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Niacin nutrition and rumen-protected niacin supplementation in dairy cows: an updated review

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Abstract

As the precursor to NAD^+ and $NADP^+$, niacin is important for catabolic and anabolic redox reactions. In addition, niacin is known for its anti-lipolytic action via a hydroxycarboxylic acid-2-receptor-dependent mechanism. The anti-lipolytic effects of traditional free niacin supplementation during transition periods had been studied extensively, but the reported effects are ambiguous. In the past decade, a series of studies were conducted to evaluate the effects of rumen-protected niacin (RPN) on production performance and metabolic status in early lactation and on heat stress in dairy cows. Feeding RPN seems more effective than free niacin regarding increasing circulating niacin concentration. The rebound of plasma NEFA was found after termination of niacin abomasal infusion. Feeding RPN or infusion of niacin via the abomasum could suppress lipolysis and reduce insulin resistance in early lactation. Additionally, RPN supplementation could possibly relieve heat stress through vasodilation during moderate to severe heat stress condition. However, these beneficial effects of niacin supplementation have not always been observed. The inconsistent results across studies may be related to dosages of niacin supplementation, rebound of plasma NEFA concentration, stage of lactation or severity of heat stress. Overall, the current review is to present updated information on niacin nutrition in dairy cows and the recommendations are given for future research.

Key words: **Niacin: Dairy cows: Transition period: Heat stress: Hydroxycarboxylic acid-2 receptor**

Niacin (vitamin B_3) is an essential water-soluble vitamin, comprising two main forms as nicotinic acid and nicotinamide. Niacin is the precursor to NAD^+ and $NADP^+$, which are important coenzymes in catabolic and anabolic redox reactions, respectively. Besides being precursor to coenzymes, nicotinic acid is also known for its anti-lipolytic action via the hydroxycarboxylic acid-2 receptor (HCA_2)[1]. On one hand, the National Research Council suggested that niacin supplementation may not be necessary for lactating dairy cows in terms of improving production performance[2]. On the other hand, a number of studies found that pharmacological doses of niacin did have some beneficial effects for cow health, especially under challenging conditions. For instance, supplementing pharmacological doses of niacin was reported to decrease the ketosis prevalence in early lactation[3] and reduce the heat stress[4] in dairy cows. These benefits are likely related to pharmacological effects of niacin on lipid metabolism or vasodilation rather than alleviating niacin deficiency, because the ruminal synthesis of niacin could cover the requirement[2]. However, the reported effects of niacin supplementation are not always consistent across studies. The synthesis and absorption of niacin, and effects of non-rumen-protected niacin (non-RPN) supplementation on milk yield and composition, and plasma metabolite concentrations in dairy cows were thoroughly reviewed by Niehoff *et al.*[5] In the past decade, the RPN was developed, which could be more efficient than free niacin. Moreover, HCA_2 was found to be widely expressed in different tissues in dairy cows[6], which provides new insight into niacin's mechanisms of action. Therefore, this aim of the current review is to present the updated information on ruminal niacin synthesis, the role of HCA_2, effects of niacin supplementation on ruminal metabolism and nutrient digestibility, metabolic status, milk yield and composition, insulin resistance during early

Abbreviations: ARS, apparent ruminal synthesis; BHBA, β-hydroxybutyrate; HCA_2, hydroxycarboxylic acid-2 receptor; HSL, hormone-sensitive lipase; RPN, rumen-protected niacin; RQUICKI, revised quantitative insulin sensitivity check index; THI, temperature humidity index.

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Table 1. Niacin contents in several feedstuffs

Feedstuff	Niacin content (mg/kg DM)	References
Maize silage	24·0–204·2	Seck *et al.*[7]; Schwab *et al.*[8]; Beaudet *et al.*[9]
Alfalfa silage	21·6–291·0	Seck *et al.*[7]; Castagnino *et al.*[10]; Castagnino *et al.*[11,12]
Orchardgrass silage	52·4–81·7	Castagnino *et al.*[10]; Castagnino *et al.*[11,12]
Grass hay	12·1	Schwab *et al.*[8]
Alfalfa hay	34·0–70·2	Schwab *et al.*[8]; Beaudet *et al.*[9]
Wheat straw	46·6	Beaudet *et al.*[9]
Soyabean hulls	44·6–228·9	Schwab *et al.*[8]; Beaudet *et al.*[9]
Maize	7·0–30·2	Seck *et al.*[7]; Schwab *et al.*[8]; Castagnino *et al.*[10]; Castagnino *et al.*[11,12]
Barley	36·9	Schwab *et al.*[8]
Beet pulp	36·9	Beaudet *et al.*[9]
Soyabean meal	34·5–58·4	Seck *et al.*[7]; Schwab *et al.*[8]; Beaudet *et al.*[9]; Castagnino *et al.*[10]; Castagnino *et al.*[11,12]
Blood meal	45·2–56·3	Schwab *et al.*[8]; Castagnino *et al.*[11]

lactation and heat stress, with emphasis on RPN and studies published in the last decade. An attempt was made to point out the problems in relation to niacin nutrition in dairy cows for future research.

Niacin sources and bioavailability

Niacin is naturally present in animal by-products and plant-source feedstuffs. The niacin content of forage is highly variable, and the niacin content of grains seems relatively low (Table 1). The daily intake of niacin in dairy cows is largely dependent on feed intake and diet composition, varying from 325 to 4434 mg/d as reported by various researchers in different studies[7–11]. In addition to the niacin supplied from feed, ruminants can synthesise niacin from tryptophan and quinolinic acid. This synthesis, however, is relatively inefficient, and tryptophan is preferentially utilised for protein synthesis[13].

Ruminal synthesis of niacin by micro-organisms is considered as the main source of niacin for ruminants. Nevertheless, the actual ruminal synthesis of niacin is difficult to measure. The apparent ruminal synthesis (ARS) of niacin, calculated as the niacin content in duodenal flow minus daily niacin intake, is often used to estimate the microbial niacin synthesis. It was estimated that ARS of niacin in a 650-kg cow producing 35 kg/d of 4 % fat-corrected milk was 1804 mg/d[2]. Niehoff *et al.*[5] reviewed six studies published between 1985 and 2006 and suggested that ARS of niacin is affected by forage to concentrate ratio or non-fibre carbohydrate content. In the last decade, several studies also investigated the influence of different types of feed on the ARS of niacin (Table 2). Niehoff *et al.*[14] observed that ARS of niacin was less in cows fed the low concentrate diet compared with the high or medium concentrate diet, which is consistent with the findings of Seck *et al.*[7] In addition, the diet with high niacin content[9,12] would likely result in negative ARS of niacin. Especially, 98·5 % of supplementary nicotinamide[15] and 88–94 % of supplementary nicotinic acid[14] disappeared in the rumen, resulting in a greater negative ARS of niacin. The exact mechanism of this phenomenon is not clear. It is suggested that a niacin homeostatic system may exist in the rumen: synthesis will occur when the niacin content is below the optimal level, and niacin is degraded by bacteria when the niacin content is above the optimal level[5]. In order to increase the bioavailability of niacin in the small intestine, RPN was developed. It was stated that the ruminal stability of RPN is approximately 90 % and could deliver daily about 40 % bioavailable niacin[16,17]. Thus, it can be expected that feeding RPN is more effective compared with the traditional free niacin.

Unlike the traditional free niacin, the reported effects of RPN supplementation on plasma niacin concentration were relatively consistent. All four studies that determined plasma niacin concentration detected a significant increase in niacin content in plasma after an RPN supplementation[16–19]. This could be related to the increased bioavailability of RPN. Rungruang *et al.*[19] reported that RPN supplementation (0, 4, 8 or 12 g/d) increased plasma and milk niacin concentrations in a linear manner. However, the niacin concentration in that study was determined by microbiological assay, which is not possible to distinguish between nicotinic acid and nicotinamide. Morey *et al.*[16] found the plasma concentration of nicotinic acid was not influenced by RPN supplementation and were about 100 times lower than plasma nicotinamide concentration. In the study by Zeitz *et al.*[17], plasma nicotinic acid concentration was below the detection limit (0·025 μg/l). Those results are in agreement with an earlier study by Kollenkirchen *et al.*[20] who reported that only nicotinamide was present in the blood of sheep after nicotinic acid or nicotinamide supplementation. It is generally accepted that nicotinic acid is readily converted into NAD^+ in the intestine and liver and then is cleaved to produce nicotinamide into blood for extrahepatic tissues[21]. It implies that nicotinamide might be the main transport form in blood, which could partly explain the undetectable or extremely low concentration of nicotinic acid in blood. Additionally, the kinetics of niacin supplementation in dairy cows is not completely clear. In humans, the maximal plasma concentration of nicotinic acid is reached after 30–60 min when administered orally[22], and the plasma half-life of nicotinic acid is about 20–45 min, whereas the half-life of nicotinamide is about 4 h[23]. In dairy cows, the half-life of nicotinamide could probably be longer than 4 h, because Zimbelman *et al.*[18] found the serum niacin concentration returned to presupplementation values by 3 d after a dose of 12 g/d RPN supplementation. In above-mentioned studies, the difference in half-life time could be another reason for the different content in blood of two niacin vitamers as the blood samples were collected before feeding. However, Campbell *et al.*[24] reported that concentrations of plasma nicotinic acid and plasma

Table 2. Apparent ruminal synthesis (ARS) of niacin in dairy cows

Reference	Diet	NS (g/d)	DMI (kg/d)	NI (mg/d)	DNF (mg/d)	ARS (mg/d)
Niehoff *et al.*[14]†	Low-concentrate diet (1/3 concentrate + 2/3 forage)	0	12·1	533	1602	1057
	Low-concentrate diet (1/3 concentrate + 2/3 forage)	6	12·4	6449	2021	–4419
	Medium-concentrate diet (1/2 concentrate + 1/2 forage)	0	12·3	325	1886	1575
	Medium-concentrate diet (1/2 concentrate + 1/2 forage)	6	12·6	6337	2221	–4089
	High-concentrate diet (2/3 concentrate + 1/3 forage)	0	12·2	476	1895	1421
	High-concentrate diet (2/3 concentrate + 1/3 forage)	6	12·4	6370	2630	–3738
Beaudet *et al.*[9]	High-N (14 % CP) + high-starch diet	0	19·9	2135	1604	–530
	Low-N (11 % CP) + high-starch diet	0	20	2143	1473	–670
	High-N (14 % CP) + high-fibre diet	0	20·4	2215	1530	–1003
	Low-N (11 % CP) + high-fibre diet	0	20·3	2170	1166	–684
Castagnino *et al.*[10]	Early-cut alfalfa silage diet	0	28·6	4434*	1268*	–3168*
	Late-cut alfalfa silage diet	0	26·8	1902*	1602*	–300*
	Early-cut orchardgrass silage diet	0	22·5	1291	1671	380
	Late-cut orchardgrass silage diet	0	22·4	1269	1712	519
Castagnino *et al.*[11]	Alfalfa silage diet	0	20·9	466*	1197*	731
	Orchardgrass silage diet	0	20	1128*	1662*	585
	Alfalfa silage diet	0	24·2	1160	1993	845
	Orchardgrass silage diet	0	23·2	1167	1879	711
Castagnino *et al.*[12]	Long-cut alfalfa silage diet	0	26·3	2667	1414	–1253
	Short-cut alfalfa silage diet	0	27·2	2550	1510	–1097
	Long-cut orchardgrass silage diet	0	21·8	715	1313	587
	Short-cut orchardgrass silage diet	0	22·7	1033	1325	293
Seck *et al.*[7]	Low-forage diet (45 % forage: 55 % concentrate)	0	27	1135*	3020*	1885*
	High-forage (61 % forage: 39 % concentrate)	0	24	1035*	3866*	2831*

NS, niacin supplementation; DMI, DM intake; NI, niacin intake; DNF, duodenal niacin flow.
* Significant difference ($P \leq 0{\cdot}05$) between groups within a study. Niehoff *et al.*[14] did not illustrate the significance.
† Organic matter intake was determined in the study of Niehoff *et al.*[14].

nicotinamide did not differ over time in 11 h after 12 g/d of free nicotinic acid or nicotinamide supplementation. Thus, further research are required to clarify the kinetics of niacin, especially RPN, in ruminants.

Effects of free niacin supplementation on ruminal metabolism and nutrient digestibility

Effects of free niacin supplementation on ruminal metabolism and nutrient digestibility have been inconsistent (Table 3). Although free niacin supplementation has been reported to increase microbial protein synthesis in two *in vitro* studies[32,25], microbial protein production increased in only two of seven *in vivo* studies. However, five *in vivo* studies reported that protozoa number (mainly *Entodinium*) in rumen fluid was increased by niacin supplementation. Ruminal protozoa are unable to synthesise niacin and the niacin from diet and ruminal bacteria might not be adequate to cover their requirement[28,29]. Thus, the free niacin supplementation may improve the growth for ruminal protozoa in the rumen and increase the protozoal predation of bacteria[33]. Aschemann *et al.*[30] proposed that the free niacin supplementation might change the composition of microbial protein by increasing the protozoal protein and decreasing the bacterial protein, which could be the reason for the unchanged microbial protein content reaching the duodenum. In addition, those authors suggested the reduced faecal N excretion in niacin supplemented animals could also be related to the increased protozoal protein because the digestibility of protozoal protein is greater than bacterial protein[34].

The ruminal protozoa digest 25–30 % of the total fibre[35]. Accordingly, the increased protozoa number during free niacin supplementation could be expected to improve the fibre digestibility. Indeed, the neutral-detergent fibre digestibility increased in two studies[34,31], but not in other studies[14,24,28,26]. This discrepancy could be related to dietary composition. Aschemann *et al.*[34] suggested that niacin may be less beneficial to fibre degradation when the N supply from the diet is optimal. In addition, the protozoa number could possibly influence the ruminal fermentation pattern, since the protozoa number was reported to be negatively related to acetic acid concentration in the rumen[36]. In line with this, free niacin supplementation led to a decrease in acetic acid proportion[30] and tended to decrease the acetic acid content[27]. Nevertheless, propionate, butyrate and total SCFA were mostly unaffected by free niacin supplementation in *in vivo* studies. In summary, free niacin supplementation increases the protozoa number in the rumen, but the changed protozoa population may not be sufficient to significantly affect the fermentation pattern and nutrient digestibility in ruminants.

Niacin supplementation during transition periods

Metabolic status

During early lactation, dairy cows typically experience a negative energy balance because the feed intake is not adequate to meet the nutrient demand for the rapidly increasing milk yield[37]. Body fat is mobilised to compensate for the energy deficit that results in a substantial increase in plasma NEFA, β-hydroxybutyrate (BHBA) and liver TAG in dairy cows[38]. The negative energy balance-induced metabolic changes have been associated with increased incidence of metabolic disorders and infectious diseases, increased culling rate and reduced

Table 3. Effects of nicotinic acid (NA) or nicotinamide (NAM) supplementation on ruminal metabolism and nutrient digestibility

Reference	Niacin supplementation	SCFA	Microbial protein	Protozoa	Digestibility		
					OM	NDF	ADF
Horner *et al.*[25]	6 g NA/d	–	–	↑	N/A	↑	–
Erickson *et al.*[26]	12 g NA/d	N/A	N/A	↑	–	–	–
	12 g NAM/d	N/A	N/A	–	–	–	–
Campbell *et al.*[24]	12 g NA/d	–	–	–	–	–	–
Christensen *et al.*[27]	12 g NA/d	–	–	N/A	–	↓§	↓
Doreau & Ottou[28]	6 g NA/d	–	–	↑	–	–	–
Kumar & Dass[29]	100 mg NA/kg feed	↑	↑	↑	N/A	N/A	N/A
	200 mg NA/kg feed	↑	↑	↑	N/A	N/A	N/A
Aschemann *et al.*[30]	6 g NA/d	–	–	↑	–	↑	–
Niehoff *et al.*[13]	6 g NA/d	↓	↑	N/A	↓	–	–
Luo *et al.*[31]	320 mg NA/kg feed	N/A	N/A	N/A	–	–	–
	480 mg NA/kg feed	N/A	N/A	N/A	–	↑	↑
	640 mg NA/kg feed	N/A	N/A	N/A	↑	↑	↑

OM, organic matter; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; N/A, not available.
↑, Significantly increased ($P \leq 0{\cdot}05$); ↓, significantly decreased ($P \leq 0{\cdot}05$); ↓§, tended to decrease ($0{\cdot}1 \geq P > 0{\cdot}05$); – , no significant effect of niacin supplementation ($P > 0{\cdot}1$).

Table 4. Effects of niacin supplementation during transition periods on DM intake (DMI), milk yield, milk composition and plasma metabolites

Reference	No. of cows	Niacin supplementation	DMI	Milk yield	Milk protein	Milk fat	Plasma metabolites				
							NEFA	BHBA	Glucose	Insulin	Liver TAG
Morey *et al.*[16]*	22	12 g RPN/d	–	–	–	–	–	–	–	–	–
Yuan *et al.*[44]	30	12 g RPN/d	–	–	–	↓	↓	–	–	N/A	–
Kenéz *et al.*[48]	20	24 g NA/d	–	–	N/A	N/A	–	N/A	N/A	N/A	N/A
Tienken *et al.*[47]	56	24 g NA/d	–	–	–	↑§	–	–	–	N/A	N/A
Hristovska *et al.*[45]	30	120 g NA/d	N/A	N/A	N/A	N/A	↓	↓	↑	N/A	N/A
Wei *et al.*[46]	40	45 g NAM/d	–	–	–	–	↓	↓	↑	N/A	N/A
Zeitz *et al.*[17]	30	55 g RPN /d	–	–	–	↓	–	↓	N/A	N/A	–

BHBA, β-hydroxybutyrate; RPN, rumen-protected nicotinic acid; N/A, not available; NA, nicotinic acid; NAM, nicotinamide.
↑, Significantly increased ($P \leq 0{\cdot}05$); ↓, significantly decreased ($P \leq 0{\cdot}05$); ↑§, tended to increase ($0{\cdot}1 \geq P > 0{\cdot}05$); –, no significant effect of niacin supplementation ($P > 0{\cdot}1$).
* Treatment × parity × time interactions were detected for plasma NEFA and BHBA concentrations.

fertility[39,40,41]. Nicotinic acid has been long known as a lipid-lowering compound due to its anti-lipolytic action[42]. Thus, nicotinic acid is expected to suppress lipolysis in early lactation to improve the health of dairy cows, but the reported effects are inconsistent.

Circulating NEFA is an important indicator of adipose mobilisation and metabolic health in dairy cows[43]. In some studies[44–46], supplementation of nicotinic acid, RPN or nicotinamide during transition periods was reported to decrease the plasma NEFA concentration, but in some other studies[17,47,48] supplementation had no influence (Table 4). Additionally, Morey *et al.*[16] reported that 12 g/d RPN supplementation in early lactation decreased peak plasma NEFA concentration but did not affect the average plasma NEFA concentration. The niacin supplementation did not affect feed intake or milk yield in early lactation in most studies, thus the discrepancy in plasma NEFA concentration among studies could not be attributed to the differences in energy input or milk energy output. It is likely that niacin supplementation level may play a role in the anti-lipolytic effect in dairy cows. In early lactation, the relatively low dosages of free nicotinic acid or nicotinamide supplementation (6–24 g/d) in studies summarised by Niehoff *et al.*[5] and two more recent studies[47,48] demonstrated only minor effects, whereas 12 g/d of RPN[16,44], 45 g/d of nicotinamide[46] or 120 g/d of nicotinic acid[45] supplementation demonstrated a greater influence on plasma NEFA concentration. The extensive degradation of free niacin in the rumen may partly explain the lack of effects when a low level of nicotinic acid was supplemented.

Pires *et al.*[49] reported that abomasal infusion of nicotinic acid delivered as a single bolus or hourly could significantly decrease the plasma NEFA concentration during feed restriction. Later, Pescara *et al.*[50] and Pires *et al.*[51] confirmed that finding and found continuous abomasal infusion of 3 mg nicotinic acid/h per kg of body weight was sufficient to depress plasma NEFA concentration during feed restriction. However, a dramatic rebound of plasma NEFA concentration was observed 2–4 h after termination of infusion, and the duration of the rebound lasted 4–9 h[49,50]. These observations indicate that a certain level of rumen bypassing niacin supplementation could suppress lipolysis, but the rebound of plasma NEFA concentration may interfere with the evaluation of niacin's anti-lipolytic effect in dairy cows. Therefore, the blood sampling time may be another reason for the differences in plasma NEFA concentrations among studies, because the plasma NEFA concentration could vary greatly depending on the time interval between feeding and blood sampling.

Plasma NEFA can be partially oxidised to form BHBA or esterified to be stored in the liver as TAG[39]. As expected, Hristovska *et al.*[45] and Wei *et al.*[46] found the plasma BHBA concentration decreased after niacin supplementation. Similar to plasma NEFA concentration, abomasal infusion of niacin

decreased plasma BHBA concentration, and the rebound of plasma BHBA concentration after termination of infusion was found in feed-restricted dairy cows[50,51]. However, plasma BHBA and liver TAG concentrations were not affected, while plasma NEFA concentration decreased after RPN supplementation in the study by Yuan *et al.*[44] It was suggested that plasma NEFA concentration may not be the only factor that determines the plasma BHBA[38]. In addition, Zeitz *et al.*[17] found plasma BHBA concentration, but not plasma NEFA concentration, decreased after supplementation with 55 g/d RPN. The authors declared that effects of nicotinic acid on ketogenesis might occur only at very high levels. In contrast, 3·5 g/d of RPN supplementation in early lactation tended to decrease ketosis prevalence from 36 to 20 %, but 14 g/d RPN supplementation had no effect on ketosis prevalence in a study involving 906 dairy cows[3]. These findings indicate that it is possible to decrease the ketosis prevalence in dairy herd by RPN supplementation, though the level of supplementation still needs to be determined.

In human studies, it was shown that niacin may decrease the rate of liver TAG synthesis by inhibition of the diglyceride acyltransferase-2 which is independent of niacin's anti-lipolytic action[52,53]. However, this was not found in dairy cows. Several studies reported that RPN supplementation had no effects on liver TAG concentration. It is not clear whether this is also related to the plasma NEFA rebound[16,17,44].

Insulin resistance

Insulin resistance is defined as a state of either decreased sensitivity or responsiveness to insulin in the insulin-sensitive body tissues[54]. Insulin resistance together with the lowered plasma insulin concentration in early lactation facilitates lipolysis in adipose tissue and spare glucose for milk synthesis[40]. It is believed that high plasma NEFA concentration is the major cause of insulin resistance in dairy cows[55], thus niacin supplementation could possibly decrease the insulin resistance in dairy cows in early lactation. Hristovska *et al.*[56] concluded that niacin supplementation had a dual influence on insulin resistance during early lactation based on the revised quantitative insulin sensitivity check index (RQUICKI), which is a surrogate index for insulin sensitivity. Another study also concluded that RPN supplementation could improve the peripheral tissue sensitivity based on RQUICKI[57]. However, RQUICKI was derived from human-based studies and may not be suitable to assess the sensitivity of the peripheral tissues to insulin in early lactation in dairy cows[58]. Several studies demonstrated no correlation between RQUICKI and the results obtained by the gold standard tests (the hyperinsulinaemic–euglycaemic clamp test) or intravenous glucose tolerance test in dairy cows[59,60]. Therefore, the reported effects of niacin on insulin sensitivity based on RQUICKI might not accurately reflect the actual insulin sensitivity states and could lead to confusing conclusions.

Pires *et al.*[49] evaluated the effects of hourly abomasal infusion of niacin on insulin resistance using intravenous glucose tolerance test in a short-term study (11 h), and these authors found niacin supplementation enhanced insulin response in feed-restricted dairy cows probably by lowering plasma NEFA concentration. Nonetheless, the same research group found that a long-term (74 h) abomasal infusion of niacin decreased plasma NEFA concentration but caused an insulin resistance state in feed-restricted dairy cows[51]. Apparently, the niacin anti-lipolytic effect did not result in an enhanced insulin response in the long-term niacin abomasal infusion, but the underlying mechanisms are still unknown.

Milk yield and composition

Milk yield and milk protein percentage were not affected by either RPN or free niacin supplement in early lactation in several studies[16,17,44,46–48], which is in line with most of the earlier studies investigating the effects of free niacin supplementation on milk yield and milk protein percentage[5]. Supplementation of RPN in early lactation decreased milk fat percentage in two studies[17,44]. It was assumed that niacin supplementation might affect milk composition by affecting ruminal microbial growth or adipose tissue lipolysis[8]. As discussed above, the niacin supplement has minor effects on microbial protein synthesis or ruminal nutrient digestibility and the RPN is unlikely to affect ruminal metabolism due to its high ruminal stability. Hence, the decreased milk fat percentage after the RPN supplementation might mainly contribute to the decreased plasma NEFA concentration because plasma NEFA could be used for milk fat synthesis by mammary glands[44]. However, decreased[17] or tendency of increased[47] milk fat percentage without affecting plasma NEFA concentration was found after niacin supplementation in early lactation. It is possibly that niacin may directly affect lipid metabolism in the mammary gland independent of the anti-lipolytic function. Further studies are needed to confirm this hypothesis.

Supplementation of niacin prepartum

Two studies evaluated the effects of supplementation of 48 g/d free nicotinic acid[61] or 12 g/d RPN[57] on colostrum quality only in prepartum or on metabolic status in early lactation. Aragona *et al.*[61] found nicotinic acid supplementation prepartum significantly increased IgG concentration but did not affect calf immunity or calf body weight. The authors suggested that the increased IgG may be related to the increased IgG transferred to colostrum due to increased blood flow or the increased microbial protein synthesis after niacin supplementation. Surprisingly, plasma NEFA concentration increased in the first week after calving in dairy cows supplemented with nicotinic acid, which the author attributed to the rebound of NEFA concentration[61]. In the study of Youssef *et al.*[57], no significant effects of RPN supplementation prepartum were observed on plasma NEFA, BHBA, glucose or insulin concentrations in early lactation. Those findings indicate that niacin supplementation may improve the colostrum quality, but niacin supplementation only in prepartum had limited or even negative effects on metabolic status in early lactation.

Niacin supplementation during heat stress

Skin flushing, resulting from a cutaneous vasodilation, is considered as the most common side effect of nicotinic acid in humans[62]. The vasodilatory reaction of nicotinic acid could be

used to improve heat dissipation through enhanced evaporative heat loss and then to alleviate heat stress in dairy cows. In addition, Rungruang *et al.*[19] found the niacin concentration in whole blood was significantly decreased in heat-stressed dairy cows, which implies that the niacin metabolism might increase and dietary supplementation of niacin might be necessary in heat stress conditions. Indeed, RPN or free nicotinic acid supplementation were reported to reduce the vaginal temperature[18,63,64] or skin temperatures[65] during moderate to severe heat stress conditions with a temperature humidity index (THI) > 72. However, Lohölter *et al.*[66] reported that 24 g/d free nicotinic acid supplementation did not affect skin or rectal temperatures during a mild heat stress (THI > 68). The relatively lower THI in that study could be the cause of the lack of niacin supplementation on body temperature. Di Costanzo *et al.*[65] found free nicotinic acid supplementation decreased skin temperatures during severe heat stress (mean maximum THI = 85·1) but not in no heat stress condition (mean maximum THI = 75). Also, a dual role of niacin in regulating heat shock protein expression was reported in a recent *in vitro* study, in which niacin decreased the mRNA expression of heat shock protein under thermoneutral conditions, but increased the mRNA expression of heat shock protein in heat stress condition in mammary and uterine cells[6]. Rungruang *et al.*[19] carried out a trial in environmentally controlled rooms during winter and found that RPN supplement did not affect the sweating rate and core body temperature during moderate to severe thermal stress (THI = 72–80). They proposed seasonal depression in sweat gland function could be the reason for that result.

Heat stress caused feed intake depression is usually related to the reduced milk yield. Supplementation of RPN during a more severe heat stress condition increased[64] or tended to increase[63] the milk yield, despite no effect observed on feed intake. Similar findings were observed for free nicotinic acid supplementation[66]. However, niacin supplementation did not affect milk yield in several other studies[4,18,65]. It seems that increased milk after niacin supplementation is not necessarily related to the alleviation of heat stress in dairy cows but the reasons remain unclear. Unlike in the early lactation, RPN supplementation in heat stress were reported to increase milk fat percentage[18,63]. Although the milk fat percentage decreased after niacin supplementation, the milk fat yield increased due to the increased milk yield in the study by Lohölter *et al.*[66] Wrinkle *et al.*[4] found milk fat percentage increased in mid-lactation but decreased in early lactation after RPN supplementation in heat-stressed dairy cows. It seems likely that different mechanisms other than anti-lipolytic effect are involved in the regulation of lipid synthesis in mammary glands of heat-stressed compared with the early lactation dairy cows. In addition, Di Costanzo *et al.*[65] and Rungruang *et al.*[19] reported no effects of niacin supplementation on milk yield or milk composition in heat stress. The discrepancy among studies could be related to the different degrees of heat stress, lactation stage, dosage of niacin supplementation or the form of niacin.

In short, niacin supplementation may alleviate heat stress in dairy cows possibly through vasodilation during moderate to severe heat stress. Niacin supplementation in heat stress seems to exert different effects on milk synthesis compared with early lactation, but the mechanism is unknown. In addition, Rungruang *et al.*[19] detected a linear relationship between RPN supplementation and water intake. It would be interesting to investigate whether the same relationship could also be detected in early lactation and to study whether the increased water intake affects ruminal metabolism, animal health and performance in dairy cows.

Role of the hydroxycarboxylic acid-2 receptor

HCA_2, formerly also known as GPR109a, is a G_i protein-coupled receptor. In 2003, HCA_2 was identified as the receptor of nicotinic acid and mediates the anti-lipolytic effect of nicotinic acid through reducing cyclic AMP (cAMP) accumulation and inhabiting hormone-sensitive lipase (HSL) in adipose tissue, independently by three research groups[67–69]. The BHBA is reported to be the endogenous ligand of HCA_2 and butyrate also possesses the property to activate HCA_2[70], but another form of niacin, nicotinamide, is inactive on HCA_2[69]. In ruminants, Titgemeyer *et al.*[71] reported that HCA_2 mRNA expression was more abundant in the liver than in adipose tissues and muscle, and HCA_2 mRNA was also expressed in the brain. Later HCA_2 mRNA expression was found even higher in skin and the udder than that in the liver, ovary and uterus[6]. The widely expressed HCA_2 mRNA in various tissues imply that HCA_2 may be involved in various physiological processes in dairy cows.

To date, studies regarding HCA_2 in dairy cows mainly focused on its role in anti-lipolytic effects of nicotinic acid. The HCA_2 mRNA abundance in adipose tissue decreased during early lactation in dairy cows, suggesting a feedback mechanism to reduce the anti-lipolytic effects of BHBA in early lactation[72]. Kenéz *et al.*[73] found nicotinic acid, but not nicotinamide, was able to suppress isoproterenol-induced lipolysis by reducing the extent of HSL phosphorylation in the isolated bovine adipose tissues. Those authors suggested that stimulating HCA_2 by nicotinic acid triggers the anti-lipolytic pathway. However, supplementing niacin in feed did not influence the HCA_2 mRNA abundance in the liver[16,17] or the extent of HSL phosphorylation in early lactation[48]. The short half-life of nicotinic acid or the ruminal degradation probably explains the lack of effect of niacin on HCA_2 mRNA abundance or HSL phosphorylation. In addition, in an *in vitro* study, nicotinic acid treatment increased adiponectin concentration via HCA_2 in differentiated bovine adipocytes, indicating an improvement in insulin sensitivity[74]. Besides mediating anti-lipolysis in adipose tissue, the expression of HCA_2 in brain, mammary glands, and liver indicates that niacin might play a role in monitoring plasma BHBA concentrations in the central nervous system[71] or allow the niacin to affect milk synthesis or lipid metabolism directly.

Recent studies demonstrated that nicotinic acid has a profound anti-inflammatory effect by an HCA_2-dependent mechanism in mouse alveolar macrophages[75], colonic macrophages, dendritic cells[76], human retinal pigmented epithelial cells, kidney cells[77], etc. This raises the question of whether

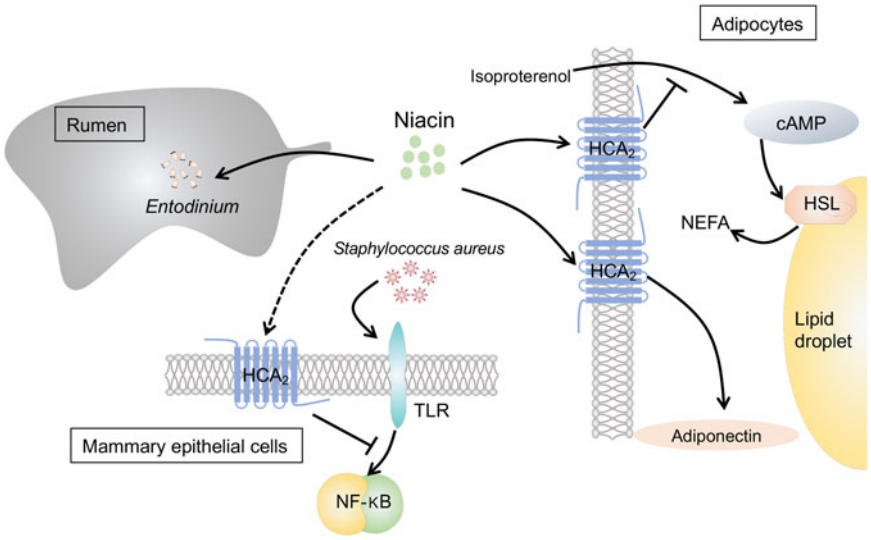

Fig. 1. (Colour online) The effects of niacin on ruminal metabolism, inflammation of mammary epithelial cells and lipolysis in adipocytes: (1) Niacin increases the number of protozoa (mainly *Entodinium*) in the rumen. (2) Niacin suppresses isoproterenol-induced lipolysis in bovine adipocytes by reducing the intracellular level of cyclic AMP (cAMP) and inhabiting hormone-sensitive lipase (HSL) via the hydroxycarboxylic acid-2 receptor (HCA_2). (3) Niacin increases adiponectin concentration in bovine adipocytes via HCA_2. (4) Niacin could reduce inflammation in bovine mammary epithelial cells, which was triggered by *Staphylococcus aureus* by suppressing the toll-like receptor (TLR)–NF-κB signalling pathway possibly via HCA_2.

nicotinic acid exerts a similar anti-inflammatory effect in ruminants. Indeed, nicotinic acid was found to inhibit *Staphylococcus aureus*-induced NF-κB activation in bovine mammary gland cells, indicating the reduced inflammatory response[78], which could be possibly related to HCA_2. By contrast, 24 g/d free nicotinic acid supplementation during the transition period did not show an effect on NF-κB mRNA abundance in blood leucocytes[79]. This could be due to the extensive degradation of free nicotinic acid in the rumen. However, Ringseis *et al.*[80] evaluated the effects of high-dosage RPN supplementation (approximately 55 g/d) on liver metabolism by analysing the liver transcriptome. These authors found that RPN supplementation may amplify the systemic inflammation-like condition in early lactation, and they suggested the markedly high-dosage RPN might be related to the indications of inflammation-like condition. To support this hypothesis, Gautam *et al.*[77] found low concentration of niacin down-regulated the pro-inflammatory cytokines, but the high concentration of niacin induced cell apoptosis.

To sum up, nicotinic acid could suppress lipolysis and inflammation in *in vitro* studies but no effects were found *in vivo* (Fig. 1). The dosage of niacin supplementation and ruminal degradation of free nicotinic acid may partly explain the discrepancy between *in vitro* and *in vivo* findings.

Conclusions and future research

Because of the high ruminal stability, RPN supplementation increased plasma niacin concentration in most studies. However, RPN supplementation did not always show beneficial effects on cow performance or health during the transition period or in heat-stressed conditions. The following issues should be addressed in the future research for better understanding of niacin's mechanisms of action and better application of niacin in practice:

1. Results from *in vitro* studies or from abomasal infusion studies clearly showed that nicotinic acid could depress lipolysis in dairy cows. Despite the high ruminal stability, RPN supplementation still led to inconclusive results in *in vivo* studies. We postulated that the short half-life of nicotinic acid, which only transiently depresses lipolysis, may be one of the reasons. Thus, further studies are required to determine the kinetics of RPN in dairy cows.
2. The rebound of plasma NEFA concentration could be another reason for the inconsistent results regarding the anti-lipolysis effects of RPN supplementation. Studies concerning the mechanism of plasma NEFA rebound are desirable. In addition, further studies should determine whether the plasma NEFA rebound aggravates the metabolic stress during early lactation.
3. Previous studies demonstrated high dosages of RPN supplementation may have adverse effects on cow performance and health. More studies are needed to elucidate the optimal dosage of niacin supplementation.
4. HCA_2 mRNA expression is relatively high in the mammary gland and liver. Studies should be conducted to test the hypothesis that niacin may directly influence lipid metabolism or milk synthesis through HCA_2.
5. A number of studies reported the anti-inflammatory effects of nicotinic acid through an HCA_2-dependent mechanism. Studies should be carried out to investigate whether nicotinic acid depresses inflammation in bovine mammary glands via stimulating HCA_2.

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