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REVIEW

Interaction between metabolic challenges and productivity in high yielding dairy cows

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Abstract

The onset of lactation in modern dairy cows is characterized by a negative energy balance, due to a drastic increase in energy requirements for milk yield and a simultaneous depression in dry matter intake around parturition. Prioritization of milk yield over maternal body functions is a universal biological strategy in all lactating mammals to buffer the newborn's nutrition from fluctuations in the dam's energetic status. Consequently, in case of an energy deficiency, the dam will mobilize fat and protein reserves in order to safeguard milk yield. During decades of one-sided selection for milk yield, man has exploited the cow's potential to prioritize mammary energy supply without an equivalent progress in dry matter intake capacity. Consequently, genetic selection for milk yield has widened the gap between energy expenditure and energy intake, and has increased the cow's inclination to respond to energy deficiencies in the transition period by aggressive body tissue breakdown. Chronically elevated concentrations of non-esterified fatty acids and ketone bodies have been demonstrated to affect multiple organ systems including the immune system, the reproductive axis and the liver and are, in contrast to absolute milk yield, closely and consistently related to the final incidence of reproductive disorders.

Key Words: dairy cow, metabolism, production

Introduction

Despite constant progress in the understanding of dairy cow physiology and metabolism, the economical and environmental benefit of high milk yield is all too often overshadowed by a high disease incidence and culling rate. The vast majority of health disorders occur during the immediate postpartum period and most of these have a significantly negative effect on productivity and profitability as diseases mainly occur during the time peak production is to be expected. In the present paper, the adaptation mechanisms of the modern dairy cow to the demands of high peak

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yields in the period after calving are reviewed in relation to the pathogenesis of ketosis. The impact of ketosis as a gateway-disease on health, productivity and fertility is discussed, as well as the major risk factors predisposing cows to develop this condition. Finally, an overview is given of tools to diagnose and monitor ketosis at cow and herd level including the ketone threshold levels that are applied to distinguish between 'normal' and 'hyperketonemic' cows.

How do modern cows succeed to produce the high amounts of milk?

Intensive genetic selection together with considerable improvements in dairy cow nutrition, housing and management have led to significant increases in average milk yield, with no signs that limits of production capacity or biological efficiency have been reached. The question may be asked whether production records represent a metabolic or physical ceiling. Based on genetic trends it is highly likely that current production records will someday be broken again, whilst the average yield of 'normal' cows will catch up with the old record holders in the intervening period. It is even more challenging to ask how these increases in milk yield have been achieved. Are 'modern' high yielding dairy cows metabolically different from their contemporaries living some decades ago?

It is clear that selection for greater milk yield has changed the intermediary metabolism of the current dairy cow substantially, resulting in the production of large quantities of milk and this for an extended period of time. Energetically, increases in milk yield can only be achieved in one of three ways. Firstly, the high yielder may simply consume, and/or acquire more metabolisable energy (ME) from her feed. Secondly, she may be more efficient in partitioning ME from her feed for milk production. And finally, she may mobilise more body energy stores to support milk yield. It is clear that copious milk production requires a copious supply of nutrients for milk synthesis, and thus nutrient requirements, appetite, and feed intake capacity have increased in tandem with increases in milk yield. In a number of comparisons of production data from low- and high-yielding genetic lines of dairy cows (Veerkamp *et al.,* 1995; Buckley *et al.,* 2000), higher yielders consume more feed, which is in part a consequence of greater appetite and selection for physical and perhaps physiological characteristics which may allow more feed intake. There is no evidence to suggest that high yielding cows obtain more nutrients for milk synthesis by digesting their diets more efficiently. To the contrary, one concern with higher feed intake is the potential for a depression in digestibility arising from higher rates of digesta passage through the gastrointestinal tract (Van Soest, 1994).

The effect of high milk yield on energy partition is furthermore exemplified by the loss of body reserves, mainly in the form of adipose tissue, in early lactation. The latter is seen as a hallmark of the rising phase of lactation in modern dairy cows when increases in milk energy output outpace increases in ME inputs, with a consequent mobilisation of body fat reserves to balance the deficit. The extent of the loss of adipose tissue varies with the magnitude and rate of increase of milk yield compared to ME intake, and thus can be exacerbated if metabolic conditions, disease or management practices impair nutrient intake. As total milk yield is proportional to peak milk yield, higher yielding cows have higher peak yields, and thus are presumed to be subject to more extensive body energy mobilisation in early lactation. Hence, it may further be questioned whether modern dairy cows lose more body reserves than their less producing predecessors under similar environmental conditions. This was covered in a study (Crooker *et al.,* 2001) in which it has been shown that if cows managed to achieve adequate ME intakes, higher milk yields of modern dairy cows do not necessarily require more mobilisation

of tissue energy and loss of body condition than occurs in lower yielding dairy cows.

Hence, a modern high yielding dairy cow seems to tolerate a certain discrepancy between in- and outputs during early lactation, and succeeds to do so by mobilizing body reserves. So, instead of simply reducing her lactational output to match available inputs- as a cow with no body reserves would have to do- modern dairy cows obviously give priority to the production of milk compared to maintaining body condition. Given that cows do have body reserves, then, when they are compromised by the nutritional environment, they clearly place higher priority on maintaining lactation than on maintaining their body reserves during early lactation (Friggens, 2003). But also when not compromised by the nutritional environment, they appear to have priorities for body tissue mobilisation (Ingvartsen *et al.,* 2003). This means that the body energy change we observe, including the one which occurs in early lactation, has both genetically driven and environmentally driven components. If we are to correctly interpret body mobilisation, we must not neglect the priorities of the animal. 'Genetically' driven body energy change is defined as that which occurs in cows kept in an environment that imposes no constraints in anyway. It then follows that 'environmentally' driven body energy change is defined as that which occurs in response to an environment that is constraining *e.g.*, an insufficient supply of nutrients. Excessive body fat mobilisation will arise in situations where environmental limitations force the cow to deplete reserves faster than (genetically) planned in order to maintain desired levels of milk production (Ingvartsen *et al.,* 2003). The latter especially occurs in cases where insufficient glucose precursors are available to sustain the genetically feasible production level.

In this respect it is important to mention that high milk production is accompanied by obvious alterations in the peripheral concentrations of 'milk driving' hormones (Bonczek *et al.,* 1988). The latter not only stimulate milk driving processes such as lipolysis and gluconeogenesis, but also regulate the uptake and utilisation of metabolites both by the udder as well as by the rest of the body tissues in overall favour of milk production. The latter is well described as the adaptation of the intermediary metabolism towards lactation (Herdt, 2000). This adaptational process of the cow, generally referred to as 'homeorhesis', is furthermore reflected by a broad range of changes of metabolites which can be measured in the blood of the cow. Among these metabolites, non-esterified fatty acids (NEFAs) and ketone bodies definitely are located in 'pole position'. Essentially, peripheral NEFA-levels reflect the breakdown of body fat reserves, while peripheral levels of ketone bodies mirror the capacity of the liver to handle the high NEFA supply (Herdt, 2000). Elevated ketone concentrations 'visualize' the incapacity of the liver to handle the overwhelming flux of NEFA. A certain amount of mobilisation of body reserves and as a consequence the formation of ketones in early lactation, should therefore be considered as a normal physiological response of the modern dairy cow.

Based on the above it is clear that during early lactation the demand of the mammary gland for energy often exceeds the amount of available energy derived from the feed, resulting in negative energy balance (NEB). During the period of NEB, the cow mobilizes her fat reserves (*i.e.* adipose tissue), as the difference between the cow's outputs and inputs, *i.e.* her metabolic (work)load, has to be met from body reserves. Therefore, mobilisation of reserves quantifies metabolic load, and extreme mobilistation is indicative of extreme metabolic load, *i.e.* metabolic stress. Modern dairy cows however do possess an adaptation system to cope with this metabolic imbalance. Adaptation is a gradual process which differs between animals. It is obvious that the majority of cows will in the long run reach the adapted stage, and a rigid final classification of cows into adapted and non adapted at a certain point in time is therefore meaningless. Providing

signals or indicators that can inform us about the degree of adaptation may contribute to a solution for this dilemma. Such signals may refer to the biochemical, endocrinological, subclinical and clinical characteristics of the cow. Moreover, these signals can be measured not only at the time of interest, but also during several days or even weeks before this time point. So far, no single specific indicator is known that adequately informs about the degree of adaptation and due to the multifactorial aspect of adaptation, it is unlikely that such a clear and obvious signal will ever be found (Jorritsma *et al.,* 2003). It should be clear, however, that a more extensive challenge, for example a high level of milk production, requires a more comprehensive adaptation and is at a greater risk of failure.

As the paramount proportion of the cow's energy throughput is going to the production of milk, other processes like reproduction and immunity, get a lower priority. Because most of these other processes require energy, their efficiency is lowered during the critical period around the moment of calving and during early lactation. Indeed, at peak lactation the nutrient needs of the mammary gland greatly exceed those of the rest of the cow's body. While the mobilisation of body reserves makes a substantial contribution to the rapid acceleration in milk production at the start of lactation, it is interesting to note that the time-course of energy mobilisation reflects the temporal pattern of disease incidence more closely than patterns of other performance rates, such as feed intake and milk yield. Hence, because of this marked coincidence of peak energy mobilisation with an elevated incidence of diseases, the most prevalent diseases occurring during this time lapse are often referred to as 'energy related diseases'. However, a higher incidence of these diseases among high producers is clearly not inevitable, because we all are aware of the existence of very high yielding herds with rather low incidences of these 'energy related diseases'.

Ketosis: definition and pathogenesis

Ketosis is a disease associated with NEB and characterized by abnormally elevated concentrations of the ketone bodies acetoacetic acid (AcAc), acetone (Ac), and beta-hydroxybutyric acid (BHBA) in the body tissues and fluids. The clinical signs of ketosis tend to be variable, subtle and nonspecific. These clinical signs include reduced appetite, excess loss of body weight, reduced milk yield, ketone odour in the breath or milk, hard or dry faeces, reduced activity, and in severe cases a variety of nervous signs. Furthermore, these clinical signs, though present, are usually difficult to observe under practical conditions (*e.g.* in cases of housing in free stall conditions where individual feed intake cannot be measured).

Ketosis is classified as clinical or subclinical on the basis of levels of ketone bodies in the blood, urine, and milk and the presence or absence of observed clinical signs. Furthermore, any disease process occurring in early lactation that reduces feed intake may cause secondary ketosis. Here, the decline in serum glucose concentration is caused by an insufficient dry matter intake caused by the primary health disorder.

In contrast to the difficulties of observing clinical ketosis, subclinical ketosis can be defined quite objectively and can hence be reliably measured across herds. A working definition of subclinical ketosis is 'a condition marked by increased levels of circulating ketone bodies without the presence of clinical signs of ketosis' (Duffield, 2000). In order to avoid the difficulties in differentiating and defining clinical and subclinical ketosis, for the reasons described above, also the terminology 'hyperketonemia' may be used (Duffield, 2000). This term objectively refers to the elevated concentrations of blood ketones and includes both the clinical as well as the subclinical condition irrespective of whether clinical signs were observed or not.

Ketosis is caused by an insufficient supply of

blood glucose to support metabolic needs associated with the sudden onset of high milk secretion after calving when feed intake is not in accordance with production. Generally, both circulating concentrations of NEFA and BHBA are stated to measure aspects of the success of adaptation to negative energy balance (LeBlanc, 2010). The concentration of NEFA reflects the magnitude of mobilization of fat from storage and hence indirectly mirrors dry matter intake (DMI). The level of BHBA however reflects the completeness of oxidation of fat in the liver. In the dairy cow, the majority of glucose is produced in the liver via the tricarboxylic acid cycle, and originates mainly from propionic acid produced by the rumen microflora. When insufficient glucose precursors are available, there is a lack of substrate to adequately process the mobilised fat resulting in incomplete oxidation of the mobilized body fat, and excessive production of ketone bodies (Herdt, 2000).

As the supply of NEFA to the liver exceeds the ability of the liver to completely oxidize the fatty acids to supply energy, the amount of ketone production increases. Ketones can be used as an energy source by many tissues and ketone utilization by cells can hence be seen as a normal part of dairy cow metabolism. In fact, individual capacity to handle elevated ketones may vary from cow to cow. Despite this, when their production exceeds their use, accumulation will occur leading to elevated blood ketone levels and these elevations are recognized as reasonable surrogate indicators of impaired energy metabolism (Duffield *et al.*, 2009). Furthermore, a lack of blood glucose induces a decline in plasma insulin, which in turn causes an increase in fat mobilisation from adipose tissue, thereby further aggravating the condition. Although glucose is the primary metabolic fuel and is absolutely required for vital organ function, fetal growth and milk production, its concentration is under tight homeostatic control and therefore a poor analyte for monitoring or investigating herd problems (Herdt, 2000b).

Ketosis: a gateway disease with major effects on general health, production and reproduction

Ketosis can be considered a gateway disease during the postpartum period, since its presence significantly increases the risk of other diseases to occur as well. Several international publications have shown that hyperketonemia or subclinical ketosis (SCK) increase the risk for metritis (Hammon *et al.*, 2004; Duffield *et al.*, 2009), mastitis (Oltenacu and Ekesbo, 1994; Duffield *et al.*, 1997) abomasal displacement (Geishauser *et al.*, 2000; Leblanc *et al.*, 2005; Duffield *et al.*, 2009), cystic ovaries (Dohoo 1984) and clinical ketosis (CK) (Duffield 2009). In some cases it was mentioned that ketosis not only increases the risk for other diseases to occur, but also increases the severity of the disease *i.e.* ketosis (Kremer *et al.*, 1993).

Impairment of neutrophil function directly after parturition is generally suggested to be one of the major causes of this higher disease incidence. Physiological indicators of NEB (like ketone bodies) contribute to this impairment in immune function as cows with low glucose, and elevated ketone and NEFA concentrations are believed to have a compromised immune function. A negative effect of ketone bodies on neutrophil function has been reported for both sheep and dairy cows (Sartorelli *et al.,* 1999 and 2000; Hoeben *et al.,* 1997 and 2000). The ketone bodies BHBA and AcAc, inhibited the proliferation of hematopoietic cells at concentrations observed after parturition (Hoeben *et al.,* 2000). The latter physiological indicators of NEB, furthermore inhibited the *in vitro* phagocytic activity of neutrophils in a study of Klucinski *et al.* (1988). A negative relation was furthermore found between chemiluminescence response of polymorphonuclear neutrophils and BHBA. All these results support the suggestion that too strong a NEB causes an impairment of neutrophil function. This means that development of infectious diseases like metritis and mastitis during the postpartum period might be attributable to an underlying

defect in the functional ability of circulating neutrophils that may exist already prior to parturition. Additionally, Cai *et al.*, (1994) demonstrated that the production of superoxide ions by the neutrophils from cows with metritis was much lower than that by the neutrophils from healthy parturient cows, and similarly that the chemotaxis of the neutrophils from cows with mastitis was much weaker than that of the neutrophils from normal cows. Also Detilleux *et al.* (1995) demonstrated altered immunologic functions in 137 periparturient dairy cows, and herewith confirmed that the innate immune functions are altered in energetically stressed periparturient cows.

The severity of experimental *E. coli* mastitis in relation to *in vitro* chemotaxis of neutrophils was investigated in cows during NEB induced by feed restriction (Kremer *et al.,* 1993). Typically, the severity of *E. coli* mastitis has been stated to depend more upon the immune response of the patient than of the pathogenicity of the bacterium (Burvenich *et al.,* 2004). The bovine mammary gland has furthermore been reported to be more susceptible to infection and clinical disease during the periparturient period than during the remainder of lactation or during the dry period (Smith *et al.,* 1985). In this context it has also been shown that *E. coli* mastitis may be life threatening in the beginning of the lactation, while it is known as a self curing disease later in lactation. The fact that the severity of clinical *E. coli* mastitis is mainly dependent on cow factors (Burvenich *et al.,* 2004) also implies that searching for a breakthrough in optimizing both the treatment and the prevention of this disease should primarily focus on the cow and not on directly destroying the pathogen. In other words, looking for a stimulation of immunity, or in other words a minimalization of the negative energy balance and circulating levels of ketone bodies, is definitely far more important than searching for more potent antimicrobials.

Conclusively, there is clear evidence to state that in many modern dairy cows ketosis is part of the adaptation process to reach the current production levels (Jorritsma, 2003). Because *in vitro* studies have shown that ketones have a detrimental effect on the leucocyte activity, ketosis probably contributes to impaired immune function and thus accounts for an important proportion of the periparturient diseases as encountered in the modern high yielding dairy cow. Infectious diseases originating in this way further decrease dry matter intake and hence aggravate the NEB, which may cause the animal to end up in a vicious circle leading to a process of non-adaptation and even death or in premature culling. The fact that infectious diseases usually cause a decrease in DMI and hence easily provoke a state of NEB and ketosis in this stage of lactation, explains the generally observed interrelationship of diseases shortly after calving in high yielding dairy cows (Correa *et al.,* 1993). Furthermore, a decrease of DMI shortly after calving obviously reduces rumen fill and causes ketosis to be an important risk factor for the occurrence of displaced abomasums (DA) (van Winden, 2002). The latter has been confirmed by the studies of Geishauser *et al.* (2000), in which it was shown that cows with ketosis were at a significantly increased risk to develop left displacement of the abomasum later on.

Duffield *et al.* (2009) concluded that hyperketonemia in the first week of lactation is an important risk factor for the subsequent diagnosis of DA, CK and metritis, together with a significant milk loss. In their study, the increased health risk and reduced milk production appear to start between a threshold of 1200 to 1400 μM of serum BHBA in the first week following calving. Elevations in serum BHBA during the second week postcalving were associated with an increased risk of DA and CK, decreased first milk yield, but greater lactational milk yield.

The increase in milk production in modern dairy cows over the last decades is known to be accompanied with a decrease in reproductive capacity (Bousquet, 2004). In most of the papers the NEB is referred to as the most important

underlying cause of this fertility decline, and this both in a direct as well as in an indirect way (*e.g.* by causing a higher incidence of metritis). Both the depth as well as the length of the NEB post partum, are stated to be crucial factors in determining the reproductive capacity on a modern dairy herd (Butler, 2003). Although it is not completely clear what the underlying pathophysiology is, an important role of ketone bodies has been suggested (Opsomer, 1999; Reist *et al.,* 2000). Events of ketosis/hyperketonemia during the first weeks after calving significantly increase the risk of an anovulatory condition (Walsh *et al.*, 2007a) and significantly reduce the first insemination conception rate later on (Walsh *et al.*, 2007b). The same authors concluded that both the relative circulating concentration of BHBA and the duration of elevated circulating BHBA were negatively associated with the probability of pregnancy at first service. In addition, ketonemic cows have been shown to have increased odds to develop cystic ovarian disease (Doohoo and Martin, 1984). All these associations postpone the establishment of a new pregnancy post partum (Ospina *et al.*, 2010), leading to a substantial prolongation of the calving interval with its financial consequences. In most European herds where all lactating cows are kept in one and the same group and are fed by total mixed ration or partial mixed ration, a prolongation of the calving interval will often end up in an elevated number of cows suffering from a too high body condition at the moment of drying off and subsequent calving. The latter implies that cows will come into a vicious circle as they will have a significantly higher risk to suffer from ketosis again.

In our own lab, we demonstrated that changes in circulating concentrations of BHBA are promptly reflected in the follicular fluid of recently calved dairy cows (Leroy *et al.,* 2004). Hence, both the granulosa cells as well as the oocyte itself have to live and mature during the first weeks after calving in an environment characterized by elevated concentrations of ketone bodies and NEFAs, in combination with lowered glucose levels. The latter has been suggested to play a decisive role in the pathogenesis of reduced fertility in the modern high yielding dairy cow (Leroy *et al.*, 2006). Additionally, Vanholder *et al.* (2005) demonstrated that BHBA can modulate granulosa cell function at physiological glucose concentrations *in vitro* and hence may be involved in the pathogenesis of the high amount of ovarian dysfunctions in modern postpartal dairy cows (Opsomer *et al.,* 1998). The latter may also be one of the underlying causes of the reduced signs of heat in modern dairy cows (Van Eerdenburgh *et al.*, 1996), as in these NEB conditions at the ovarian level, granulosa cells simply produce less oestrogens.

Diagnostic tools and monitoring programs

Each of the three main ketones, BHBA, AcAc and/or Ac is present in blood, milk and urine and can hence be measured. BHBA is relatively stable in blood samples and although it is well known that it can originate from dietary sources like poorly fermented silage, and that there is some diurnal variation due to feeding, it is generally accepted as the gold standard test for hyperketonemia, (LeBlanc, 2010; Van Saun, 2010). Therefore, the standard test to which other tests are compared, is serum BHBA measured in a diagnostic laboratory (Tyopponen and Kauppinen, 1980). Other ketone bodies are known to be less suitable to be accurately measured. Acetoacetate for example is unstable and volatile as it readily decomposes to acetone and carbon dioxide.

The most commonly used cut of point for subclinical ketosis used in serum is $\geq 1400\ \mu M$ (14.4 mg/dl), while clinical ketosis generally involves much higher serum levels of BHBA (2600 to 3000 μM or more) (Duffield, 2000; Oetzel, 2004). However, other threshold concentrations for defining ketosis have also been used, including 0.9 mM (Sauer *et al.,* 1998), 1.0 mM (Blood and

Radostits, 1989; Lean *et al.,* 1992; Lean, 1994), ≥ 1.2 mM (Duffield *et al.,* 1997; Duffield *et al.,* 1998; Geishauser *et al.,* 1998; Green *et al.,* 1999; Jorritsma *et al.,* 1998, and Enjalabert *et al.,* 2001). Prior to calving, BHBA concentrations generally do not exceed 575–750 μM, unless the animal is in negative energy balance or consuming ketogenic silage (Van Saun, 2010). Practitioners in the field currently mention a very wide variety in symptoms associated with (elevated) serum levels of ketobodies in periparturient dairy cows. Very often, these practitioners at the same time state that modern cows seem to be able to cope better with high serum ketolevels in comparison with their lactating predecessors some decades ago.

In the field, the use of cheap and easy to perform 'cowside' tests has become increasingly popular for both the diagnosis of individual sick cows and the routine monitoring of fresh cow groups. These cowside tests measure either acetoacetate (AcAC) or BHBA in urine, milk or blood. Ketones are excreted in urine, resulting in higher concentrations in urine than in blood and giving rise to elevated levels of false positive reactions when used to find hyperketonemic patients (Nielen *et al.*, 1994). BHBA concentrations in milk reflect the concentration in serum, but are only 10 to 15% as high (Duffield, 2000).

Recently, the Precision Xtra™ meter, using the Precision XtraTM blood ketone test strips has gained a lot of attention. This tool was originally designed for use by human Type I diabetics at risk for ketoacidosis to monitor their blood BHBA concentrations while its use has currently been extended to ketosis diagnosis in cows. It is a small handheld meter that measures the BHBA concentration in a drop of blood that has to be applied to a test strip. Although the procedure is a little more complicated, the accuracy of this cowside test is excellent (sensitivity and specificity both $> 90\%$). Furthermore, very high correlations were found with 'conventional' laboratory BHBA results. Therefore the electronic hand-held BHBA measuring system using whole blood is a useful and practical tool to diagnose subclinical ketosis (Iwersen *et al.*, 2009).

The selection of which test to use, has been the subject of an excellent review (Oetzel, 2004). Basically, in practice this decision is mainly based on cost, availability and sampling ease. An often overlooked but at least as important criterion, is the reason why the test is used. Tests having a high sensitivity are more valuable for diagnosing individual cows for example to confirm a suspected case of clinical ketosis or identify truly subclinically ketotic cows. Unfortunately, these tests will fail to accurately identify a substantial number of subclinically ketotic cows. If the goal is to identify as many subclinically ketotic cows as possible for treatment, such as during routine fresh cow monitoring, tests known to have a high specificity should be preferred in order to avoid treating high numbers of false positives.

Monitoring and prevalence

Testing herds for the prevalence of subclinical ketosis in early lactating cows has become more or less common practice, mainly as part of a monitoring program to monitor the metabolic health of dairy cattle in the postpartum period. Furthermore, clinical impressions of metabolic disease problems can be confirmed by herd-based metabolic testing. Several authors have proposed different protocols for monitoring early lactating cows to test the herd incidence of ketosis. Differences exist in the test used, the number of animals tested, the moment relative to (expected) calving that the animals are tested. Programs to monitor the management of the transition and postpartum periods usually include BHBA determination in the first week after calving (Oetzel, 2004; LeBlanc, 2010).

The number of samples required for group or herd-level interpretation basically depends on 3 factors: the prevalence of affected animals that is judged important to detect, the certainty of detection that is desired (generally known as the

confidence interval), and the size of the group of interest (LeBlanc, 2010). A more or less generally accepted protocol is to test 12 or more cows for blood BHBA concentrations. Mainly based on clinical experience, Oetzel (2004) suggested that if more than 10% of the cows tested have concentrations $\geq$ 1400 μM, the group is considered to have a ketosis problem (Oetzel, 2004). It is indeed important to interpret the test results as the proportion of animals above a meaningful threshold because this best describes the biology of the condition. It is misleading and even statistically incorrect to calculate the average from a group of samples.

Other cut off points have been proposed, but are not very well defined, partly because most of the ketone bodies are unstable. An overview of the proposed threshold levels is given by Fleming (1996). However, critical threshold values reported vary widely and may be dependent upon the method of measurement used. An interesting approach is to define cut off levels using the results of studies that examined the association between serum concentrations and the subsequent incidence of health problems later on. Studies showed that both serum NEFA and BHBA concentrations and this both before as well as shortly after parturition are associated with an elevated risk to suffer from clinical diseases like clinical ketosis, metritis and a displaced abomasum. For example, cows with serum BHBA concentrations of $\geq$ 1.4 mM during the first or second week after calving were respectively 4 and 8 times more at risk to suffer from a displaced abomasum 1 to 3 weeks later (Geishauser *et al.,* 2000). Van Saun (2006) in his study demonstrated that cows having a BHBA concentration $\geq$ 960 μM or $\geq$ 1340 μM between 3 and 21 days after calving have a respectively 2.8 to 4.2 higher risk to suffer from 'any disease'. Based on the tables given by Van Saun (2010) that review the results of recently carried out field studies, it can be concluded that the most appropriate time to measure BHBA levels to estimate the risk to suffer health disorders in the postpartum period is between 1 and 21 days after calving. The determination of serum NEFA levels both pre- (starting from −21 days relative to calving) and post partum (3 to 21 days), may further contribute to indicate cows to be at a higher risk level.

While some stated that a lot of information is lost if samples from multiple animals are pooled (LeBlanc, 2010), others recently proposed herd monitoring strategies using pools of samples, as pooled sample concentrations of BHBA (and NEFA) were very accurate for herd-based detection of subclinical ketosis (Van Saun, 2010; Borchardt and Staufenbiel, 2012). Results of a recent study indicate that the concentrations of NEFA and BHBA in pooled serum samples can be successfully used for herd-based detection of SCK in cows that have recently calved (Borchardt and Staufenbiel, 2012). In this study, a sample size of 10 cows/herd was deemed adequate for a herd based metabolic testing. By use of the pooled sample approach with a sample size of 10, the costs of laboratory analyses can be reduced by 90%. Based on the assumption that a high proportion of cows with high circulating concentrations of NEFA and BHBA after calving indicates an increased risk of disease and impaired production, pooled sample values of NEFA and BHBA from 10 randomly selected apparently healthy cows during the first week after parturition can be regarded as a continuous measure of risk and can provide useful information about the metabolic health of the herd. Authors showed that if SCK for an individual cow is defined by a serum BHBA concentration $>$ 1400 μM, a herd can be classified as positive for SCK (*i.e.*, $>$ 2/10 cows having a BHBA concentration of $>$ 1400 μM) during the first week after parturition with a sensitivity of 86% and a specificity of 85%, when the pooled sample value is $>$ 1180 μM (Borchardt and Staufenbiel, 2012).

Besides the use of pooled serum samples, there are currently some studies going on to test novel and cheap methods to determine the threat

of hyerketonemia in a dairy herd. Using a combination of readily available traits like body condition scoring and the analysis of the milk constituents of individual cows during the first milk recordings is a nice example. In a recent study, researchers found that in SCK cows, elevated proportions of C18: 1 *cis*-9 are visible in the milk fat, making the fatty acid analysis of milk fat an interesting tool for prediction of the disease (Van Haelst *et al.*, 2012). More research is however necessary to further explore the possibilities to use these tools in the field.

The fact that many different tests and criteria for establishing the prevalence of ketosis in a dairy herd are used, makes it difficult to come to a general conclusion regarding the importance of ketosis with regard to its prevalence and incidence. Each study probably defines ketosis a little bit differently, which makes it impossible to compare the incidence and prevalence of ketosis in different settings. Different publications do however indicate that when monitoring of fresh cows is performed, ketosis is still all too common showing prevalence levels varying between 8.9 and 43 % (Dohoo and Martin, 1984; Dirksen *et al.*, 1997; Duffield *et al*, 1997; Duffield *et al.*, 1998; Borchardt and Staufenbiel, 2012). It should furthermore been mentioned that the above stated prevalence numbers are mainly retrieved from cross sectional studies in which the cows were only sampled once at a certain moment in the postpartum period. In a recent study carried out in 16 dairy herds in Iran (Asl *et al.*, 2011), and in which a cut off level of 1200 μM was used, it was found that in total 97% of the tested cows (97/100) were considered positive in at least one of the 3 sampling periods being at respectively week 2, 4 and 6 post parturition. The latter suggests the cumulative lactational incidence to be much higher than generally realized.

Underlying causes and intervention

Elevations of ketone body levels indicate a stressed metabolism, and therefore indirectly point to an adaptation problem during the postpartum period, and especially a failed adaptation towards NEB. As mentioned above, NEB is essentially universal among current dairy cows in the first weeks of lactation. In spite of this, the majority of cows do not develop ketosis, as most cows cope with NEB through an intricate mechanism of metabolic adaptation. Hence, ketosis occurs not because of NEB, but because of the failure of these adaptive mechanisms.

The key factor for a successful adaptation process during the transition/postpartum period is feed intake. Peripartum energy metabolism and immune function will be favoured when cows have unrestricted access to diets formulated to meet nutrient requirements and to water during the transition/postpartum period. There is general consensus nowadays that a constraint in feed intake both at herd as well as at individual cow level, is the main causative factor for ketosis problems to occur in a herd. At herd level, these factors are: lack of cow comfort, overcrowding leading to competition at the feed bunk, heat stress, an unbalanced ration, stress caused by changes in the social groups. In a recent Swedish study, it was concluded that a large herd size, a high maximum daily milk yield in multiparous cows, keeping all dry cows in one group, and not cleaning the feeding platform daily are risk factors for a high herd incidence of DA or CK (Stengärde *et al.*, 2012). At cow level, main reasons for insufficient feed intake during the transition/postpartum period are known to be: body condition score ≥ 3.5, twin pregnancy, any other disease event like lameness, retained placenta, an acute infection (like an acute mastitis or metritis), extended length of the dry period and in first calvers an extended age at first calving.

Pro-active management and investigation of problems should focus on minimizing nutritional,

housing, social, and environmental factors that may impair feed and resting access for all or some members of the groups of peripartum cows (LeBlanc, 2010).

Conclusions

As a conclusion, ketosis appears to be an expensive disease due to a decrease in feed intake and milk production. Furthermore the disease also has negative effects on several immunity parameters, predisposing ketotic cows to a higher risk to suffer from any infectious disease, and suboptimal reproductive performance. The main problem of subclinical ketosis is that it all too frequently remains undiagnosed and/or incorrectly diagnosed, which prevents the implementation of appropriate corrective measures. The diagnostic of ketosis can however be relatively easy organized using well defined protocols. Results of analytic blood data should be of supportive evidence together with other information related to the incidence of diseases, reproduction and production data. The diagnosis and the implementation of corrective measures should allow improving animal performance and welfare.

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