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Changes in the oxidative biochemical status in dairy cows during the transition period affecting reproductive and health parameters

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Abstract: Negative energy balance (NEB) after calving leads to unique metabolic changes in cows and it provides important information on the period when nutrient output prevails over nutrient intake associated with a number of physiological changes in the organism suitable for detection using biochemical blood analyses. The aim of this paper is to summarise current knowledge of the changes in blood parameters during the period of NEB in relation to non-esterified fatty acids, ketone bodies, and immunologic and stress indicators. The impact of these changes on bovine reproduction and health is also discussed in the sequel. The period of NEB is still a very pertinent area of research as it provides a new insight into connections between physiological systems, to wit, immunology, manifestation of various stress indicators, including oxidative stress, and heat shock proteins *inter alia*. Understanding the physiological changes during NEB is crucial for successful management of the transition period and subsequent overall good animal health and productivity.

Keywords: health; immunity; ketosis; reproduction; stress

INTRODUCTION

Negative energy balance (NEB) is a significant problem for dairy cows on current high-production farms. It is a situation with an imbalance between the intake and the output of energy and nutrients in the period around calving. This is due to the high energy and hence nutritional requirements at the

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beginning of lactation compensated by the intensive mobilisation of fat reserves. Cows remain in a period of NEB for about 10–15 weeks following the calving, with a peak between the 2nd and the 3rd week. Dairy cows experience multiple metabolic changes in the period of 3 weeks before calving and 3 weeks after it – the transition period (Grummer 1995).

In the case of dairy cows, NEB is studied very intensively but it must be said that it is not a special feature of cattle. However, it is a special problem in dairy cows due to very intensive milk production which greatly exceeds the needs of the young calf. Thus, NEB in dairy cows may lead to a variety of problems including metabolic diseases. Their basic examples are ketosis, hepatic steatosis and displacement of the abomasum (Aslan et al. 2022). The metabolic status of dairy cows affects their resilience, overall health, productivity as well as reproductive capabilities (Stadnik et al. 2002, 2024; Vacek et al. 2007; Kasna et al. 2022, 2023).

Other phenomena associated with NEB in cows are immune changes and detection of stress markers including indicators of oxidative stress and heat shock proteins (HSP). This is a salient topic reflected in the health of the animals as well as in their production and reproduction. The relationship between NEB and both immune system sequelae and oxidative stress has been intensively studied in recent years and a number of new findings have been made.

The impact of NEB on different metabolic, including biochemical, indicators in blood

A negative energy balance is associated with several physiological or pathophysiological processes which can clearly be detected in the blood of the animals as anintegral part of biochemical analyses. These processes mainly include: (*i*) formation and increased metabolism of non-esterified fatty acids (NEFA); (*ii*) greater production of ketone bodies which become an alternative source of energy; or there may be a situation of (*iii*) reemergence of triacylglycerols which are stored (accumulated) in the liver and the situation can also arise that the cow may be (*iv*) deficient in some micro/macroelements (primarily calcium and magnesium). These processes also lead to manifestations of (*v*) immune changes and the production of (*vi*) some stress indicators [reactive oxygen and nitrogen species (RONS); antioxidants; HSP, etc.] which are also measurable from the blood analysis.

A number of these changes can lead to pathological conditions and diseases such as those already mentioned: acidosis, ketosis, displacement of the abomasum, hepatic steatosis and other disorders related to the metabolism of minerals (hypocalcaemia, hypomagnesaemia). These are subsequently reflected in milk production, including changes in fat and protein content, their ratio in the milk and/or fatty acid proportions (Duchacek et al. 2014, 2020), in reproduction (Beran et al. 2013), in the health status of the animals (Yenuganti et al. 2016; Sharma et al. 2019; Bezdicek et al. 2020; Stadnik al. 2022) and also in subsequent diseases such as mastitis, dystocia and endometritis (Zhang and Burim 2020).

Thus, the negative energy balance in the transition period in cows is a very complex situation that can be studied from various aspects, not only from the viewpoint of external subjective evaluation (e.g. change in body condition), but also through blood analysis (increased NEFA metabolism, increased ketone bodies, immune changes, oxidative stress etc.) and also by evaluating the ratio of milk components, specifically fat *vs* proteins (Stadnik et al. 2017; Duchacek et al. 2020). In this review we focus on key biochemical changes that can be detected in blood analyses. The latter mainly include determination of: (*i*) non-esterified fatty acids; (*ii*) ketone bodies, and (*iii*) immunological and stress indicators. The results of these analyses provide valuable and precise information on the degree of the negative energy balance and its impact on the reproduction and health of the animals.

Consequences of NEB and increased concentration of NEFA for the health and reproduction in cows

Adipose tissue cells (adipocytes) and their lipids (triacylglycerols) are an important source of energy during fasting or starvation. Their intensive breakdown is, in general, easily detectable in the postpartum period by an increase of fatty acids in the blood. At the same time, an increased concentration of NEFA is associated with a number of health, reproductive and production problems.

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Basic relationship between NEB and NEFA

The liver is the main organ of fat metabolism. Dissociated adipose tissue contains mainly palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) and NEFA, whose blood concentration increases during NEB (Vanholder et al. 2005). An increase in fatty acids in the blood is dangerous. The limit for NEFA is approximately 0.6 mmol/l in healthy animals.

Successful reproduction is based on the oocyte quality, and also on the quality of cumulus cells surrounding the oocytes. For this reason, a number of studies focus on the relationship between the increased NEFA content in a period of negative energy balance and reproductive issues in cows as reflected in the granulosa cells, oocyte and embryo quality as well as this is where reproduction obviously begins (Yenuganti et al. 2016; Sharma et al. 2019). There is a relationship between increased fatty acids in the blood and increased content in relevant tissues – in relation to this article in the ovary, specifically in follicular fluid, granulosa cells and in oocytes. The majority of the authors agree that an increase in the fatty acid level in the blood is in general associated with a change in ovarian tissue (in follicular fluid) even ifthis may not correspond to the actual fatty acid level or quantity. E.g. Leroy et al. (2005) reported that total NEFA concentrations (in blood plasma *vs* follicular fluid) were different in cows on day 16 after calving (0.4–1.2 *vs* 0.2–.6 mmol/l), but on day 44 after calving, these differences were equalized and reached the same levels (0.1–0.3 *vs* 0.1–0.3 mmol/l). On the other hand, it is not only the quantity of fatty acids but also particular fatty acids in follicular fluid in comparison with the blood that is crucial. E.g. the level of oleic acid is generally higher in follicular fluid than in blood plasma (Aardema et al. 2015). At the same time, lipotoxicity (a negative effect of palmitic and stearic acid) on oocyte development has been proven (Leroy et al. 2005). A major question of the research of this time period in dairy cows is to determine how these lipids are eliminated from the cells in the case of increased concentrations. Basically, there are two strategies, namely storage lipids as lipid droplets or their conversion into other non-lipotoxic acids. Lipid droplets are prime subcellular organelles which, along with oocyte and granulosa cells, are also common components of other eukaryotic cells. In general, during starvation, energy is released from lipid droplets, which is evidenced by their increased interactions with mitochondria and lysosomes (Valm et al. 2017). On the one hand, the primary utilisation of lipid droplets is associated with the provision of energy. On the other hand, the function of lipid droplets is much broader; e.g. with an increased concentration of lipids, lipids can be stored in lipid droplets and thus prevented from causing lipotoxicity and oxidative stress (Li et al. 2023).

Lipid droplets are also salient during embryonic development, as they provide key lipid components for embryo development, as evidenced by their lower volume density in morulas and blastocysts than at the beginning of embryonic development (Li et al. 2023). Of course, the essential cell is the oocyte, which must be protected from the negative effects of fatty acids. The cumular and theca cells play a fundamental protective role here and these create a very significant protective barrier that can correct the increase in NEFA in the blood (follicular fluid) and protect the oocyte. An example of oocyte protection from lipid toxicity through cumular cells (e.g. through stearic acid) is not only the formation of lipid droplets, but also the presence of the enzyme stearoyl-CoA desaturase in cumular cells, which converts lipotoxic palmitic and stearic acid into monounsaturated oleic and palmitoleic acid. Cumular cells thus acquire another important function in the protection and support of oocyte development. The length of the negative energy balance also plays a significant role in terms of the effect of increased NEFA concentrations in the blood and subsequently in the follicular fluid (Aardema et al 2017).

The concentration of triacylglycerols (TAG) in oocyte, plasma and granulosa cells is currently a specific and well-studied topic. At the same time, there are interspecies differences in the concentration of TAG and other lipids in healthy, functional oocytes. This concerns e.g. the concentration of TAG in pig oocytes compared to oocytes of mice and cows (e.g. mouse *vs* pig: 4 *vs* 156 ng). Slower development to the blastocyst stage is probably the reason for the higher concentration of TAG in pig oocytes; thus, pig oocytes need a higher content of reserve substances. This is the generally accepted explanation. For this reason, it is not possible to compare the oocyte lipid concentration across different animal species, including their energy metabolism (Melo-Sterza and Poehland 2021). It is also necessary to take into account the fact that lipid concentration and lipid storage are changed

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in oocyte and granulosa cells in relation to development stage, nutritional status and season as well (Shi and Sirard 2022). E.g. lower fertility of cows during the summer is generally known not only from the animal husbandry point of view, but also from results of embryo transfers and *in vitro* processes (Bezdicek et al. 2015; Stadnik et al. 2017). A whole range of causes may contribute to the reduced fertility of cows during the summer season including heat stress (Bezdicek et al. 2021). An interestingly higher concentration of saturated fatty acids was found in oocyte and granulosa cells during the summer season than in winter (saturated acids have more damaging effects than unsaturated fats). This may be related to the lower fertility of cows in summer (Shi and Sirard 2022).

As mentioned above, NEB can occur a few weeks before calving, with a dramatic decrease in dry matter intake before calving. At this time, NEFA concentrations in the blood begin to rise and reach their maximum during the calving period. Subsequently, the concentrations of NEFA should decrease but they may increase due to the negative energy balance. As the concentration of fatty acids in the blood increases, the feed intake decreases (Kaufman et al. 2016); this fact further deepens the negative energy balance and energy deficiency. E.g. Kaufman et al. (2016) reported that multiparous cows with ketosis tended to ruminate less than healthy cows during weeks –1, +1 and +2 related to calving. Other authors also provided evidence that the rumination time is affecting the milk yield and health of the animal in the period around calving and so it is crucial to pay great attention to this period (Codl et al. 2023).

On the other hand, if the mobilization of fat reserves is adequate, NEFAs are metabolized and NEFAs cover the energy requirement of the organism; the organism is able to cope with this difficult period. However, a very common case in dairy cows is the higher concentration of NEFAs and their association with a variety of reproductive and heatlh issues, as will be described below.

Relationship of increased TAG and NEFA concentrations to reproduction

From the foregoing, the increased concentration of NEFA in blood plasma is associated with a number of reproductive issues in cows, which are reflected, for example, in the poorer quality of granulosa cells, oocytes and embryo quality (Yenuganti et al. 2016; Sharma et al. 2019; Stadnik et al. 2022).

Cumulus and granulosa cells in antral follicles play an important role in correct development of the oocyte, as they significantly support its growth and metabolism through the supply of nutrients including energy (pyruvate), cholesterol, etc. (Su et al. 2008). At the same time, increased concentrations of NEFA negatively affect the proliferation of granulosa and theca cells (Vanholder et al. 2005); in addition, the increased NEFA concentration has been shown to decrease progesterone production (Vanholder et al. 2006). It was also found that NEFA stimulated RONS production and induced apoptosis in granulosa cells (Wang et al. 2020). Similarly, Sharma et al. (2019) found that granulosa cells were significantly negatively affected by the supplementation of fatty acids during *in vitro* experiments, mainly apropos their morphological and physiological activity. These authors also concluded that palmitic and stearic acids are involved in expression of genes connected with steroidogenesis and 17β-estradiol production (Sharma et al. 2019). Similar conclusions were also reached by Marei et al. (2010), who focused on the concentration of linoleic acid (18:2n-6) as the most frequent fatty acid in follicular fluid (Marei et al. 2010). These authors demonstrated that the increased concentration of linoleic acid during oocyte maturation is connected with altered oocyte maturation and also with altered embryo development. They also related these conclusions to the importance of monitoring fertility in relation to feed supplements.

The lipomobilisation syndrome (hepatic steatosis) is a major problem associated with excessive lipomobilisation. It occurs in fat cows that are overfed with carbohydrates before calving and where NEB is connected with an excessive intake of nitrogenous substances after calving when fat reserves are intensively broken down, the liver cannot manage it and the hepatocytes are irreversibly damaged. Jorritsma et al. (2001) found a prevalence of fatty liver in 54.1% of cows in dairy herds in the Netherlands. In this experiment, the concentration of TAGs in the liver varied between 12.7 and 208.6 mg/g of wet liver tissue (mean 61.2 mg/g). In percentage terms, the normal fat content in the liver is generally reported to be around 5%; with steatosis of the liver it is 20–45%. At this increased value, the liver function is impaired by worsening

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or even arresting the metabolic activity. Some authors report that the regular daily walking activity of cows may burn NEFA and minimise lipid accumulation in liver issue at early lactation.

In sum, the issue of optimum concentration of fatty acids (in blood, follicular fluid, oocyte etc.) and their lipotoxicity are still a very topical theme, as they are related not only to fertility and oocyte quality, but also to metabolic diseases and health status. As mentioned above, a number of papers have shown that the addition of NEFAs (mainly palmitic and stearic acid) during *in vitro* cultivation reduces oocyte quality, embryo development and is also connected with negative changes in preantral follicles (Leroy et al. 2005; Marei et al. 2010). Similarly, research has also demonstrated a detrimental effect of NEFA on follicle development in *in vivo* experiments, e.g. through direct intrafollicular injections of 200 μM of stearic, oleic and palmitic acid (Ferst et al. 2020). On the other hand, it should be noted that a significant barrier, probably also a selective one, is formed by the cumulus and theca cells. Cumulus cells protect the oocyte from the negative effects of fatty acids, e.g. through the accumulation of many fatty acids as lipid droplets, through their enzymatic conversion, etc. (Aardema et al. 2017; Warzych and Lipinska 2020). Increased metabolism of NEFA is also connected with higher mitochondrial activity and subsequent RONS production (Meulders et al. 2023). In general, lipotoxicity at the cellular level (oocytes, granulosa cells, etc.) is for these reasons a very germane topic albeit higher concentrations of cytoplasmic fatty acids in the oocyte are typical of some animal species. Hence, it is paramount to pay attention to the optimal conditions of the animals in the relevant period. A number of *in vivo* experiments have shown that a deteriorated condition in the direction of fattening is associated with poorer fertility in cows, manifested e.g. in the number of viable embryos (Bezdicek et al. 2015).

Consequences of NEB and increased concentration of ketone bodies in cows for health and reproduction

To recapitulate, mobilisation of fat reserves during the NEB in cows is associated with an increase in fatty acids in the blood. On reaching a critical level, reproduction and health are impacted. Ketone bodies play an important role in this regard. These are also commonly detectable in blood tests and are another salient feature of NEB in the postpartum period.

Basic relationship between NEB and ketone bodies

Due to the NEB there is a greater release of free fatty acids or NEFAs into the blood. However, this may exceed the capacity of the liver to esterify them (the availability of oxaloacetate is also limited). For this reason, NEFAs are oxidized in hepatocytes to ketone bodies mainly as β-hydroxybutyrate acid (BHBA), acetoacetate and acetone. Ketones enter the blood, urine and milk and they are a usable form of energy, an alternative source of ATP production during periods of low glucose availability. Ketone bodies have been revealed as an energy source for the nervous system, intestinal cells and the heart in some mammals. Ketogenesis is an important tool in intestinal cells where it plays a major role in the enzymatic activity of 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) which is the key ketogenesis enzyme (Puchalska and Crawford 2021).

In general, ketone bodies in mammals (*i*) are a clue to a number of physiological processes and at the same time they (*ii*) support survival in a time of energy deficiency (Youm et al. 2015), e.g. during starvation or during NEB in the case of dairy cows. On the other hand, however, an increased concentration of ketone bodies in the blood is associated with a number of health, reproductive and production problems.

Relationship of increased concentration of ketone bodies to reproduction and health including ketosis

Ketosis, the most serious and most common metabolic disorder in dairy cows after calving, has an incidence of almost 40% in North America and on some farms it may be as high as 80% (Zhang and Burim 2020). It is associated in cows primarily with (*i*) negative energy balance in the postpartum period due to the intensive start of lactation and thus high energy output. However, it can also occur (*ii*) independently of lactation, e.g. in the case of animal disease and significantly reduced feed intake or when low-quality silage with a higher butyric acid

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content is fed. In general, the aetiology of ketosis is associated with intensive lipomobilisation in a period of imbalance between energy intake (feed) and output primarily at the start of lactation and subsequently higher formation and release of ketone bodies into the blood. Higher concentration of ketone bodies (in the blood serum of cow the threshold concentration of BHB was found to be 1.2 mmol/l; Bach et al. 2016). For the cow metabolism this is damaging, mainly in relation to reproduction, milk yield, intake of food and in general the health of the animals and their susceptibility to other diseases. Zhang and Burim (2020) reported an association between ketosis and susceptibility of cows to infectious diseases (e.g. mastitis, metritis) and immune response. Zarrin et al. (2014a) showed that the increased plasma concentration of ketone bodies (β-hydroxybutyrate) is connected with higher susceptibility of dairy cows to mastitis in the period of early lactation. In the next study Zarrin et al. (2014b) presented that β-hydroxybutyrate can be used as an alternative source of energy (for mammary tissues and immune system). The negative relation of ketosis to reproduction has also been confirmed (Beran et al. 2013). Rutherford et al. (2016) demonstrated that cows with detected subclinical ketosis have 4.3 times lower probability of the successful first insemination than healthy cows. The negative effect of higher concentrations of ketone bodies on reproduction has also been shown in *in vitro* experiments. E.g. Hildebrand et al. (2023) stimulated bovine uterine cells (bovine caruncular epithelial cells) with different concentrations of β-hydroxybutyrate (as a marker of ketosis). The authors found that higher concentrations of this ketone body (1.8 and 2.4 mmol/l) negatively affected the cell function and increased the inflammatory response. Also Melendez et al. (2000) demonstrated that breeding of Holstein cows with a higher concentration of milk urea nitrogen during summer months is associated with a higher risk of nonpregnancy, compared to cows bred in winter months with a lower level of urea nitrogen.

Overall, the increased concentration of ketone bodies (and possibly subclinical or clinical ketosis) is subsequently reflected not only in animal health, but also in production and reproductive indicators; therefore, some authors tried to express these problems as total economic loss. Of course, it is very difficult to investigate the economic cost of ketosis, because there are several factors that must be taken into account; not only production and reproduction losses but also costs of veterinary treatment which can be economically an important part of total costs. Steeneveld et al. (2020) calculated costs of ketosis based on the Dutch market in 2018. These costs can vary according to different regions and countries, but the reality is a significant economic loss in the case of both clinical and subclinical ketosis.

These examples show a direct link between increased concentrations of ketone bodies and NEFA with respect to cow reproduction and diseases such as ketosis and steatosis of the liver. Of course, there are impacts of other conditions (e.g. acidosis, displacement of abomasum, etc.) as well as related diseases, such as mastitis, dystocia and endometritis among others. The rapid breakdown of fat reserves directly connected with the formation of ketone bodies and NEFA at this time is a thoroughly researched topic.

Consequences of NEB and different stress indicators (oxidative status; HSP) and immunologic changes in the health and reproduction of cows

Negative energy balance is a very important situation that fundamentally affects the physiology and pathophysiology of animals. In recent years, for this reason a number of studies have also focused on the relationship of NEB to: (*i*) immunologic changes, (*ii*) oxidative stress and other factors (proteins), such as (*iii*) HSP. These stress indicators are measured in the blood and they are often analysed together. Thus, many authors focus on monitoring changes in both the immune response and the oxidative status (Catalani et al. 2010; Shen et al. 2019; Sun et al. 2020) as both are significant characteristics of the metabolic state during NEB.

Basic characteristics of changes in immunity and oxidative stress during the NEB period

In general, there is an agreement on the activation of the immune system and changes in the cytokine profile in the transition period. In particular, proinflammatory cytokines (e.g. IL1; IL6; IL12; IL19; TNFα – tumour necrosis factor alpha; IFNϒ – interferon gamma and others) are activated. A number of studies have been focused on these

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pro-inflammatory and also on anti-inflammatory cytokines (e.g. IL-4; IL-10; IL-11; Il-13; TGF-β). These studies evaluated the level of the immune response to stress due to NEB (Shen et al. 2019; Karimi et al. 2021; Sun et al. 2020). Oxidative stress is a significant manifestation of the imbalance between the production of RONS in the cell and their antioxidant defence capacity. Disequilibrium is connected with the impaired reproductive function. However, RONS play not only a negative role, e.g. by damaging DNA, plasma membranes of cells or through involvement in the activation of apoptosis, but also they are very important in a number of common physiological processes such as ovulation, angiogenesis and activation of the *corpus luteum* (Fraser et al. 2000), its regression (Park et al. 2018) and in other processes. In the absence of RONS, these processes cannot take place. However, overproduction of RONS, beyond the function of antioxidant cell protection, is connected with oxidative stress in cells and/or in the whole organism. This situation also occurs during the NEB in cows.

HSP is a key protein in cell stress. This protein is generally involved in the protection of cells against stress, not only heat tress, but also, for example, stress caused by UV radiation, chemical substances, infection, etc. Among the most studied of these proteins are HSP60, HSP70 and HSP90. Jee (2016) characterised HSPs as factors with a major role in homeostasis. On the cellular level, HSPs prevent denaturation of various molecules and thus they ensure protection in stress periods (Jee 2016; Bezdicek et al. 2021); HSPs are shown to be significantly involved in the regulation of apoptosis (Kennedy et al. 2014) and extracellular HSPs also appear to be involved in the immune response (Albakova and Mangasarova 2021) and in the protection of stem cells (Shende et al. 2019). The activity of HSPs is further connected with a number of other signalling proteins, such as hypoxia-inducible factor 1-alpha (HIF1α), vascular endothelial growth factor (VEGF), etc., which influence the response to various stresses (Tang et al. 2021).

Manifestation of immunity and oxidative stress during the NEB period in relation to health and reproduction

As mentioned above, the NEB in cows is a parameter that can be detected in blood as changes in immunity and stress characteristics, e.g. oxidative stress and various proteins, HSPs in particular. For example, Mezzetti et al. (2019) found in Holstein cows that detected subclinical ketosis (β-hydroxybutyrate; BHB> 1.4 mmol/l) was associated with the higher activity of their immune system manifested as higher concentration of plasma proinflammatory cytokines, myeloperoxidase, oxidant species and greater IFN-γ responses to *Mycobacterium avium*. The authors also found that the cows with subclinical ketosis showed higher blood concentrations of γ-glutamyl transferase (higher level is connected with liver disease) and lower concentrations of minerals in blood plasma. As for the oxidative stress biomarkers, the authors stated that reactive oxygen metabolites and advanced oxidation protein products were higher in cows with subclinical ketosis than in healthy cows (Mezzetti et al. 2019). Similarly, Shen et al. (2019) reported that in cows with ketosis the serum levels of pro-inflammatory cytokines (IL18, IL1B and TNF-α – tumour necrosis factor) were elevated. At the same time, the anti-inflammatory cytokine IL-10 concentration was lower. The authors also found the over-activation of hepatic NF-κB and NLRP3 in ketotic cows. Overall serum levels of pro-inflammatory biomarkers were higher in ketotic than in healthy cows. Other authors (e.g. Lingappan 2018; Sun et al. 2020) also focused on monitoring the factors NF-κB (nuclear factor-κB) and NLRP3, which are activated in response to a number of cellular signals including oxidative stress or other signals such as pathogen antigens, stress, etc. Activation of NF-κB and NLRP3 starts the immune response to these stimuli through activation of pro-inflammatory cytokines IL1B and IL6. These cytokines were also observed by Sun et al. (2020), who studied the relationship between oxidative stress and ketosis in mammary tissue in Holstein cows. They found the higher activity of NF-κB and the NLRP3 inflammasome in cows with detected subclinical or clinical ketosis. In ketotic cows the higher concentration of hydrogen peroxide and malondialdehyde was revealed in contrast to the lower activity of glutathione peroxidase, catalase and superoxide dismutase and also greater abundance of TNF-α, IL6, and IL1B. The authors concluded that cows with detected clinical or subclinical ketosis are affected by oxidative stress (Sun et al. 2020). Similarly, Karimi et al. (2021) reported significantly higher levels of IL-4, IL-10, TNF-α and haptoglobin in cows with subclinical ketosis in the

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transition period compared to control (healthy) cows. The authors concluded that subclinical ketosis in the transition period may be related to inflammatory and immunological changes (Karimi et al. 2021). Bernabucci et al. (2005) also confirmed that cows can be affected by oxidative stress during the peripartum period. These authors found that Holstein cows after calving had a lower concentration of superoxide dismutase and thiol groups while they showed an increase of reactive oxygen metabolites, glutathione peroxidase and thiobarbituric acid reactive substances. In general, cows are exposed to oxidative stress in the transition period (Bernabucci et al. 2005).

In addition to ketosis, an important disease related to NEB in high-producing cows is displacement of the abomasum which has also been diagnosed in relation to oxidative stress. The increased occurrence of malignancy dislocations is a multifactorial disease associated primarily with nutrition and metabolism but also with changes in the body form (angularity) of dairy cattle during breeding, increased milk yield, genetic predisposition, reduced animal movement and accurate diagnosis of this disease in recent years. The latter has been disclosed in research to have a close relationship to oxidative stress. This was demonstrated by Fiore et al. (2019), who reported an imbalance between the concentrations of antioxidants and oxidants in blood plasma in Holstein cows with the diagnosed left displacement of abomasum (LDA). Other authors have provided evidence that oxidative stress and lipid parameters can be used in dairy cows in diagnosis, prognosis, and treatment in cows with LDA (Aslan et al. 2022). On the other hand, defences against stress are diverse and these are not just antioxidants. At the cellular level, heat shock proteins play an important role. However, Kristensen et al. (2004) pointed out that HSPs, primarily considered intracellular proteins, can be detected in blood plasma (e.g. in cows). The authors also reported that the concentration of heat shock protein (HSP72 in this study) is affected by stage of lactation and age (Kristensen et al. 2004). Further, Catalani et al. (2010) showed that the concentration of heat shock proteins increases significantly after calving. Specifically, that the plasma concentration of HSP72 was the highest 35 days after calving, while from calving this value gradually increased over the observed days (+7; +14; +28) compared to the values before calving. The authors also found changes in the concentration of TNFα after calving. The value was higher than within the pre-calving period, especially on day 14 (Catalani et al. 2010). Similarly, Petrovic et al. (2022) found a relationship between NEB and higher concentration of extracellular HSP70, TNF-α, beta-hydroxybutyrate and non-esterified fatty acids. The intracellular role of HSP70 can be characterised as protective and extracellular HSP70 has a pro-inflammatory role. There are a number of papers focused on the concentration of HSPs in dairy cows. This is due to the wide range of heat shock protein activity, including their involvement in immunology, protection of stem cells, angiogenesis, in hypoxia and in many other processes (Catalani et al. 2010; Tang et al. 2021; Vaidya et al. 2023). This is a broad area of research.

However, evaluation of HSP is linked to other factors, including the season (summer *vs* winter), breed and level of milk production *inter alia*; these parameters were included in the monitoring of changes in HSP72 by Vaidya et al. (2023). Plasma concentration of HSP72 was higher in summer, on the day of calving and in high yielding breeds. In general, a number of factors must be considered in relation to the concentration of stress proteins – indicators.

The NEB is an expected event in cows during the transition period, therefore the breeder must be prepared. This is because negative factors can overturn the delicate balance in favour of various metabolic diseases. These factors can mainly be problems in animal nutrition before and after calving, poor condition in the transition period, concomitant disease, animal stress, etc. A direct manifestation of NEB is increased NEFA and ketone bodies which have been studied in a number of papers in relation to worsened reproduction. Recent research has also shown the connection of NEB with changes in the immune system as well as the manifestation of various stress factors, such as proteins of oxidative stress and heat shock proteins (Sun et al. 2020; Karimi et al. 2021; Vaidya et al. 2023).

CONCLUSION

The transition period in cows is unique in view of interesting metabolic changes. Serious NEB is often manifested in immune changes resulting in the activation of pro-inflammatory cytokines mainly in ketotic cows. In this period, there is also greater production of stress factors such as heat shock proteins and overproduction of RONS. It is of paramount importance to maintain a precise balance in the organism between production and elimination of stress factors. This period is also characterised by an increase in ketone bodies and NEFA, whose significant increase in concentration is associated with negative health consequences in terms of reproduction and animal health. The transition period is an interesting physiological phenomenon. However, research of this period, given its uniqueness, is providing new knowledge in the field of fasting and starvation which are a common part of the life of many animals.

Conflict of interest

The authors declare no conflict of interest.

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