproduction and health performance.

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Table 1 *Description of reviewed studies on hyperketonemia in Holstein cows detected from blood or milk analysis*

References	Country[1]	Herds (*n*)	Cows (*n*)[2]	Parity	Observation period
Blood					
Walsh *et al.* (2007)	CDN	25	796	2, 3	−3 to 9 weeks from calving
van Haelst *et al.* (2008)	NL	1	16	2, 3, 4	9 weeks from calving
Duffield *et al.* (2009)	CDN	25	1010	1, 2, 3	2 weeks from calving
Ospina *et al.* (2010a)	USA	100	2758	1, ⩾2	−2 to 2 weeks from calving
Ospina *et al.* (2010b)	USA	91	2290	1, ⩾2	−2 to 2 weeks from calving
McArt *et al.* (2011)	USA	4	1717	1, 2, ⩾3	3 to 16 days in milk
Seifi *et al.* (2011)	CDN	16	849	1, 2, ⩾3	3 weeks from calving
Chapinal *et al.* (2012a)	CDN/USA	45	1919	1, ⩾2	−1 to 3 weeks from calving
Chapinal *et al.* (2012b)	CDN/USA	55	2365	1, ⩾2	−1 to 1 weeks from calving
McArt *et al.* (2012)	USA	4	1717	1, 2, ⩾3	3 to 16 days in milk
Roberts *et al.* (2012)	CDN/USA	69	5979	1, 2, ⩾3	−1 to 2 weeks from calving
van der Drift *et al.* (2012a)	NL	118	1678	1, 2, 3, ⩾4	5 to 60 days in milk
van der Drift *et al.* (2012b)	NL	122	1615	1, 2, 3, ⩾4	5 to 60 days in milk
Suthar *et al.* (2013)	Several[4]	528	5884	1, 2, 3, ⩾4	2 to 15 days in milk
Vanholder *et al.* (2015)	NL	23	1715	1, 2, ⩾3	7 to 14 days in milk
Kaufman *et al.* (2016)	CDN	4	339	1, 2, ⩾3	−2 to 4 weeks from calving
Mann *et al.* (2016)	USA	1	84	2, ⩾3	3 to 14 days in milk
Rutherford *et al.* (2016)	GB	3	203	1, 2, ⩾3	7 to 21 days in milk
Song *et al.* (2016)	CHN	1	45	>1	–
Stangaferro *et al.* (2016)	USA	1	1080	1, ⩾2	−2 to 4 weeks from calving
Belay *et al.* (2017b)	N	2828	179 691	1, 2, 3, 4	11 to 120 days in milk
Rathbun *et al.* (2017)	USA	1	570	1 to ⩾6	5 to 18 days in milk
Ruoff *et al.* (2017)	D	6	621	1, ⩾2	1 to 42 days in milk
Weigel *et al.* (2017)	USA	3	1453	1 to ⩾5	5 to 18 days in milk
Chandler *et al.* (2018)	USA	16	1005	1, ⩾2	5 to 20 days in milk
Milk					
van der Drift *et al.* (2012b)	NL	122	1615	1, 2, 3, ⩾4	5 to 60 days in milk
Buitenhuis *et al.* (2013)	DK	20	371	1, 2, 3	129 to 228 days in milk
Berge and Vertenten (2014)	D, F, GB, I, NL	131	4709	1 to 12	7 to 21 days in milk
Koeck *et al.* (2014)	CDN	–	61 331	1	5 to 100 days in milk
Moyes *et al.* (2014)	DK	1	30	1	4 to 6 weeks from calving
Kayano and Kataoka (2015)	J	50	693	1 to 12	7 to 30 days in milk
Penasa *et al.* (2015)	I	299	19 980	1, 2, 3	5 to 305 days in milk
Jamrozik *et al.* (2016)	CDN	–	35 575	1 to 5	5 to 40 days in milk
Lee *et al.* (2016)	ROK	–	7895	1, 2, 3	4 to 305 days in milk
Santschi *et al.* (2016)	CDN	4242	498 310	1, 2, ⩾3	5 to 35 days in milk
Rathbun *et al.* (2017)	USA	1	570	1 to ⩾6	5 to 18 days in milk
Parker Gaddis *et al.* (2018)[3]	USA	–	23 865	1 to 5	1 to 60 days in milk

[1]CDN = Canada; CHN = China; D = Germany; DK = Denmark; F = France; GB = United Kingdom; I = Italy; J = Japan; NL = The Netherlands; N = Norway; ROK = South Korea; USA = United States.
[2]Berge and Vertenten (2014) (German Black Pied and other breeds); Suthar *et al.* (2013) (Holstein-Friesian crossbreds, Jersey and Brown Swiss); Belay *et al.* (2017b) (Norwegian Red); Chandler *et al.* (2018) (Jersey); Parker Gaddis *et al.* (2018) (Jersey).
[3]Producer-recorded cases.
[4]Denmark, Spain, Croatia, Hungary, Italy, Poland, Portugal, Slovenia, Serbia, Turkey.

with an observation period that started 1 to 3 weeks before calving, this review focussed more on the *postpartum* period. Table 1 also shows that in recent years several papers have dealt with BHB concentration in milk, which is a more practical matrix than blood.

The keywords reported in each paper by their authors were extracted by adapting the Bibliometrix package (Aria and Cuccurullo, 2017) for R v. 3.4 (R Core Team, 2017), which produced the network of all recurrent keywords weighted by their frequency (Figure 2). The closer two terms are located in the map, the stronger the relationship between the terms, and the bigger the nucleus, more frequently the word has been used. The total number of keywords retrieved after editing of synonyms was 75 and 'dairy cows', 'β-hydroxybutyrate', 'ketosis', 'non-esterified fatty acids' and 'hyperketonemia' were the most recurrent keywords.

Methods and thresholds used to define hyperketonemia

The tested matrix, method of determination and diagnostic threshold affect the results about relationships between BHB

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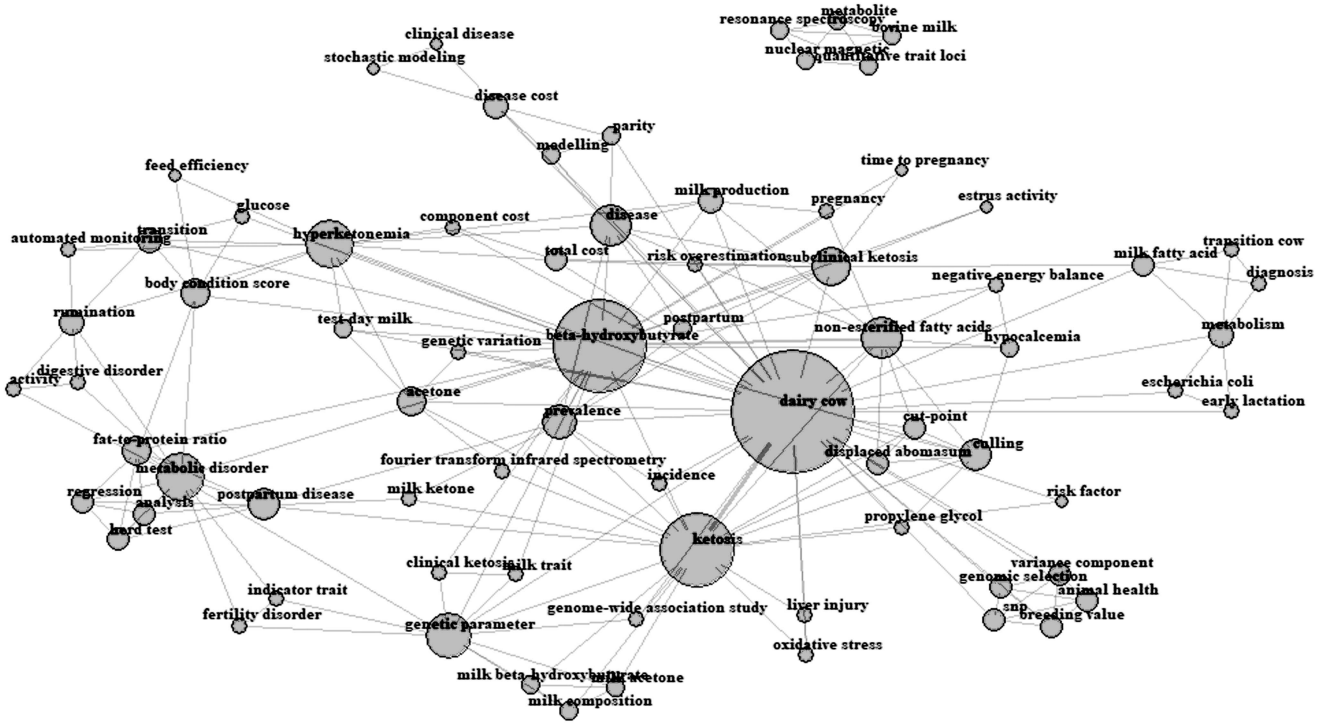

Figure 2 Author's keywords occurrence map for reviewed studies in dairy cattle. The closer two terms are located in the map, the stronger the relation between the terms and the bigger the nucleus, the more frequent the word has been used.

concentration and HYK and, consequently, the impact on cow performance. However, universal method and threshold for defining HYK and linking it to ketosis have not been established yet, probably because of the difficulty of accurately diagnose CK, and therefore discriminate between CK and SCK. Moreover, the cut-offs used in the reviewed papers could increase the error of HYK detection because BHB concentration in blood and milk fluctuates during the day (Nielsen *et al*., 2003). Most reviewed studies did not specify sampling times, which could lead to unpredictable differences between outcomes. However, a clear pattern of the diurnal variation of BHB concentration in blood and milk has not been described yet, even if a relationship with the energy content of the diet has been reported by Nielsen *et al*. (2003). In particular, cows fed a total mixed ration with low-energy content showed a decrease of blood and milk BHB concentration in the evening and lower variation among cows, whereas cows fed a total mixed ration with high-energy content showed an increase of blood and milk BHB concentration in the evening and greater variation among cows (Nielsen *et al*., 2003). In the same study (Nielsen *et al*., 2003), BHB concentrations in blood of a cow fed a total mixed ration with high-energy content exceeded the most common threshold used to define HYK in blood (1.2 mmol/l) for 37.5% of the day, which could lead to a misclassification of positive HYK. Therefore, the tested matrix, method of determination and threshold need to be taken into account to correctly interpret the results. Table 2 summarises the methods of determination of BHB concentration and the cut-offs of BHB used to establish HYK, or SCK and CK, in the reviewed papers.

The BHB can be detected in blood and milk. In particular, blood BHB concentration is the most common indicator to diagnose HYK and this explains why the majority of reviewed papers dealt with blood sampling (Table 2). The laboratory determination of BHB concentration is based on a colorimetric enzymatic reaction followed by a spectrophotometric analysis. Moreover, handheld blood ketone meters have been developed and validated in order to provide a more practical tool for on-field data collection. These on-farm testing systems include handheld devices and test strips based on an electrochemical reaction with a small amount of blood to determine the ketone concentration with acceptable specificity and sensitivity for HYK diagnosis (Bach *et al*., 2016; Sailer *et al*., 2018). However, it should be taken in consideration that blood BHB concentration is usually based on a single blood sample and it represents the status of the animal at that sampling point of the day.

Blood sampling is a labourious and time-consuming procedure, and it is stressful for the animals. The possibility of using milk BHB concentration to diagnose HYK has been investigated (Table 2) because milk recording is already a routine and non-invasive procedure, and it facilitates monitoring at herd level. Moreover, it differs from blood measurement of BHB status of the animal as a milk sample represents a period of time. Although laboratory analyses such as photometrical method or enzymatic assays (Chandler *et al*., 2018) and cowside strip tests (Keto-Test; Berge and Vertenten, 2014) exist in this field, mid-IR spectroscopy (MIRS) is the most promising tool for the determination of milk BHB (Grelet *et al*., 2016). Indeed, the use of chemical analysis or handheld meter to measure BHB in

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Table 2 *Thresholds of β-hydroxybutyrate (BHB) concentration in blood and milk (mmol/l) used in the literature to determine hyperketonemia (HYK), also defined as subclinical ketosis (SCK) and clinical ketosis (CK) in some cases, in dairy cattle*

References	Method of analysis	HYK/SCK	CK
Blood BHB			
Walsh *et al.* (2007)	Automated analyser: Dacos 2 Analyzer (Coulter Electronics)	⩾1.0–1.4[1]	–
van Haelst *et al.* (2008)	Automated analyser: Unicel DxC 600 (Beckman Instruments B.V.)	⩾1.2	–
Duffield *et al.* (2009)	Automated analyser: Dacos 2 Analyzer (Coulter Electronics)	⩾1.4	–
Ospina *et al.* (2010a and 2010b)	Automated analyser: Hitachi 917 (Roche Diagnostics)	⩾1.0	–
Seifi *et al.* (2011)	Automated analyser: Hitachi 911 (Roche Diagnostics)	–	⩾1.2
Chapinal *et al.* (2012a and 2012b)	Automated analyser: Hitachi 911 (Randox Laboratories)	⩾ 1.4	–
McArt *et al.* (2011 and 2012), Weigel *et al.* (2017)	Handheld meter: Precision Xtra (Abbott Laboratories)	1.2 to 2.9	⩾3.0
van der Drift *et al.* (2012a and 2012b)	Kit test: Ranbut kit (Randox Laboratories)	⩾1.2	–
Suthar *et al.* (2013)	Handheld meter: Precision Xtra (Abbott Laboratories)	⩾1.2	⩾1.1
Vanholder *et al.* (2015)	Handheld meter: Precision Xceed (Abbott Laboratories)	1.2 to 2.9	⩾3.0
Kaufman *et al.* (2016), Mann *et al.* (2016), Rathbun *et al.* (2017)	Handheld meter: Precision Xtra (Abbott Laboratories)	⩾1.2	–
Ruoff *et al.* (2017)	Handheld meter: NovaVet (Nova Biomedical)	⩾1.2	–
Rutherford *et al.* (2016)	Handheld meter: Optium Xceed (Abbott Laboratories)	1.2 to 2.9	⩾3.0
Song *et al.* (2016)	Not specified	1.2 to 1.5	⩾1.5
Belay *et al.* (2017b)	FT-MIR spectrometer: Milkoscan Combifoss 6500 (Foss Electric)	⩾1.2	–
Chandler *et al.* (2018)	Colorimetric assay	⩾1.2	–
Milk BHB			
van der Drift *et al.* (2012a)	FT-MIR spectrometer: MilkoScan FT6000 (Foss Electric)	⩾0.08	–
Berge and Vertenten (2014)	Keto-Test: Ketolac test strip (Sanwa Kagaku Kenkyusho Co. Ltd.)	⩾0.10	–
Koeck *et al.* (2014), Santschi *et al.* (2016)	FT-MIR spectrometer: MilkoScan FT6000 (Foss Electric)	⩾0.15–0.20	–
Lee *et al.* (2016)	FT-MIR spectrometer: CombiFoss FT + (Foss Electric)	0.01 to 0.19	⩾0.20

[1]Thresholds for 1st and 2nd week of lactation, respectively.

routine milk recording is not feasible because it is too expensive and time consuming, whereas MIRS allows to collect data at population level cheaply and provide routine reports to farmers for monitoring their herd. Currently, the accuracy of the developed prediction equations for BHB concentration in milk is not high enough to quantify the exact content but it has proved to be useful for screening purposes to detect cows with elevated BHB concentrations in milk (Grelet *et al.*, 2016; Lee *et al.*, 2016; Santschi *et al.*, 2016). Moreover, predicted milk acetone and BHB have been used to develop models for the prediction of blood BHB from test-day milk and performance traits (Chandler *et al.*, 2018), and the use of MIRS milk spectra has been proposed to directly predict blood BHB concentration (Belay *et al.*, 2017a and 2017b). Nevertheless, for this approach, we have to consider that usually blood BHB concentration is determined using a single blood sample that represents the status of the animal at the time of blood sampling, whereas a milk sample represents the BHB concentration of a period of time, which could interfere with the accuracy of the developed calibration model. To increase the accuracy of the prediction models using milk samples, several blood samples during the same period of time that the milk sample would represent could be collected.

For both blood and milk, different thresholds have been used (Table 2). In general, the optimum thresholds of BHB concentration for *postpartum* diseases occurrence (Duffield *et al.*, 2009; Ospina *et al.*, 2010a; Suthar *et al.*, 2013), fertility indicators (Chapinal *et al.*, 2012a), and change in milk production and composition traits (Duffield *et al.*, 2009) have been identified based on the highest combination of sensitivity and specificity of the analysis performed. However, most of reviewed studies have defined the cut-offs before starting the experiment (Koeck *et al.*, 2014; Vanholder *et al.*, 2015; Rutherford *et al.*, 2016). The main difficulty was to unify terminology as some authors clearly specified a distinction between SCK and CK, whereas others considered only HYK. In general, regarding BHB cut-offs reported in the papers, HYK and SCK were defined from the same threshold. In blood, BHB ⩾1.2 mmol/l is generally used as cut-off to identify cows with HYK, or affected by SCK. A deeper analysis of the adequacy of this cut-off in blood has been discussed in McArt *et al.* (2013). A less uniform BHB cut-off for CK has been proposed in literature. Although most papers have reported a direct relationship between blood BHB concentrations and CK incidence and established the threshold at blood BHB ⩾3.0 mmol/l, some authors observed that CK occurrence can be associated to lower BHB concentration (e.g. BHB ⩾1.1 mmol/l) (Seifi *et al.*, 2011; Suthar *et al.*, 2013; Song *et al.*, 2016).

Only few papers have used milk BHB concentration to detect HYK (Table 2) and, considering the limitations of strip tests and MIRS prediction models, there is not a clear cut-off point. Ranges of milk BHB concentrations to classify cows with suspect HYK (0.15 to 0.19 mmol/l) or positive HYK (⩾0.20 mmol/l) have been recently proposed by Koeck *et al.*

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(2014) and Santschi *et al*. (2016). On the other hand, Lee *et al*. (2016) considered cows as affected by SCK with milk BHB concentration between 0.01 and 0.20 mmol/l, and affected by CK with milk BHB concentration ⩾ 0.20 mmol/l. However, even with elevated concentrations of milk BHB, a blood test and/or a veterinary visit is necessary to help on the diagnosis of HYK.

Frequency and risk factors of hyperketonemia

A disease frequency can be described through two different measures, prevalence and incidence, usually expressed as a percentage. Prevalence is defined as the number of existing cases of a specific disorder divided by the number of sampled animals at a given time, that is, a single test is required. Incidence is calculated as the number of new cases of a specific disease divided by the number of animals at risk during a defined period of time. Thus, to estimate incidence is necessary to test animals frequently enough to ensure that all cows that develop the disease during the observation period will be correctly identified. Moreover, incidence can be expressed as cumulative incidence or as incidence rate. Cumulative incidence is the most common one and provides a measure of risk in a given period of time. Incidence rate is the proportion of new cases calculated per unit of time and the result should be expressed per unit of time. Therefore, the ease of prevalence calculation explains why there is a lower number of papers that computed incidence (Table 3). Even if not clearly expressed in most of the cases, incidence values reported in reviewed studies corresponded to cumulative incidence, meaning that some misleading use of terminology to express frequency exists in literature. For instance, cumulative incidence was wrongly referred as rate in Weigel *et al*. (2017), whereas prevalence and incidence rate were used as synonyms in Lee *et al*. (2016), leading to a difficult interpretation of the results.

Prevalence and incidence of HYK reported in reviewed papers are displayed in Table 3. Prevalence ranged from 11.2% (van der Drift *et al*., 2012b) to 47.2% (Vanholder *et al*., 2015) when papers considered HYK or SCK. On the other hand, the percentage dropped off considerably when CK was considered, ranging from 3.7% (Seifi *et al*., 2011) to 11.6% (Vanholder *et al*., 2015). A similar situation was observed in the studies reporting incidence, showing a range between 19.7% (McArt *et al*., 2012) and 44% (Kaufman *et al*., 2016) for HYK or SCK and a value of 2.4% for CK (Weigel *et al*., 2017).

As reported above, to interpret and compare correctly the measures of frequency, several factors such as method of detection, threshold used and biological fluid analysed should be taken into account. Moreover, these frequency expressions greatly depend on stage of lactation (observation period) and parity (Table 1). As HYK is mainly the consequence of NEB experienced by the cow after calving, the highest HYK prevalence has been detected in the first 2 weeks of lactation, declining greatly thereafter (van der Drift *et al*., 2012a; Koeck *et al*., 2014; Santschi *et al*., 2016).

Table 3 *Prevalence and incidence (%) of hyperketonemia (HYK), also defined as subclinical ketosis (SCK) and clinical ketosis (CK) in some cases, in dairy cattle*

References	HYK/SCK	CK
Prevalence		
Walsh *et al*. (2007)	18.8 to 36.2	–
Duffield *et al*. (2009)	16.6 to 18.6	
Seifi *et al*. (2011)	–	3.7
Chapinal *et al*. (2012a)	12 to 20	–
van der Drift *et al*. (2012a)	11.2	–
Suthar *et al*. (2013)	21.8	–
Berge and Vertenten (2014)	39	–
Koeck *et al*. (2014)	14	–
Vanholder *et al*. (2015)	47.2	11.6
Mann *et al*. (2016)	30.5	–
Rutherford *et al*. (2016)	17	–
Santschi *et al*. (2016)	22.9	–
Chandler *et al*. (2018)	14 to 19	–
Incidence		
McArt *et al*. (2012)	43.2	–
Kaufman *et al*. (2016)	44	–
Rathbun *et al*. (2017)	19.7	–
Weigel *et al*. (2017)	24	2.4

For example, a decrease of prevalence by 60% (from 18% to 7%) between the 1st and the 2nd month of lactation has been reported by van der Drift *et al*. (2012a). Moreover, both HYK peak incidence (22.3% of cows with their first positive test) and prevalence (28.9% of cows with a positive test) have been detected at 5 days in milk in McArt *et al*. (2012). Considering these high frequencies in 1st days of lactation, the calculation of the risk of disease occurrence through multiple tests during early lactation would be an appropriate approach to measure HYK frequency.

Several authors observed a higher frequency of HYK in multiparous than primiparous cows (Santschi *et al*., 2016; Rathbun *et al*., 2017; Chandler *et al*., 2018), and suggested a direct relationship between increasing parity and HYK occurrence. Cumulative incidence from 8.6% to 26.2% (Rathbun *et al*., 2017) and prevalence from 18.8% to 27.6% (Santschi *et al*., 2016) have been detected moving from first to third or greater lactation. The increase of HYK occurrence with parity may be due to the concurrent needs of gestation and lactation, as indicated by Berge and Vertenten (2014). For this reason, it could be appropriate to record separately HYK risk-estimates for different parity orders and then compute a herd level incidence risk by standardising parity-specific percentages. Conversely, the same pattern has not been observed in Jersey breed, as HYK was more prevalent in primiparous than multiparous cows (Chandler *et al*., 2018).

Other factors that should be considered for interpreting HYK occurrence are season of calving, breed and herd management. Authors generally agreed to identify spring as the season with greater prevalence of HYK, whereas contrasting results have been reported for late autumn and winter (Vanholder *et al*., 2015; Santschi *et al*., 2016) or summer (van

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der Drift *et al*., 2012a; Suthar *et al*., 2013). However, in most cases, no biological reason or evidence has been reported to justify the greater HYK prevalence in spring (Santschi *et al*., 2016). It has been suggested for Dutch farmers that the lower quality of the silage used during the first half of the year could explain the greater HYK prevalence in spring (Vanholder *et al*., 2015). Concerning breed, higher overall HYK prevalence in Jersey (19%) than Holstein cows (14%), with values that ranged from 11.4% to 25% in Jersey herds and 0% to 28% in Holstein herds, has been observed by Chandler *et al.* (2018). Management and feeding of gestating heifers, dry cows and cows in early lactation, as well as on-farm prevention approaches and incidence of other diseases contribute to HYK prevalence (Santschi *et al*., 2016). A negative association between the increase of herd size and HYK prevalence has been reported (Berge and Vertenten, 2014) as bigger herds usually implemented strategies such as grouping cows based on milk production to better meet nutritional requirements. Berge and Vertenten (2014) also observed a lower prevalence of HYK in systems with cubicles, cubicles and yards, or tie-up bars than in systems with straw yards, and a slightly greater frequency in systems in which cows were on pastures rather than housed indoor. Moreover, a lower prevalence of HYK was reported in herds feeding forage and concentrate separately or total mixed ration compared with herds using partial mixed ration. Mixed ration refers to cows fed a total mixed ration between grazing periods. These differences in prevalence might be due to the fact that farmers cannot easily control animals in terms of nutritional level and health status when they are on pasture or housed in straw yards.

Associations of β-hydroxybutyrate with dairy cow performance

Health

Cows with BHB concentration $\geqslant 1.2$ or 1.1 mmol/l in blood (Seifi *et al*., 2011; Suthar *et al*., 2013) or $\geqslant 0.10$ mmol/l in milk (Berge and Vertenten, 2014) are from 4.7 to 14.7 times more likely to manifest clinical signs of HYK. In these studies, CK was diagnosed by veterinarians (Seifi *et al*., 2011; Suthar *et al*., 2013; Berge and Vertenten, 2014) or herd managers (Suthar *et al*., 2013) according to the following definitions: decreased feed intake or appetite, decreased milk production, a positive urine or milk ketone test, low rumen fill, reduced activity or demeanour, excessive loss of body condition, constipation or hard/dry faeces, ketone odour in breath/milk and nervous signs. However, in the reviewed papers, it is not clear how authors used all those variables to establish CK. Moreover, it is commonly agreed that HYK increases the risk of the onset of other early lactation diseases, such as displaced abomasum (odds ratio (OR) = 1.6 to 19.3; Seifi *et al*., 2011; McArt *et al*., 2011 and 2012; Suthar *et al*., 2013; Berge and Vertenten, 2014), metritis (OR = 1.5 to 1.7; Suthar *et al*., 2013; Berge and Vertenten, 2014) and lameness (OR = 1.7 to 1.8; Suthar *et al*., 2013; Berge and Vertenten, 2014), and the risk increased with blood BHB concentration (Ospina *et al*., 2010a; McArt *et al*., 2012; Suthar *et al*., 2013). Overall, reviewed studies supported the hypothesis of Roberts *et al*. (2012) that cows with high blood BHB concentrations, especially multiparous animals, had greater probability of being removed from the herd in early lactation. In particular, in McArt *et al*. (2012) cows diagnosed with HYK were three times more likely to die or be culled than non-hyperketonemic cows, observing also that each 0.1 mmol/l increment of blood BHB concentration during the 1st month of lactation increased the risk of culling by 1.4 times. Although most studies have focussed on the very early lactation ($\leqslant$21 days in milk), consequences of elevated BHB levels on health can be observed until 60 days in milk. Some authors noted that a greater disease risk occurred for cows diagnosed with HYK in the 1st week after calving (Seifi *et al*., 2011; McArt *et al*., 2012). For instance, in McArt *et al*. (2012) cows diagnosed with HYK from 3 to 5 days in milk were 6.1 times more likely to develop displaced abomasum than cows diagnosed with HYK after the 1st week. Furthermore, several authors highlighted that the risk of HYK occurrence (Berge and Vertenten, 2014; Kaufman *et al*., 2016) or being culled after its detection (Roberts *et al*., 2012) was more likely for multiparous cows, probably due to the greater milk yield and to possible problems experienced during the previous lactation and dry period.

The relationships between early lactation disorders are complex. From the outcomes of reviewed papers, displaced abomasum appears as a result of HYK. Despite this, in the studies of McArt *et al*. (2011 and 2012) some cows developed displaced abomasum before being diagnosed positive for HYK. Displaced abomasum and HYK are both generated by a poor adaptive response to early lactation requirements. Indeed, after calving cows (especially high-producing animals) do not assume the appropriate amount of energy to face the requirements of high production, mainly because the maximum intake capacity is reached 7 to 8 weeks *postpartum*. In addition, since HYK leads to hypoglycaemia in multiparous cows (Ruoff *et al*., 2017), and to reduced rumination time and activity (Duffield *et al*., 2009; Kaufman *et al*., 2016; Stangaferro *et al*., 2016), HYK can be considered as a cause of displaced abomasum.

In a recent study, high levels of blood BHB have been described to be significantly correlated to oxidative stress and liver apoptosis damage (Song *et al*., 2016). Thus, it is reasonable to conclude that the NEB status in early lactation and the physiological stress occurring during HYK have a role in the depression of the immune system. As a consequence, cows with HYK are more likely to be affected by metritis and lameness during the early lactation (Duffield *et al*., 2009; Ospina *et al*., 2010a; Suthar *et al*., 2013; Berge and Vertenten, 2014). Regarding mastitis, controversial results have been reported. Although Berge and Vertenten (2014) found that cows diagnosed with HYK were almost twice as likely to have a mastitis event in the 1st month of lactation compared with healthy cows and Moyes *et al*. (2014) observed that udder inflammation caused an increase of milk BHB

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concentration, Duffield *et al.* (2009) and Suthar *et al.* (2013) did not detect any association between mastitis and HYK.

Milk production

The effects of elevated blood or milk BHB levels on milk yield are controversial. While a decrease of daily milk production between 1% and 18% has been observed in several studies, an increase of daily milk yield from 5% to 11% in hyperketonemic cows has been reported by other authors (Figure 3). Besides, van der Drift *et al.* (2012a) and Chandler *et al.* (2018) did not report significant differences between cows with or without HYK. Generally, HYK affects more negatively milk production of multiparous than primiparous cows (Ospina *et al.*, 2010b; Chapinal *et al.*, 2012b; Kayano and Kataoka, 2015; Santschi *et al.*, 2016), which is reasonable because first lactation cows do not have NEB status of the previous lactation as potential risk factor, and on average they have better body condition and yield less milk than multiparous animals. However, Rathbun *et al.* (2017) reported that the onset of HYK is not related to milk yield in previous lactation or to genetic potential for milk production. They suggested that HYK in high-producing cows is indeed due to energy requirements of current lactation. Further research is needed to confirm this hypothesis which seems contradictory to the results observed for primiparous and multiparous cows. The negative impact of HYK on milk yield is more pronounced when detected in the 1st week rather than in the 2nd week of lactation, even if cows show the same blood BHB concentration (Duffield *et al.*, 2009; Chapinal *et al.*, 2012a; McArt *et al.*, 2012). The difference of milk yield between cows with HYK and without HYK increases during lactation (Kayano and Kataoka, 2015; Santschi *et al.*, 2016), probably due to the cumulative NEB in hyperketonemic cows.

Milk composition

Hyperketonemia has been associated with greater milk fat content, fat-to-protein ratio (F : P) and energy-corrected milk and lower protein, lactose and urea nitrogen in milk (Table 4; Kayano and Katatoka, 2015). An increment in fat content between 2.4% (Vanholder *et al.*, 2015) and 23.9% (Santschi *et al.*, 2016) has been reported in hyperketonemic compared with healthy cows. Generally, greater differences of fat content between hyperketonemic and healthy cows have been observed in very early lactation (Koeck *et al.*, 2014; Rathbun et al., 2017). However, a greater increment of fat percentage for cows with HYK in the second rather than in the 1st week of lactation was reported in the study of Duffield *et al.* (2009). This discrepancy could be related to the fact that Duffield *et al.* (2009) defined a greater BHB concentration threshold for the 2nd week (2 mmol/l) than for the 1st week of lactation (1.2 mmol/l). Moreover, while Santschi *et al.* (2016) reported that differences in milk fat content between hyperketonemic and healthy cows increased with parity, no significant differences were observed by Chandler *et al.* (2018). Hyperketonemia negatively affects milk protein content; hyperketonemic animals produced milk with 0.3% (Santschi *et al.*, 2016) to 11.6% (Chandler *et al.*, 2018) less protein compared with healthy animals. Moreover, the greatest differences between hyperketonemic and healthy cows were detected in primiparous animals (Santschi *et al.*, 2016; Chandler *et al.*, 2018) and, when the week effect was considered, in the 2nd week of lactation (Rathbun *et al.*, 2017).

The F : P has been reported to be 10% to 32.8% higher in hyperketonemic than in healthy animals (Chandler *et al.*, 2018). As it has been indicated for protein, those differences were greater for primiparous than multiparous cows. In addition, F : P and fatty acids (FA) have been proposed as indicators of HYK in early lactation (van Haelst *et al.*, 2008; Mann *et al.*, 2016). A significant decrease of several *de novo* (C6:0, C8:0, C10:0, C12:0, C14:0) and a medium-chain (C15:0) FA has been observed in milk of cows with HYK (Mann *et al.*, 2016). Moreover, Chandler *et al.* (2018) reported increased concentrations of long chain as well as total unsaturated and trans-FA in hyperketonemic Jersey cows. In both studies, monounsaturated FA increased with HYK. The decrease of the synthesis of *de novo* FA in milk might suggest a less metabolically active mammary gland, while the increment of long-chain FA and total unsaturated FA could be related to a greater acidogenic ruminal fermentation due to lower dry matter intake and higher passage rate. Overall, the association between milk FA and elevated concentrations of BHB needs further investigation.

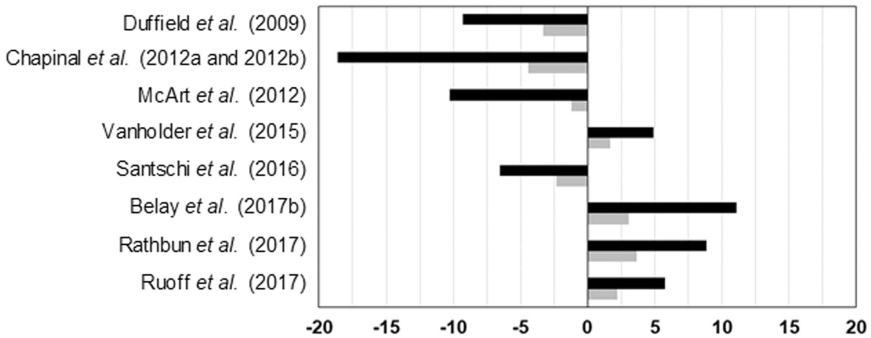

Figure 3 Greatest significant differences between normal and hyperketonemic cows for daily milk yield. Black bars express milk in %/day per cow and grey bars express milk in kg/day per cow. Negative and positive values indicate lower and higher values in hyperketonemic cows, respectively.

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Table 4 *Significant differences between normal and hyperketonemic cows for milk composition*[1]

References	Fat (%)	Protein (%)	F : P	Lactose (%)	MUN (mg/dl)	SCC	ECM (kg/day)
Duffield *et al.* (2009)[2]	+0.22 to +0.48	−0.09	–	–	–	–	–
van der Drift *et al.* (2012a)	+0.66	−0.11	+0.26	–	–	–	–
Koeck *et al.* (2014)[2,3]	–	–	0 to +0.33	–	–	–	–
Vanholder *et al.* (2015)[4]	+0.10 to +0.31	−0.10 to −0.22	–	–	–	–	–
Santschi *et al.* (2016)[3,5]	+0.46 to +0.98	−0.01 to −0.10	+0.17 to +0.33	−0.05 to −0.17	−0.50 to −1.70	+55 to +184[6]	+0.60 to +2.10
Belay *et al.* (2017b)[2]	+0.40 to +0.50	−0.13 to −0.15	–	−0.03 to −0.06	–	–	–
Rathbun *et al.* (2017)[2]	+0.25 to +0.36	−0.16 to −0.24	–	–	–	−0.06[7]	+3.29 to +5.51
Chandler *et al.* (2018)[5]	+0.59	−0.12 to −0.39	+0.12 to +0.43	–	–	–	–

Negative and positive values indicate lower and higher values in hyperketonemic cows, respectively. Values are mean, or minimum and maximum.
[1]F : P = fat-to-protein ratio; MUN = milk urea nitrogen; SCC = somatic cell count; ECM = energy-corrected milk calculated as in National Research Council (2001).
[2]Values represent differences between days or weeks of lactation.
[3]Values represent differences between suspect or positive cows for hyperketonemia.
[4]Values represent differences between subclinical and clinical ketosis.
[5]Values represent differences between parities.
[6]SCC expressed as SCC × 10^3/ml.
[7]SCC expressed as $\log_{10}$ of SCC.

High-producing cows suffer a pronounced lipomobilisation in early lactation, concurrently with low serum concentrations of glucose, total proteins and urea which could explain the results of Table 4. Hyperketonemic cows had from 4.6% to 16.6% less milk urea nitrogen compared with healthy animals. The greatest decrease between hyperketonemic and healthy cows has been observed in multiparous cows (Santschi *et al.*, 2016). The decrease of milk urea nitrogen could be related to the reduced feed intake, the oxidative stress and the liver apoptosis damage in cows affected by HYK, which leads to a low dietary protein availability and a lower protein biosynthesis in the liver, respectively (Duffield *et al.*, 2009; Song *et al.*, 2016). Energy-corrected milk has been reported to increase from 2% (Santschi *et al.*, 2016) to 12.6% (Rathbun *et al.*, 2017) in hyperketonemic compared with healthy cows. Moreover, the greatest differences in energy-corrected milk between hyperketonemic and healthy cows were observed in the 1st week of lactation (Rathbun *et al.*, 2017) and in pluriparous cows (Santschi *et al.*, 2016). The increase of energy-corrected milk in cows with HYK within the 1st month of lactation is mostly influenced by elevated fat percentage (Santschi *et al.*, 2016; Rathbun *et al.*, 2017) and in some cases by greater milk yield (Rathbun *et al.*, 2017). Regarding lactose content, the inverse relationship between circulating BHB and glucose at metabolic level reduces the availability of this fundamental precursor for lactose synthesis in epithelial cells of the mammary gland. Thus, for lactose content a reduction between 0.6% (Belay *et al.*, 2017b) and 3.7% (Santschi et al., 2016) has been reported in hyperketonemic compared with healthy cows. As indicated for the other traits, the difference in lactose concentration between hyperketonemic and healthy cows was greater in primiparous than pluriparous cows (Santschi *et al.*, 2016). As hyperketonemic cows have higher incidence of clinical mastitis compared with healthy cows (Berge and Vertenten, 2014), greater somatic cell count is expected in hyperketonemic animals. Although results of Santschi *et al.* (2016), who reported a 61.3% increment of mastitis incidence in hyperketonemic multiparous cows compared with healthy animals, supported this hypothesis, HYK and somatic cell count were uncorrelated in Vanholder *et al.* (2015) and Chandler *et al.* (2018), and were negatively associated in Rathbun *et al.* (2017), who observed that the incidence of mastitis decreased by 3.2% in hyperketonemic compared with healthy cows. In addition, milk acetone concentration has shown a coefficient of correlation with blood BHB between 0.50 and 0.79, and thus it has been proposed as an additional milk indicator of HYK, similarly to milk BHB (van der Drift *et al.*, 2012a and 2012b; Chandler *et al.*, 2018).

Reproductive performance

The cow has to be in positive energy balance to fully express oestrus behaviour and become pregnant (Rutherford *et al.*, 2016). All reviewed studies on the effect of HYK on reproductive performance dealt with HYK diagnosed with the analysis of blood BHB (Table 5). Animals with elevated blood BHB in the first 2 weeks after calving had lower pregnancy success at first artificial insemination than healthy cows (OR = 0.47, $P = 0.003$; Walsh *et al.*, 2007), whereas no effects were observed by Chapinal *et al.* (2012a) and McArt *et al.* (2012). However, a decrease of pregnancy success within 70 days post-voluntary waiting period with a hazard ratio of 0.87 ($P = 0.10$) was reported by Ospina *et al.* (2010b). Moreover, greater number of inseminations per pregnancy (2.8 *v.* 2.0, respectively; $P < 0.05$), lower peak activity (35% less activity), shorter activity at oestrus (14% less hours) and longer interval from calving to first observed oestrus in HYK than healthy cows were observed by Rutherford *et al.* (2016), who reported also prolonged days open for multiparous cows.

Genetic aspects

In recent years, genetic investigations on BHB have become more relevant, leading to an increased number of papers on this topic (Figure 1). Several authors described blood and milk BHB as heritable traits, with estimates that ranged from

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0.09 to 0.37 and 0.04 to 0.29, respectively (Table 6). On average, estimates of heritability of both traits increased during lactation (Koeck *et al*., 2014; Lee *et al*., 2016; Belay *et al*., 2017b), probably because the environmental and residual factors play a stronger role in early rather than in mid or late lactation. Some authors (Penasa *et al*., 2015; Jamrozik *et al*., 2016) observed that heritability of milk BHB decreased with increasing parity. On the other hand, an increase of heritability from first to second parity, and a slight decrease in third parity have been reported by Lee *et al*. (2016).

Heritability estimates of blood and milk BHB were greater than estimates of CK assessed by Koeck *et al*. (2014), Jamrozik *et al*. (2016) and Belay *et al*. (2017b) using linear animal models (Table 7); this could depend not only on the less variability of CK, which is a dichotomous variable (presence/absence of disease), but also on the possible discrepancy of health data, which were recorded by more than one observer in the studies of Koeck *et al*. (2014), Jamrozik *et al*. (2016) and Belay *et al*. (2017b). The greater heritability of blood and milk BHB compared with CK, coupled with positive moderate to strong genetic correlations between these traits suggest that BHB is a useful indicator to select against ketosis (Koeck *et al*., 2014; Jamrozik *et al*., 2016; Belay *et al*., 2017b). Although BHB has been reported as a relatively good genetic indicator for metabolic disorders, it did not exhibit any potential as a predictor of fertility problems (Table 7; Jamrozik *et al*., 2016).

Considering genetic correlations of BHB with milk yield and composition traits (Table 7), some controversial results have been reported in studies that considered the early (van der Drift *et al*., 2012b; Koeck *et al*., 2014; Jamrozik *et al*., 2016) or entire lactation (Penasa *et al*., 2015) rather than mid lactation (Buitenhuis *et al*., 2013). Weak to moderate positive (Buitenhuis *et al*., 2013; Belay *et al*., 2017b) and negative (Penasa *et al*., 2015) relationships were observed between milk or blood BHB and milk yield. Negative genetic associations with milk protein, lactose and urea content were generally consistent in literature (Buitenhuis *et al*., 2013; Belay *et al*., 2017b), and a positive genetic correlation with milk fat percentage has been reported by Belay *et al*. (2017b), probably due to the larger fat mobilisation required in early lactation by selecting for high milk production. A general consensus in the literature described milk BHB and F : P as positively genetically correlated (Koeck *et al*., 2014; Penasa *et al*., 2015; Jamrozik *et al*., 2016) and blood or milk BHB to be strongly positively correlated with acetone (van der Drift *et al*., 2012b).

Table 6 *Heritability of blood and milk β-hydroxybutyrate (BHB) in dairy cattle*

References	Breed	Blood BHB	Milk BHB
van der Drift *et al*. (2012b)	Holstein	0.17	0.16
Koeck *et al*. (2014)	Holstein		0.14 to 0.29[1]
Penasa *et al*. (2015)	Holstein		0.08 to 0.14[2]
Jamrozik *et al*. (2016)	Holstein		0.07 to 0.13[2]
Lee *et al*. (2016)	Holstein		0.04 to 0.17[1,2]
Belay *et al*. (2017b)	Norwegian Red	0.25 to 0.37[1]	
Weigel *et al*. (2017)	Holstein	0.09	

[1]Values in different stages of lactation.
[2]Values in different parities.

On average, genetic correlations of BHB with diseases and milk production or composition traits were stronger in early lactation (Penasa *et al*., 2015; Belay *et al*., 2017b) and for primiparous cows (Penasa *et al*., 2015; Jamrozik *et al*., 2016) compared with later stages of lactation and parity orders. Nevertheless, a clear explanation for the stronger correlations in early lactation and primiparous cows has not been provided yet.

Genetic perspectives for analysis of HYK and ketosis have been emerging in recent years. For instance, Weigel *et al*. (2017) reported that the incorporation of genomic data to pedigree-based analyses enhanced estimates of HYK heritability, breeding values and predicted phenotypes. Parker Gaddis *et al*. (2018) observed that susceptibility to ketosis in Jerseys was affected by numerous regions along the genome, involving genes related with several pathways as immune system, insulin regulation and lipid metabolism. However, in this study ketosis events were retrieved from a voluntary producer-recorded database which increases the error of diagnosis because of the several producers involved and the declarations collected on a voluntary basis.

Economic aspects

Recent studies have been conducted to evaluate the economic impact of HYK in the dairy herd. The HYK cost in European countries has been evaluated using a stochastic model with distribution laws as input parameters (Raboisson *et al*., 2015) and a dynamic stochastic simulation model (Mostert *et al*., 2018). The economic impact of HYK in United States has been assessed using a deterministic model (McArt *et al*., 2015; Liang *et al*., 2017), even if in the deterministic

Table 5 *Associations between hyperketonemia and reproductive performance in dairy cattle*

References	Days open	Successful to first insemination	Oestrus duration	Oestrus activity
Walsh *et al*. (2007)	Prolonged	Reduced	–	–
Ospina *et al*. (2010b)	–	Reduced	–	–
Chapinal *et al*. (2012a)	–	No difference	–	–
McArt *et al*. (2012)	No difference	No difference	–	–
Rutherford *et al*. (2016)	Prolonged	Reduced	Reduced	Reduced

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Table 7 *Heritability and genetic correlations (r_g) of early lactation diseases, milk yield, fat, protein and lactose percentages, fat-to-protein ratio (F : P), and acetone with blood and milk β-hydroxybutyrate (BHB) in dairy cattle*

Traits	Heritability	r_g with blood BHB	r_g with milk BHB	References
Disease				
Clinical ketosis	0.02	–	0.48	Koeck *et al.* (2014)
	0.02 to 0.04	–	0.25 to 0.63	Jamrozik *et al.* (2016)[2]
	0.08	0.18 to 0.47	–	Belay *et al.* (2017b)[3]
Displaced abomasum	0.04	–	0.07	Koeck *et al.* (2014)
	0.02 to 0.06	–	0.05 to 0.36	Jamrozik *et al.* (2016)[2]
Metritis	0.02	–	0.09 to 0.37	Jamrozik *et al.* (2016)[2]
Retained placenta	0.02 to 0.03	–	0.12 to 0.16	Jamrozik *et al.* (2016)[2]
Milk trait				
Milk yield (kg/day)	0.31	–	0.45	Buitenhuis *et al.* (2013)
	–	–	−0.21 to −0.09	Penasa *et al.* (2015)[2]
	0.16 to 0.23	0.05 to 0.19/−0.03 to 0.28[1]	–	Belay *et al.* (2017b)[3]
Fat (%)	0.39	–	−0.94	Buitenhuis *et al.* (2013)
	0.10 to 0.17	−0.01 to 0.17/0.03 to 0.08[1]	–	Belay *et al.* (2017b)[3]
Protein (%)	0.27 to 0.44	−0.28 to −0.23/−0.36 to −0.22[1]	–	Belay *et al.* (2017b)[3]
F : P	0.12	–	0.49	Koeck *et al.* (2014)
	–	–	0.28 to 0.33	Penasa *et al.* (2015)[2]
	0.10 to 0.16	–	0.15 to 0.49	Jamrozik *et al.* (2016)[2]
Lactose (%)	0.41 to 0.46	−0.23 to −0.15/−0.19 to −0.16[1]	–	Belay *et al.* (2017b)[3]
Acetone (mmol/l)	0.10	0.52	0.90	van der Drift *et al.* (2012b)

[1]Correlations within and across stages of lactation.
[2]Values represent differences between parities.
[3]Values represent differences between stages of lactation.

model of Liang *et al.* (2017) several variables were modelled stochastically. While McArt *et al.* (2015) calculated the cost for HYK with blood BHB concentration ⩾1.2 mmol/l, Liang *et al.* (2017) did not clearly define the diagnostic method.

All the studies observed that the cost of HYK, which can be increased twice considering diseases related to HYK (mastitis, metritis, displaced abomasum, lameness and CK), is mainly due to impaired reproductive performance and milk loss. Despite the variation of prices (e.g. milk, feed, replacement and slaughter prices) on the market of each region and year, observed results followed the same trend. The total average cost of HYK has been estimated between $77 (Liang *et al.*, 2017) and $289 (McArt *et al.*, 2015) per case and year in United States, and between €130 (Mostert *et al.*, 2018) and €257 (Raboisson *et al.*, 2015) per case and year in Europe. In general, the cost is at least twice greater in multiparous than primiparous cows (Liang *et al.*, 2017; Mostert *et al.*, 2018). However, a higher cost for primiparous ($374) than multiparous animals ($256) has been reported by McArt *et al.* (2015). Although cost distribution was difficult to compare among studies due to the different variables considered in each formula, McArt *et al.* (2015) and Mostert *et al.* (2018) clearly reported that the most important cost was related to impaired reproductive performance (34% to 36%) and milk production loss (24% to 26%). Interestingly, the cost of the milk that was discarded following the treatment of HYK-related diseases (14%) has been additionally considered by Mostert *et al.* (2018). Most of HYK total costs (80%) were attributable to several HYK-related diseases (e.g. displaced abomasum, lameness, clinical mastitis and metritis) and consequently early culling, allocating less relative importance to milk production loss (11%) and prolonged days open (9%) in Raboisson *et al.* (2015). On the other hand, Liang *et al.* (2017) assigned most of the total costs to veterinary interventions and treatments (68%) or to extended days open (47%) for primiparous or multiparous cows, respectively.

Conclusions and perspectives

The present review summarised the major impacts of elevated blood or milk BHB concentrations on productive, fertility and economic aspects in early lactation dairy cows. A general consensus defined HYK as cause of increased risk of health problems during early lactation, with consequent negative effects on herd profitability. Nevertheless, controversial results have been observed for milk production and somatic cell count. The associations between BHB concentrations and milk yield are still not well defined both from a phenotypic and genetic point of view, and further studies are necessary to better understand the mechanisms underlying these relationships. Although the reviewed literature is consistent in reporting that elevated blood BHB concentration is detrimental to reproductive performance, Ospina *et al.* (2010a and 2010b) highlighted that NEFA concentration is a stronger predictor of fertility depletion than BHB. A debate about the most convenient indicator concerns also milk

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composition traits, for which routinely predicted traits as acetone, F : P and FA profile have been assuming increasing importance. Moreover, the interest of using multiple measurable indicators to determine HYK has been emerging because until now most papers established HYK on the basis of a single predictor.

Therefore, the following relevant points deserve further investigations:

(i) Identification and validation of trustworthy methods to discriminate between cows affected by HYK and healthy animals in field conditions. Although the determination of blood BHB concentration is the most common method to identify HYK, it is not a useful tool in field conditions both from the economic and animal welfare point of view. The possibility to predict BHB in milk using MIRS is currently the most concrete and feasible way to collect phenotypes at population level and some studies have demonstrated that this approach is useful for monitoring HYK in dairy cows. Nevertheless, MIRS does not allow to collect detailed individual milk BHB concentrations with enough accuracy; this issue has been widely discussed in several papers and it is one of the main topics that lead researches on MIRS and metabolic-related indicators. The combination of several milk test-day predicted traits (e.g. BHB, acetone, F : P, FA) and performance variables has been proposed as an interesting strategy to predict HYK (Chandler *et al*., 2018). Another approach that has been recently suggested is the use of BHB concentration in blood rather than in milk as reference method to calibrate MIRS devices (Belay *et al*., 2017b). The possibility to predict the metabolic profile of cow by MIRS is a great challenge for the dairy sector.

(ii) Some of the controversial results highlighted in this review between the effect of elevated BHB concentration and health, milk production and composition, and reproductive performance, as well as HYK economic cost and genetic aspects could be related to data quality. Considering that ketosis is a complex metabolic disease, the diagnosis by using a single cut-off could be not enough to correctly discriminate between healthy and hyperketonemic animals, or to properly separate between SCK and CK. Further research should focus on BHB variability within and between cows, in order to provide essential information for the development of a more accurate diagnosis method. Moreover, when dealing with health events, results could vary if they come from a voluntary declaration, if the declaration comes from the farmers or the veterinarians, or if the health event is registered by only one person or different persons. In addition, further investigation using ordinal or multinomial logistic regression to assess the relation between HYK (or CK) and various predictors is needed. A more detailed review focussed on data quality could help to better understand how the quality of recorded data may affect the impact of HYK on cow's health and performance.

(iii) Quantification of phenotypic and genetic variation of HYK in different breeds and environmental conditions. This issue is very relevant for scientists and technicians, and the possibility of recording BHB concentration or other predicted traits at population level, as mentioned above, would help investigate this topic. Several impacts of HYK on cow performance are controversial or not quantified yet, and the difficulties to have large and accurate data in the 1st days after calving is one of the main concerns for future research. Moreover, the possibility of combining metabolic information of dairy cows in early lactation with milk production and composition will support farmers to achieve an early detection of metabolic problems minimising the economic losses.

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Declaration of interest

The authors declare that they have no conflicts of interest.

Ethics statement

No ethical concerns.

Software and data repository resources

None of the data were deposited in an official repository.

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