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Niacin, Metabolic Stress and Insulin Resistance in Dairy Cows

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Abstract

The periparturient period in cows is associated with metabolic stress and a state of negative energy balance, which are characterized by increased lipolysis, ketogenesis, hepatic steatosis, oxidative stress and insulin resistance. Such metabolic changes may exert adverse effects on the health and milk yield of lactating cows. The pharmacokinetics of niacin in ruminants is specific as rumen microorganisms facilitate both the synthesis of tryptophan and the degradation of niacin. Niacin administration to cows leads to an increase in the coenzyme activity, encompassing the activity of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes are actively involved in the metabolism of lipids and carbohydrates, whereas NAD protects the organism from oxidative stress. In periparturient cows, the supplementation of niacin has been found to induce depressed lipolysis and a limited impact of nonesterified fatty acids on all metabolic processes. It also results in decreased lipid peroxidation regardless of the magnitude of lipolysis in the periparturient period. Furthermore, niacin reduces the concentration of ketone bodies, thus preventing the development of fatty liver disease and ketosis in cows. The anti-inflammatory effect of niacin is manifested in stimulating the secretion of adiponectin and inhibiting immune cells.

Keywords: transition period, nonesterified fatty acid, insulin, glucose, oxidative stress

1. Introduction

Metabolic stress in dairy cows occurs around the time of parturition as a consequence of heightened milk production requirements, accompanied by depressed feed intake and

a negative energy balance. Accordingly, the organism enters a state of increased lipolysis, oxidative stress and insulin resistance. Niacin is a well-known antilipolytic vitamin which enhances gluconeogenesis and insulin concentrations in the blood [1]. A review of the literature has revealed that the effect of niacin is dependent upon the dosage administered, pharmaceutical form, duration of administration and biological features of cows [2, 3]. There is a limited body of information on the relationship between niacin and insulin productions and efficiency in cows, as well as on the NAD and NADP response to niacin administration. This chapter will elucidate the mechanism of metabolic stress in cows, the pharmacokinetics of niacin, the physiology of the niacin-containing NAD and NADP coenzymes, as well as the biological effect of niacin on lipolysis, lipolysis-dependent metabolic adaptations and insulin resistance in dairy cows.

2. Metabolic stress in cows

In addition to a number of metabolic and physiological adaptations, the periparturient period in cows is associated with a dramatic increase in the nutritional requirements essential for foetal growth and milk synthesis. The nutritional requirements of the placenta and foetus are highest in the last 3 weeks of pregnancy, whereas the dry matter intake (DMI) is reduced by 10–30% relative to the DMI in the early dry period. As milk production surges from the onset of lactation to the yield required to sustain the calf, the ongoing adaptations occur rapidly, resulting in a marked discrepancy between the varying nutritional requirements and concomitant adaptations. The peak of lactation is expected to be reached in weeks 4–8 postpartum, whereas the highest dry matter intake is achieved in weeks 10–22 after parturition [1, 2]. The greatest negative energy balance in dairy cows is recorded around day 14 of lactation, continuing even to day 72 of lactation (as reported by the same author) [4]. During early lactation, the energy requirements for milk production and proper tissue function exceed the amount of energy ingested. To compensate for a negative energy balance, energy and protein body reserves are mobilized, expending to approximately 600 g/d of fat and 40 g/d of protein in the first 8 weeks after parturition [5]. The failure of these adaptive mechanisms has been implicated in the occurrence of common metabolic disorders in early lactation. Postpartum metabolic disorders are interrelated and concurrent, greatly influencing the fertility of cows. Fatty liver syndrome and degeneration, ketosis, parturient paresis, mastitis, hypomagnesaemia, rumen acidosis, displaced abomasum, laminitis, postpartum infections and fertility problems are the predominant diseases of dairy cows in the periparturient period [6]. A glucose mass of 72 g is required to produce 1 kg of milk [7]. In ruminants, the largest amount of carbohydrates ingested is fermented in the rumen, whereas very little glucose is absorbed from the digestive tract. Consequently, glucose requirements of dairy cows are, for the most part, met by gluconeogenesis, i.e. the synthesis of glucose from propionates, amino acids, glycerol and liver lactates. A postpartum increase in the expression of key enzymes, i.e. pyruvate carboxylase and phosphoenolpyruvate carboxylase, enhances the magnitude of gluconeogenesis in the liver. Substantial discrepancies between the depressed feed intake and elevated energy requirements of the mammary gland in this period incite the organism to provide sufficient energy to

the mammary gland in order to maintain the persistence of lactation. This is achieved through a number of metabolic adaptations induced by the hormonal changes and associated tissue responses. Increased liver gluconeogenesis, liver glycogen depletion, increased lipolysis, protein catabolism and limited glucose utilization by all tissues other than the mammary gland represent some of the alternative means by which the organism meets the energy requirements of the mammary gland. Growth hormone levels increase around parturition, resulting in the increased responsiveness of adipose tissue to lipolytic signals such as norepinephrine. An increased release of NEFAs from adipose tissue subsequently ensues, which are converted by the liver to ketone bodies and used as alternate fuels for extramammary tissues. The ketones serve as alternate fuels which can replace glucose in many tissues, thus conserving glucose for milk synthesis. Elevated somatotropin levels also enhance gluconeogenesis [8]. An increase in corticosteroid concentrations around parturition enhances the responsiveness of adipocytes to the action of catecholamines and stimulates glycogenolysis as well as gluconeogenesis [9]. Depressed plasma insulin concentrations and decreased insulin sensitivity enable the insulin-independent uptake of nutrients by the mammary gland, whereas insulin-dependent tissues increase the oxidation of fatty acids and reduce the utilization of glucose.

Adipose tissue has a pivotal role in homeorhesis and metabolic stress in cows. In the dry period and late lactation, anabolic processes predominate as the cow's body stores triglycerides in adipose tissue, which is thereafter sensitive to insulin (the key antilipolytic hormone reducing the degradation of triglycerides in adipose tissue cells and facilitating the synthesis of fatty acids and glycerol). A negative energy balance in early lactation is associated with a number of metabolic changes, leading to increased lipid catabolism in adipose tissue and the mobilization of body fat stores. The degradation of triglycerides stored in adipocytes also ensues, accompanied by the release of NEFAs and glycerol. The mobilization of adipose tissue fat is mediated by the following similarly functioning enzymes: monoglyceride lipase (MGL), hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) [10]. ATGL initiates lipolysis followed by the actions of HSL on diacylglycerol and MGL on monoacylglycerol. An increase in the action of triglyceride lipase is recorded at low insulin levels in the blood. The name of hormone-sensitive lipase itself suggests that hormones such as catecholamines, adrenocorticotrophic hormone (ACTH) and glucagon stimulate the action of this intracellular neutral lipase [11]. The mobilization of fatty acids from body stores is induced by both energy deficits and changes in neuroendocrine regulation. Hormonal changes such as low insulin and glucagon concentrations significantly contribute to initiating and maintaining the mobilization of depot fat, whereas reduced insulin resistance, as an indicator of decreased insulin functional capacity, is of paramount importance [12]. Low plasma insulin concentrations enhance the action of triacylglycerol lipase and inhibit the entry of NEFAs, glycerol and glucose into adipocytes by reducing the action of lipoprotein lipase (the enzyme which hydrolyzes triacylglycerols in chylomicrons and very-low-density lipoproteins) as well as the expression/translocation of GLUT4 molecules. Lipolysis occurs in a state of reduced insulin sensitivity or low serum insulin concentrations (which is characteristic of early lactation), resulting in increased serum NEFA concentrations. In ruminants, acetate is a major substrate for the de novo synthesis of fatty acids, and adipose tissue is of overriding importance to the process. The degree of in vitro incorporation of acetate in the de novo synthesis of fatty acids

in adipose tissue is significantly reduced in late pregnancy (15 days antepartum), compared to days 120 and 240 of lactation, and completely impeded in early lactation [13]. Depressed lipogenesis is mainly attributable to hypoinsulinemia and decreased insulin sensitivity of adipose tissue, i.e. increased insulin resistance.

3. Pharmacokinetics of niacin, NAD and NADP

Niacin is a vitamin essential to energy metabolism. Physiologically, niacin is incorporated into the coenzyme nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These cofactors are involved in numerous metabolic processes: (1) anabolic pathways (NADPH/NADP) such as the syntheses of lipids and nucleic acids which require reducing equivalents provided by NADPH and (2) catabolic pathways (NADH/NAD). NAD is involved in a great many oxidation-reduction reactions as an electron carrier capable of accepting and donating electrons. NAD⁺, the oxidized form of NAD, can accept electrons in the reduction of NAD⁺ to NADH, whereas it can donate electrons in the oxidation of NADH to NAD⁺. Moreover, NAD is a source of adenosine diphosphate ribose (ADP-ribose) for protein modification. It is also a precursor of two second messenger molecules (cyclic ADP-ribose and nicotinamide adenine dinucleotide phosphate), which augment intracellular calcium concentrations and have a central role in a number of metabolic pathways. Another physiological effect of nicotinic acid is the potential to suppress lipolysis when administered in higher doses [14, 15]. Niacin is a generic descriptor for two vitamers: nicotinic acid and nicotinamide. Both forms of niacin are nutritionally equivalent and can be used for the synthesis of NAD. However, their biological proportions vary, and only nicotinamide can act as a reactive component [16].

In addition to ingested feed as a niacin source, niacin can also be synthesized in animals by the enzymatic conversion of tryptophan and quinolinic acid to niacin. Furthermore, rumen microorganisms synthesize niacin as well, using aspartates and dihydroxyacetone phosphate [17]. Previous research suggested that dairy cows did not require an exogenous supplementation of vitamin B due to a sufficient supply of this vitamin from the feed ingested and the synthesis of niacin in the rumen. However, milk production in high-yielding dairy cows has significantly increased, accompanied by increased vitamin B requirements. Depressed feed intake in the periparturient period, often continuing long after the onset of lactation, impedes the inflow of feed precursors (which are of immense importance to the ruminal synthesis of niacin), thus further increasing the need for niacin supplementation.

As an oral supplement, niacin can be rumen-protected or not rumen-protected. Niacin supplements which are not ruminally protected are less stable in the rumen and are readily degraded and, thus, should be administered in higher pharmacological doses [18]. The rumen-protected form of niacin is often found encapsulated and is commonly referred to as encapsulated niacin. These products are practically small pellets with niacin placed in the centre and covered by several layers of lipids. As encapsulation enhances the bioavailability

of niacin in the small intestine, the lipid layers in the pallet are relatively undegradable in the rumen and thereby prevent the degradation of niacin by rumen microorganisms [19].

The pharmacokinetics of orally administered medications in ruminants depends on the form of the medication (rumen-protected vs. not rumen-protected), whereas the pharmacokinetics of niacin is further influenced by two niacin vitamers: nicotinic acid and nicotinamide. The metabolism of nicotinic acid and nicotinamide, which are involved in the biosynthesis of NAD, may differ markedly. Unlike differences found in the concentration of each niacin vitamers [20], there is no difference in the total amount of niacin in the rumen between roughage and concentrate rations in a 40:60 to 60:40 ratio, respectively. Rumen microorganisms also synthesize niacin. The ruminal synthesis of niacin exceeds 2.2 g/d [21]. Increased amounts of non-fibrous carbohydrates facilitate the synthesis of niacin, whereas the roughage-to-concentrate ratio of the diet exerts no effect [22]. The duration of niacin administration greatly affects nicotinic acid concentrations in ruminal and intestinal fluids. One hour after administering niacin which is not rumen-protected, nicotinic acid concentrations in the rumen reach the peak by the conversion of nicotinamide to nicotinic acid or other forms (Campebell et al. [23] failed to detect nicotinamide in the ruminal fluid). The results of Santschi et al. [20] suggest that a considerable portion of both niacin vitamers are synthesized in the rumen, whereas the largest portion of nicotinic acid and the entire portion of nicotinamide are bound within the microbes.

Although direct absorption from the rumen is possible, the absorption of niacin from the small intestine appears to be the main route by which niacin is made available to the host. Only 17% of niacin administered is found in the duodenum as free nicotinic acid [23]. According to Santschi et al. [21], as much as 98.5% of niacin is degraded in the rumen of dairy cows. Nicotinic acid concentrations were found to be elevated in the duodenum of nicotinamide-supplemented cows (12 g/d) compared to cows supplemented with nicotinic acid [23]. However, this research focused solely on the analysis of duodenum fluids although niacin can also be found in solid intestinal contents. The niacin administered was not ruminally protected. Owing to the extensive ruminal degradation of niacin, considerably higher doses of niacin were administered to cows (12–36 g/d) in a number of studies [18]. When higher doses of niacin are administered, a surplus of undegraded niacin is more likely to reach the lower parts of the digestive tract. Increased niacin concentrations have been found in the duodenum of niacin-supplemented cows [23, 24]. A loss of niacin occurs even after abomasal infusion, to a lesser extent (approximately 85%), which corroborates the presence of both abomasal and duodenal niacin absorptions [21].

Duodenal niacin concentrations are essentially dependent on the pharmaceutical form of niacin (rumen-protected vs. not rumen-protected), the amount of niacin available and the roughage-to-concentrate ratio of the diet. According to Niehoff et al. [25], the total amount of niacin (nicotinic acid + nicotinamide) reaching the duodenum increases with an increase in the dietary share of concentrates and nicotinic acid supplements, whereas the amount of nicotinamide is solely dependent on nicotinic acid supplementation. Unsaturated nicotinic acid is of low rumen stability. Santschi et al. [21] reported that unsaturated nicotinic acid has a bioavailability of 5%. The administration of rumen-protected niacin in dairy cows leads to

augmented free niacin concentrations in the blood [19]. Morey et al. argue that encapsulated niacin treatments increase plasma nicotinamide concentrations (24 g/d of encapsulated niacin provides 9.6 g/d of bioavailable niacin).

The intestinal absorption of nicotinic acid and nicotinamide approximates to 73% and 94%, respectively, with an overall average niacin absorption of 84% from the duodenum [21]. Nicotinic acid is mostly absorbed from the duodenum. The intestinal mucosa is rich in niacin conversion enzymes such as NAD glycohydrolase. It is highly unlikely that nicotinic acid is directly converted to nicotinamide. However, nicotinic acid is readily converted to NAD in the intestinal mucosa, and excessive amounts of NAD are subsequently hydrolyzed to nicotinamide by NAD⁺ glycohydrolase [17]. NAD⁺ glycohydrolase is an enzyme that catalyzes the hydrolysis of NAD⁺ to produce ADP-ribose and nicotinamide [26]. Morey et al. [26] found that plasma nicotinamide concentrations decreased in niacin-treated cows 50 h after niacin administration but still exceeded those of control animals. Nicotinamide is the primary circulating form of niacin and is converted into its coenzyme forms (NAD and NADP) in the tissues. The transport of niacin in the blood is mainly associated with erythrocytes. Niacin rapidly leaves the blood stream and enters the kidney, liver and adipose tissue. There is a considerable dispute over the presence of nicotinic acid in the blood. Therefore, nicotinamide is considered the primary circulating form of niacin [17]. Nicotinic acid, which is not metabolized by the liver, can be transported to different tissues in the body by administering higher pharmacological doses of niacin.

Nicotinamide is a reactive part of NAD and NADP, which are involved in numerous oxidation-reduction reactions as coenzymes, i.e. cofactors. Enzymes containing NAD and NADP are important links in a series of reactions associated with carbohydrate, protein and lipid metabolism [27]. NAD and NADP act as an intermediate in most of the H⁺ transfers in metabolism, including more than 200 reactions in the metabolism of carbohydrates, fatty acids and amino acids. The most important metabolic reactions catalyzed by NAD and NADP are summarized as follows: carbohydrate metabolism (glycolysis, i.e. the anaerobic and aerobic oxidation of glucose, and the TCA (Krebs) cycle), lipid metabolism (the synthesis and breakdown of glycerol, the oxidation and synthesis of fatty acids and the synthesis of steroids) and protein metabolism (the degradation and synthesis of amino acids and the oxidation of carbon chains via the TCA cycle). The NAD and NADP coenzymes can be synthesized from niacin vitamers, tryptophan and quinolinic acid. The primary function of the liver is to synthesize NADP from tryptophan by hydrolysis in order to release niacin for its use in extrahepatic tissues. The brain, muscles and, to a lesser extent, testicles can take up nicotinamide from the bloodstream and utilize it without the previous deamination. The nicotinamide nucleotide coenzymes are catabolized from four enzymes: NAD pyrophosphatase, NAD glycohydrolase, ADP-ribosyltransferase and poly (ADP-ribose) polymerase. Under normal conditions, there is little or no urinary excretion of either nicotinamide or nicotinic acid as both vitamers are actively reabsorbed from the glomerular filtrate. Such excretion only occurs when nicotinamide and/or nicotinic acid concentrations are so high that the transport mechanism is saturated. N¹-Methylnicotinamide and N-methyl-2-pyridone-5-carboxamide are the two principal urinary metabolites of nicotinamide in humans, rats and pigs. In herbivores, niacin is seemingly not metabolized by methylation but is mostly excreted unchanged.

Our results suggest [28] that blood NAD and NADP concentrations are a sensitive indicator of the niacin status of cows. The NAD concentrations obtained ranged from 860 to 895 pmol/mL in the control group in the weeks before and after calving. In niacin-supplemented cows, the following NAD concentrations were obtained: 1724.6 pmol/L in the week of calving (week 0), 1968.6 pmol/mL in the first week after calving and 1771.8 pmol/L in the second week after calving. The NADP concentrations obtained in the control group ranged from 385.09 to 425.62 pmol/mL during the entire period under consideration. In niacin-supplemented cows, the following NADP concentrations were obtained: 704.45 pmol/L in the week of calving (week 0), 778.36 pmol/L in the first week after calving and 796.18 pmol/L in the second week after calving.

4. Effects of niacin administration on lipolysis, ketogenesis and oxidative stress

NEFAs are the major component of triglycerides (the fat stores in the body), which consist of three fatty acids linked to glycerol. The hydrolysis of stored triglycerides (fat) in adipose tissue by hormone-sensitive lipase liberates NEFAs and glycerol. Plasma NEFA concentrations are elevated in periparturient dairy cows. Accordingly, cows mobilize fatty acids from adipose tissue as a means of adapting to a number of metabolic changes and a negative energy balance in the periparturient period. The large influx of NEFAs into the liver exceeds its fatty acid oxidation capacity and results in storing NEFAs as triglycerides in hepatocytes and muscles. Depressed feed intake and metabolic changes subsequently ensue.

In their review paper on the administration of niacin which is not rumen-protected, Niehoff et al. [3] argue that nicotinic acid can decrease NEFA concentrations under certain conditions, whereas nicotinamide does not exert the same effect. When the effect of nicotinic acid is minimized, a rebound of NEFAs above basal values occurs, followed by a return to normal concentrations. To induce these effects, the amount of niacin reaching the duodenum should be large, which can be achieved by high-dose niacin supplementation. High doses of nicotinic acid can suppress the release of fat from adipose tissue [14].

GPR109A (HM74A in humans and PUMA-G in mice) is a G-protein-coupled receptor for nicotinic acid, which has been shown to mediate the nicotinic acid-induced antilipolytic effects [15, 29]. The high-affinity receptor for nicotinic acid HM74A enhances the therapeutic effect of nicotinic acid by inhibiting adenylyl cyclase and reducing the intracellular level of cAMP in adipocytes. In vivo studies suggest that administering pharmacological doses of nicotinic acid decrease plasma NEFA concentrations by inhibiting lipolysis in cattle [14, 26, 30]. This antilipolytic potential of nicotinic acid is most likely realized by the activation of GPR109A [31–35]. The GPR109A antilipolytic pathway, already described in other mammal species, has only recently been shown to exist in a functioning form in bovine tissues under in vitro conditions. Conversely, nicotinamide has a low affinity for binding to GPR109A. The activation of GPR109A by nicotinic acid results in decreased cellular cAMP concentrations and the inhibition of adenylyl cyclase. Decreased cAMP concentrations in adipocytes lead to the inactivation of protein kinase A and decreased phosphorylation of hormone-sensitive lipase, thus inducing

a reduction of lipolysis. The GPR109A receptors are found primarily in adipose tissue and immune cells, as well as in the brain, liver and muscles of cattle. BHB is the endogenous ligand of the human GPR109A, whereas nicotinamide acts as a very weak agonist at GPR109A producing no alterations in plasma lipoprotein profiles. Nicotinic acid, nicotinamide and BHB, as the ligands of the cattle GPR109A, exhibit different levels of efficiency in the induced antilipolysis under in vitro conditions. Nicotinic acid decreases the phosphorylation of hormone-sensitive lipase, thereby reducing the lipolytic response. However, nicotinamide does not exert a suppressing impact on the lipolytic activity in bovine tissues under in vitro conditions, whereas only extremely high BHB concentrations can significantly reduce the release of glycerol and phosphorylation of hormone-sensitive lipase.

Pires and Grummer [14] administered abomasal infusions of nicotinic acid (0, 6, 30 or 60 mg of NA/kg of body weight (BW)) to feed-restricted Holstein cows as a single bolus 48 h after the initiation of feed restriction. Plasma NEFA concentrations decreased from 546 to 208 ± 141 $\mu\text{Eq/L}$ at 1 h after the infusion of 6 mg of NA/kg of BW and to less than 100 ± 148 $\mu\text{Eq/L}$ at 3 h after the abomasal infusion of the two highest doses of NA. Upon the termination of NA infusions, a rebound occurred following the initial decrease of plasma NEFA concentrations. The rebound lasted up to 9 h for the 30 mg dose of NA and up to 6 h for the 6 mg dose. On balance, nicotinic acid was shown to be a potent antilipolytic agent in feed-restricted cattle with a negative energy balance. Sustained reductions in plasma NEFA concentrations are achieved as long as there is a constant supply of nicotinic acid to the lower parts of the digestive tract. The antilipolytic effect of nicotinic acid may be favourable to dairy cows provided that niacin is administered in optimal doses and forms, accompanied by a postruminal source of nicotinic acid. Nevertheless, the optimal dose of nicotinic acid should be determined, exerting a moderately inhibiting effect on lipolysis and NEFAs (adipose tissue NEFAs are an important energy source and precursors for the synthesis of fatty acids at the onset of lactation). In their study on the administration of rumen-protected niacin, Morey et al. [26] found that 24 g/d of encapsulated niacin (providing 9.6 g/d of bioavailable niacin) inhibited lipolysis in postpartum cows by decreasing postpartum NEFA concentrations. The treatment protocols used in this study are unequivocally associated with suppressing lipolysis in cattle, causing no rebound lipolysis. A total of 24 g/d of encapsulated niacin provides a source of bioavailable niacin which modifies lipid metabolism [36].

Notwithstanding the large influx of NEFAs into hepatocytes of early-lactation cows, decreased triglyceride concentrations were found in the liver of cows supplemented with rumen-protected niacin. As the accumulation of hepatic triglycerides is directly related to blood NEFA concentrations, reductions in blood NEFA concentrations lead to decreased triglyceride accumulation in postpartum niacin-supplemented cows [36]. In addition to fatty liver, the occurrence of ketosis is another negative consequence of elevated NEFA concentrations, i.e. the incomplete metabolism of NEFAs which are converted to ketone bodies. To prevent ketosis, cows should be supplemented with niacin alongside glycogen precursors such as propylene glycol and sodium propionate [37, 38]. The previous research suggests that niacin supplementation decreases plasma BHBA and NEFA concentrations with an increase in serum glucose [39, 40]. Erickson et al. [41] report significant effects of niacin on plasma BHB concentrations in niacin-supplemented cows compared to control animals. Relative to the control group, a

marked decrease in plasma BHB concentrations was recorded in cows supplemented with 12 g/d of niacin (in a crystal powder form), whereas a slighter decrease in plasma BHB concentrations was found in cows receiving 6 g/d of niacin [42].

In addition to lipolysis and ketogenesis, niacin exerts a major effect on lipid peroxidation and oxidative stress. Oxidative stress occurs when excess prooxidants (free radicals) overwhelm the antioxidant capacity of the organism. Such a state is associated with metabolic stress in periparturient cows [43]. It most commonly occurs when there is an imbalance between the increased production of free radicals and the decreased ability of the organism to neutralize them. Oxidation is part of the biochemical regulatory processes of the organism responsible for generating the energy required to sustain life. During these processes, free radicals are formed, having positive physiological functions. However, a physiological imbalance between excess free radicals and the ability of the organism to neutralize them changes the oxidative status of the organism, which thereafter enters a state of real oxidative stress (conducive to a number of various disorders and diseases). The degree of oxidative stress is determined by measuring the concentration and activity of prooxidants and antioxidants. Prooxidants are reactive oxygen metabolites containing an unpaired electron in the outermost electron shell, thereby participating in oxidation-reduction reactions. These reactive molecules can integrate into genetic and/or anatomical cell structures, inducing significant changes in cellular function [44, 45].

The reactive molecules most essential to periparturient cows are formed in the process of increased lipid mobilization. Nonesterified fatty acids are fairly reactive molecules susceptible to oxidation and free radical reactions. Fats are considered the best indicator of oxidative stress. Malondialdehyde (MDA) results from the reaction between free radicals and polyunsaturated fatty acids. It readily reacts with thiobarbituric acid to form thiobarbituric acid reactive substances (TBARS). MDA and/or TBARS concentrations are significantly increased in dairy cows after parturition. Moreover, a positive correlation has been found between MDA and TBARS concentrations and NEFA and BHB concentrations [46, 47].

In ruminants, antioxidants are divided into three major categories: (1) intracellular antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), (2) non-enzymatic protein antioxidants in plasma such as protein thiol groups in albumin and (3) nonenzymatic low-molecular-weight antioxidants such as glutathione, alpha-tocopherol, beta-carotene, etc. The antioxidant capacity of the cow's body is greatly dependent on the energy balance of the body. Therefore, ketotic cows, due to a negative energy balance, have been found to exhibit decreased antioxidant activity and increased concentrations of reactive lipid molecules. Antioxidant protection is also influenced by the diet and milk yield of cows. Calving has a significant impact on the antioxidant system of the cow, leading to a decrease in antioxidant concentrations in early lactation [15, 17, 18]. Niacin has been shown to exert the following antioxidant effects: decreasing lipolysis and lipid peroxidation, participating in the conversion of oxidized glutathione (GSSG) to the reduced form (GSH) by glutathione reductase (GR), decreasing the NADH+H⁺/NADP⁺ ratio and increasing the NAD⁺ content. As previously mentioned herein, niacin administration increases NAD concentrations [27].

In the study of Hristovska et al. [48], niacin-supplemented cows were found to exhibit lower NEFA concentrations in the periparturient period. NEFA concentrations remained invariant

in the niacin group within the first 2 weeks after calving, whereas a significant increase in NEFA concentrations was recorded in the control group in the same period. Niacin administration exerted a significant effect on metabolic adaptations in early-lactation cows. Cows supplemented with niacin exhibited considerably lower BHB concentrations, higher cholesterol and triglyceride concentrations, lower MDA concentrations, higher glucose concentrations, lower total bilirubin concentrations, lower liver enzyme activity (AST, ALP and GGT), higher albumin concentrations and lower urea and phosphorus concentrations. As the magnitude of lipolysis increases, niacin administration greatly reduces ketogenesis and lipid peroxidation. Niacin also exerts a substantial impact on the relationship between NEFA concentrations and other metabolic parameters; thus, a weak regression relationship was found between NEFA values and glucose, cholesterol, triglycerides, total bilirubin, AST, albumin, urea and phosphorus values. Niacin reduces the dependence of metabolic adaptations in early-lactation cows on the degree of lipid mobilization. Furthermore, niacin administration to periparturient cows positively affects lipid metabolism in early lactation, i.e. decreased lipid mobilization (decreased NEFA concentrations), ketogenesis (decreased BHB concentrations) and liver lipidosis (higher triglyceride and cholesterol concentrations in the blood and higher cholesterol concentrations per unit NEFA) [49].

5. Effects of niacin administration on insulin resistance

Insulin resistance is a state of reduced biological effect of insulin, leading to a compensatory increase in insulin concentrations [12]. It is associated with a diminished insulin response to glucose, i.e. insulin hyporesponsiveness (reduced beta cell function) and/or insulin sensitivity (depressed insulin-regulated glucose uptake in tissues). From the receptor's perspective, insulin resistance is referred to as pre-receptor (decreased insulin secretion and/or increased insulin degradation), receptor (a decreased number of receptors and/or their affinity for binding insulin) and post-receptor (defects in cell signalling and translocating glucose transporters). Insulin resistance in periparturient cows is attributed to the primary glucose requirements essential for foetal growth, udder development and lactation. In Holstein cows, insulin resistance is further influenced by plasma NEFA concentrations.

As niacin decreases lipolysis and increases glycaemia, it can facilitate insulin production and efficiency, as well as reduce insulin resistance. Some studies have failed to show significant effects of either niacin treatments or niacin treatment duration on blood glucose in cows receiving either rumen-protected or not rumen-protected niacin [26, 36, 50, 51]. Thornton and Schultz [52] reported the following changes in the metabolism of glucose in ruminants upon administering pharmacological doses of nicotinic acid: increased plasma glucose and insulin concentrations, reduced tolerance to glucose and reduced insulin resistance. Di Costanzo et al. [18] found a significant increase in blood glucose concentrations in cows supplemented with 36 g/d of nicotinic acid. Such an increase in blood glucose concentrations can enhance the cellular gluconeogenic activity, induced by the partial suppression of lipogenesis. Feed-restricted cows, abomasally infused with pharmacological doses of nicotinic acid, were found to exhibit elevated insulin concentrations 4–8 h after the termination of NA infusions.

Increased glucose concentrations were recorded during a rebound of plasma NEFA concentrations (upon the initial decrease), whereas insulin concentrations followed a similar pattern to that of the NEFA rebound [14]. Pires et al. [14] argue that decreased NEFA concentrations in feed-restricted Holstein cows infused with nicotinic acid enhance the insulin response and glucose uptake with an increase in insulin sensitivity (suggesting that blood NEFA concentrations are a relevant factor in the occurrence of insulin resistance in dairy cows with a negative energy balance). These results are consistent with the results obtained in a study involving human subjects infused with acipimox (a long-acting nicotinic acid analogue). Acipimox was shown to decrease blood NEFA concentrations, increase the response to the oral glucose tolerance test and enhance the insulin-stimulated glucose uptake in peripheral tissue (using the hyperinsulinemic-euglycemic clamp technique) [53, 54].

Niacin has been shown to greatly affect glucose concentrations. An increase in glucose concentrations is dependent upon the niacin dose administered and treatment duration. Pescara et al. [55] claim that the mechanism by which nicotinic acid increases plasma glucose concentrations is unelucidated, but it may be attributable to the increased hepatic production of glucose or reduced blood glucose clearance or both. Blood insulin concentrations followed a similar dynamic pattern to that of blood glucose concentrations. An increase in glucose concentrations was recorded on days 10 and 12 of nicotinic acid infusions, continuing 1 day after treatment termination, whereas blood insulin concentrations increased during the entire treatment process [30]. According to Titgemeyer et al. [30], it is inconclusive whether an increase in glucose concentrations leads to an increase in insulin concentrations or insulin resistance causes elevated glucose concentrations. Their model is at variance with those stating that increased NEFA concentrations are associated with insulin resistance during nicotinic acid treatments. Differences in the results obtained can partially be accounted for by different energy supplies and degrees of lipolysis, indicating that both insulin and glucose concentrations in the blood are affected by niacin. Titgemeyer et al. [30] also found that glucagon concentrations were not significantly altered, inferring that glucagon was of little or no significance to the effect of niacin on blood glucose concentrations.

One of our studies has hypothesized that niacin administration to dairy cows in the transition period can influence insulin responsiveness and resistance in adipose tissue by virtue of niacin-induced changes in NEFA, glucose and insulin concentrations [56]. A total of 30 clinically healthy, multiparous Holstein-Friesian cows in late gestation were enrolled in the study. Insulin resistance was calculated on the basis of the following insulin resistance indicators: the glucose-to-insulin (G:I) ratio and the Revised Quantitative Insulin Sensitivity Check Index (RQUICKI). The formula for the glucose-to-insulin ratio is as follows: $G:I = \text{glucose (mg/dL)} / \text{insulin } (\mu\text{U/ml})$. The RQUICKI is calculated on the basis of plasma concentrations of glucose (mg/dl), insulin ($\mu\text{U/ml}$) and free fatty acids (mmol/l), using the following formula: $RQUICKI = 1 / [\log(\text{glucose mg/dL}) + \log(\text{insulin } \mu\text{U/ml}) + \log(\text{NEFA mmol/l})]$. The RQUICKI is a good indicator of insulin resistance in dairy cows. Although lipolysis-dependent, the RQUICKI correlates with numerous metabolic parameters [57, 58]. The influence of niacin supplementation, in the week of calving and the first week after parturition, on glucose, insulin and NEFA concentrations, as well as RQUICKI values, was analyzed using the analysis of variance (ANOVA). According to the RQUICKI values obtained, niacin-supplemented and

control cows ($n = 15 \text{ cow} \times 3 \text{ week} = 45$) were allocated to two groups: a more resistant group ($\text{RQUICKI} < 0.5$) and a less resistant group ($\text{RQUICKI} \geq 0.5$). Differences in glucose, insulin and NEFA concentrations between the two groups were determined using paired t-tests. Moreover, a linear regression analysis ($Y = bXi + a$) was performed on the basis of all the parameter values obtained in the niacin-supplemented and control groups in order to determine differences in the slope of regression lines (differences in the b parameters). Cows in the niacin group, which were more resistant to insulin ($\text{RQUICKI} < 0.5$), exhibited higher concentrations of nonesterified fatty acids compared to more sensitive cows in the same group but still lower than those recorded in control animals. The regression analyses performed suggest the following characteristics of niacin-supplemented cows relative to the control group: increased insulin response to glucose, decreased antilipolytic effect of insulin and increased insulin efficiency (expressed as the glucose-to-insulin ratio) with a decrease in NEFA concentrations. Niacin was found to exert a dual influence on insulin resistance in early-lactation dairy cows: decreased NEFA concentrations led to a decrease in insulin resistance (due to an increase in insulin efficiency and the insulin sensitivity index), whereas elevated insulin and glucose concentrations most likely caused an increase in insulin resistance in dairy cows (due to the lower insulin sensitivity index and antilipolytic effect of insulin).

6. Effects of niacin administration on the inflammatory response following metabolic stress

Inflammation is the common denominator of a number of processes occurring in cows during the periparturient period. Therefore, increased lipolysis may precipitate a substantial release of proinflammatory cytokines within adipose tissue, i.e. adipokines, the most important of which is tumour necrosis factor alpha ($\text{TNF-}\alpha$) [59]. Ohtuska et al. reported increased serum $\text{TNF-}\alpha$ activity in cows with moderate-to-severe fatty liver syndrome [60]. The organism protects itself from inflammation by secreting acute-phase proteins. Plasma haptoglobin and serum amyloid A concentrations have been found to be elevated in cows with fatty liver [61]. In addition to decreased albumin and cholesterol concentrations, Bertoni et al. recorded increased bilirubin, AST and GGT concentrations in cows with a high inflammatory index, which is indicative of the biochemical profile of fatty liver [62]. Inflammatory mediators were directly implicated in metabolic changes by Trevisi et al. upon the peroral administration of interferon- α during the last 2 weeks of gestation, which led to liver inflammation and the release of acute-phase proteins [63]. Relative to the control group, cows treated with interferon- α were found to exhibit significantly higher plasma ketone concentrations during the first 2 weeks after parturition. A number of experimental studies have shown a direct impact of NEFAs on inflammatory processes such as the regulation of peroxisome proliferator-activated receptors (PPARs). PPARs modulate the inflammatory response in many cells such as adipocytes. In monocytes, PPARs activate certain polyunsaturated fatty acids such as α -linolenic acid and docosapentaenoic acid, which can suppress the inflammatory response. Another instance of the effect of lipids on receptor binding is the activation of Toll-like receptors (TLRs), especially TLR4. In addition to the

ascending regulation of proinflammatory cytokines, the activation of TLR4 can lead to the inflammatory response [64–67]. The activation of the innate immune response is incited by the activation of TLR receptors present on immune and non-immune cells (able to identify pathogens). TLR4 identifies lipopolysaccharides (endotoxins), which are the major component of the outer membrane of Gram-negative bacteria [68]. The typical proinflammatory response to lipopolysaccharides entails the expression of several acute-phase cytokines (TNF, IL-1 and IL-8) and leukocyte-endothelial adhesion molecules, as well as the influx and activation of neutrophils in inflamed tissues. Furthermore, increased lipid hydroperoxide concentrations, associated with oxidative stress, have been found to induce an increase in the proinflammatory phenotype of endothelial cells [69, 70]. TNF- α , IL-1, IL-6 and IL-8 have been implicated in the occurrence of coliform mastitis in periparturient cows in a state of oxidative stress [71].

Niacin reduces adipose tissue inflammation by increasing adiponectin concentrations, thereby regulating the metabolism of carbohydrates and insulin sensitivity of adipose tissue. Such results have been obtained in cows and laboratory mice [72, 73]. Nicotinic acid increases adiponectin secretion through G-protein-coupled receptor signalling in cattle.

Niacin administration reduces TNF- α and IL-6, as well as the activation of NF- κ B in the lungs and kidneys of rats [74, 75]. In monocytes, niacin suppresses the NF- κ B signalling pathway, thus reducing proinflammatory mediators (namely, TNF- α , IL-6 and MCP-1) [76, 77] and inhibiting monocyte chemotaxis [78]. It also decreases C-reactive protein (CRP) concentrations, as well as macrophage accumulation in the liver and hepatocyte inflammation, which results in reducing acute-phase protein production [79, 80]. The anti-inflammatory effect of niacin is associated with the activation of niacin receptors [76, 77].

7. Conclusion

Niacin should be administered to ruminants in adequate pharmacological doses and forms on account of their complex stomach. The antilipolytic effect of niacin reduces metabolic stress in periparturient cows. Moreover, metabolic adaptations in the periparturient period are significantly less dependent on the magnitude of lipolysis provided niacin is administered. Niacin reduces lipid peroxidation and the degree of oxidative stress in cows by the NAD and NADP coenzymes. The antilipolytic effect of niacin decreases insulin resistance in cows. However, its potential to elevate glucose and insulin concentrations may attenuate the antilipolytic effect of insulin due to increased insulin resistance in a state of metabolic stress. Niacin exerts its anti-inflammatory effect by stimulating the secretion of adiponectin and inhibiting immune cells.

Conflict of interest

The authors declare no conflict of interest.

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