# Investigations on the effects of niacin supplementation to different rations on rumen fermentation, duodenal nutrient flow and several serum and milk parameters of dairy cows

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# Investigations on the effects of niacin supplementation to different rations on rumen fermentation, duodenal nutrient flow and several serum and milk parameters of dairy cows

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## Contents

## Abbreviations

| | |
|---|---|
| ADF | acid detergent fibre |
| ARD | apparent ruminal digestibility |
| AS | apparent synthesis |
| BHBA | ß-hydroxybutyrate |
| BW | body weight |
| CF | crude fibre |
| CONC | effect of concentrate level |
| CP | crude protein |
| d | day |
| DM | dry matter |
| DIM | days in milk |
| DMF | dry matter flow |
| DMI | dry matter intake |
| EE | ether extract |
| e.g. | exempli gratia, for example |
| EP | endogenous protein |
| F:C ratio | forage-to-concentrate ratio |
| FCM | fat-corrected milk |
| FOM | fermented organic matter |
| HC | high concentrate |
| HPLC | high performance liquid chromatography |
| LC | low concentrate |
| MAX | maximum |
| MC | medium concentrate |
| ME | metabolisable energy |
| MIN | minimum |
| MP | microbial crude protein |
| n.a. | not analysed |
| n.d. | not determined |
| NA | nicotinic acid |
| NAD | nicotinamide adenine dinucleotide |
| NADP | nicotinamide adenine dinucleotide phosphate |

| | |
|---|---|
| NAM | nicotinamide |
| NAN | non-ammonia N |
| NDF | neutral detergent fibre |
| NEFA | non-esterified fatty acids |
| NFC | non-fibre carbohydrates |
| OM | organic matter |
| OMI | organic matter intake |
| ppm | parts per million |
| RDP | rumen degradable crude protein |
| resp. | respectively |
| RUP | rumen undegradable crude protein = UDP |
| SCFA | short-chain fatty acids = VFA |
| TCA | trichloroacetic acid |
| uCP | utilizable crude protein |
| UDP | undegradable feed crude protein = RUP |
| VDLUFA | Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungs-anstalten |
| VFA | volatile fatty acids = SCFA |

# Introduction

Vitamins are defined as a group of low-molecular, organic components which are present in small amounts in foods and feedstuffs and are essential for metabolism, but without a nutritive or structure-bearing function (Schweigert, 2000). They are classified as fat and water soluble. Among water soluble vitamins, a heterogeneous group is summarised as B-vitamins. This group consists of thiamine ($B_1$), riboflavin ($B_2$), pyridoxine ($B_6$), cobalamine ($B_{12}$), biotin, folate, niacin and pantothenic acid (Ball, 2006). A common trait of B-vitamins is that they all have important coenzyme functions in the metabolism (Schweigert, 2000; Bässler et al., 2002). But the classification of these vitamins in one group is purely historical and ignores the diversity of the chemical structure and metabolic purpose (Girard and Matte, 2005). B-group vitamins have substantial relevance for health and performance of dairy cows.

Especially niacin is of great importance, because it is incorporated into the two coenzymes NAD(H) and NADP(H). The coenzymes participate in a large number of oxidative and reductive reactions in the glycolytic pathway, the citric acid cycle, the degradation of fatty acids and proteins, gluconeogenesis, synthesis of free fatty acids and proteins, urea biosynthesis and pentose phosphate pathway (Harmeyer and Kollenkirchen, 1989). Thus, they are intimately involved in energy metabolism on the cellular level as well as in the whole organism.

Apart from feed, dairy cows possess additional niacin sources due to microbial synthesis in the rumen and endogenous synthesis from tryptophan. Microbial synthesis in the rumen is believed to cover the niacin requirements of dairy cows with an average performance level (Girard, 1998; GfE, 2001) and a general supplementation of dairy cows could not be advised (GfE, 2001; NRC, 2001). However, niacin requirements have not been determined experimentally and are extrapolated from data of lactating sows (NRC, 2001). It was also concluded that ruminal synthesis may not always be sufficient (Flachowsky, 1993; Girard, 1998; Girard and Matte, 2005; Santschi et al., 2005a). Hence, a supplementation could be beneficial. In fact, diets were fortified with niacin in 42% of the analysed high producing dairy herds in the USA (Kellogg et al., 2001). But results of experiments with niacin supplementation have been “consistently inconsistent” (Schwab and Shaver, 2005). However, usually the animal is not regarded in total, e.g., the impact of a niacin supplementation only on ruminal fermentation or only on performance was measured. Thus, possible conjunctions and explanations about niacin’s

mode of action in the whole metabolism could not easily be found. Furthermore, niacin concentrations in various body fractions are very often not determined. To the author's knowledge, at present duodenal niacin flow was measured in seven experiments, blood niacin concentrations were determined in eight trials, while milk niacin concentrations were reported only twice. Simultaneous determination of niacin flows and concentrations in all three matrices was not done in any of the experiments.

As ruminal synthesis of niacin is of importance for dairy cows, the cognition of influences on this parameter seems valuable. For example different diets seem to cause differences in ruminal niacin metabolism. Santschi et al. (2005a) stated that dietary concentrate level could affect ruminal synthesis or use of B-vitamins. But research concerning the effect of different forage-to-concentrate ratios on ruminal niacin metabolism also leads to inconsistent results and only few studies can be found. Ruminal niacin concentrations have been increased in cows fed diets containing higher proportions of concentrate (Hayes et al., 1966; Nilson et al., 1967). But in another study, total niacin concentration was only numerically enhanced (Santschi et al., 2005b). However, the amounts reaching the intestine may be more important than ruminal concentrations as the small intestine is assumed to be the main absorption site (Rérat et al., 1959). Duodenal niacin flow in cows fed different diets was only measured in two trials, where it was not affected by different forage-to-concentrate ratios in one study (Miller et al., 1986) but enhanced in rations with higher concentrate proportions in another experiment (Schwab et al., 2006b). Thus, definitive deductions cannot be made. Furthermore, supplemental niacin was not provided in any of these studies. If different diets modify niacin synthesis in the rumen it seems also possible that they alter the fate of supplemental niacin as well. But studies examining the impacts of different diets on the metabolism and effects of supplemental niacin are not available.

Therefore, the objective of the present study was to contribute to a better understanding of niacin metabolism in the rumen and the whole animal as will be described in detail in the following section.

## Scope of the thesis

Considering the gaps of knowledge on niacin effects and metabolism mentioned in the introduction, the scope of this thesis is to answer the following questions:

> What is the impact of a niacin supplementation on rumen fermentation and duodenal nutrient flow?
>
> What is the impact of a niacin supplementation on blood and milk variables?
>
> What is the impact of a niacin supplementation on duodenal niacin flow and its concentration in blood and milk?
>
> Are these responses influenced by the forage-to-concentrate ratio fed?

For this purpose, literature on niacin effects in dairy cows is summarised in Paper I.

The aim of Paper II is to examine the impact of a niacin supplementation on ruminal fermentation and duodenal flow of nutrients inclusive niacin and to analyse if these response differ when diets vary in forage-to-concentrate ratio. Double fistulated cows were fed rations containing either 1/3 concentrate and 2/3 forage, 1/2 concentrate and 1/2 forage or 2/3 concentrate and 1/3 forage on dry matter basis. These rations were fed either with or without 6 g supplemental nicotinic acid per day.

In Paper III, the same experimental design was applied to characterize the influence of a niacin supplementation on several blood metabolites as well as milk production and composition. Emphasis was placed on niacin concentrations in blood and milk. The impact of the forage-to-concentrate ratio on these measurements was also examined.

## PAPER I

# Niacin for dairy cattle: a review

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## Abstract

Due to the incorporation of niacin into the coenzymes NAD and NADP, niacin is of great importance for the metabolism of man and animals. Apart from niacin in feed and endogenous formation, microbial niacin synthesis in the rumen is an important source for dairy cows. But the amount synthesized seems to differ greatly, which might be influenced by the ration fed. Many studies revealed a positive impact of a niacin supplementation on rumen protozoa, but microbial protein synthesis or volatile fatty acid production in the rumen showed inconsistent reactions to supplemental niacin. The amount of niacin reaching the duodenum is usually higher when niacin is fed. But not the whole quantity supplemented reaches the duodenum, indicating degradation or absorption before the duodenal cannula. Furthermore, supplementation of niacin did not always lead to a higher niacin concentration in blood. Effects on other blood parameters have been inconsistent, but might be more obvious when cows are in a tense metabolic situation, for example, ketosis or if high amounts are infused post-ruminally, since ruminal degradation appears to be substantial. The same is valid for milk parameters. In the few studies where blood niacin and milk parameters have been investigated, enhanced niacin concentrations in blood did not necessarily affect milk production or composition. These results are discussed in the present review, gaps of knowledge of niacin's mode of action on the metabolism of dairy cows are identified and directions for future research are suggested.

**Keywords:** Dairy cows, nicotinic acid, nicotinamide

## Introduction

Niacin is of great importance in the metabolism due to its incorporation into the coenzymes NAD and NADP[1]. Both forms of niacin, nicotinic acid (NA) and nicotinamide (NAM) can be converted into the coenzymes, although they contain only NAM as a reactive component.

Apart from feed as a source of niacin, nearly all species are able to synthesize the vitamin[1-3] from tryptophan[3] and quinolinate[4]. Since micro-organisms are able to produce niacin as well, ruminants have an additional supply due to their rumen microbes[5]. Ruminal synthesis of niacin was estimated to be 1804 mg/d for a 650 kg cow producing 35 kg of 4% fat-corrected milk/d[6]. This seems to cover the requirement definitely, which was assumed to be 256 mg/d for tissues and 33 mg/d for milk production, thus 289 mg/d in total[6]. Therefore, it was concluded that a general supplementation could not be advised[6,7]. But tissue requirements are estimated based on data from lactating sows and have not been experimentally determined[6]. Furthermore, synthesis might vary, for example, when different feeding regimens are applied[8]. Indeed, numerous studies showed positive responses to a niacin supplementation. On the other hand, a lot of research has been done where administration of niacin did not have any effect. Therefore current literature is reviewed here to distinguish the vitamin's impact on cow performance and metabolism. The aim of this review is to present the state of knowledge on niacin synthesis in the rumen and the amount of niacin arriving at the duodenum, niacin's mode of action on ruminal and several blood parameters as well as its influence on milk production and composition. Where possible, conclusions are drawn from experiments and gaps of knowledge are identified. Cognition of these processes would facilitate a decision on necessity and time of a niacin supplementation.

To our knowledge, the last detailed review available on niacin (NA and NAM) in dairy cow nutrition was done in 1993[9]. Therefore in this review studies newer than 1990 are used to show developments. But in some cases (rumen, duodenum), older literature was included as a comparison with few new results available. Only significant effects ($p < 0{\cdot}05$) and tendencies ($p < 0{\cdot}10$) are mentioned, unless otherwise noted. In all studies, supplemental niacin was not rumen-protected.

## Rumen

*Niacin in the rumen*

In Table 1, niacin concentrations in ruminal contents from several studies are summarised. In interpretation of the results, it has to be kept in mind that different analytical methods for niacin determination exist (for example, colorimetric, microbiological and HPLC methods[10]). This could lead to different results as was proven for cereal-based foods analysed by microbiological and HPLC method[10].

Niacin concentration in the rumen was enhanced if pure NA or NAM were supplemented[11,12], while the highest intake via feed components did not necessarily force the highest concentration in the rumen[8,13]. Santschi *et al.*[8] found no difference in total niacin content in the rumen when comparing rations with a forage-to-concentrate ratio (F:C ratio) of 60:40 or 40:60. However, they noticed an effect on the concentrations of each vitamer. Although no NAM was present in the feed, it was found in the rumen. Furthermore, NAM was significantly increased with the low-forage ration. NA decreased numerically and hence total niacin content was not affected. Earlier work showed an effect of the F:C ratio on ruminal niacin concentrations, which was highest in the all-concentrate ration[14] (data not shown). Thus, there is evidence that ruminal niacin concentrations and/or the concentrations of each vitamer are influenced by niacin supplementation and the F:C ratio.

Some studies have been conducted to measure ruminal synthesis of niacin. Microorganisms use aspartate and dihydroxyacetone phosphate for niacin production[4]. It is extremely difficult to measure real synthesis; therefore apparent synthesis is calculated by subtracting the intake from the amount reaching the duodenum. Some data are given in Table 2. It can be assumed that there is an influence of type of feed. Zinn *et al.*[15] mentioned a stimulating effect of starch on the ruminal synthesis of all B-vitamins. Schwab *et al.*[16] found a significant effect of the non-fibre carbohydrate (NFC) content of feed on niacin synthesis, while the F:C ratio had no effect. But the effect of NFC might also reflect large differences in niacin intake (Table 2). In the above-mentioned studies where an effect of the F:C ratio on ruminal niacin concentrations was found[8,14], duodenal niacin flow was not measured, therefore it was not possible to calculate apparent synthesis to compare these values.

In all studies listed in Table 2, the ration with the highest niacin content within a study resulted in the lowest apparent niacin synthesis. It was stated that there seems to be an optimal concentration. Synthesis will occur below this level and above it, excess niacin

**Table 1: Niacin concentrations in the rumen of cattle**

| Reference | Feeding ration | Niacin content of feed (mg/kg DM) | Niacin intake (mg/d) | Niacin concentration in the rumen | Vitamer | Studied fraction |
|---|---|---|---|---|---|---|
| Riddel *et al.*(1985)[11] | 55% wheat straw, 45% concentrate (corn starch, dextrose, soyabean meal) | | | | NA and NAM † | Whole rumen content |
| | without niacin | 6 | 50 | 102 – 114 mg/kg DM [a*] | | |
| | with 6 g NA | 697 | 6060 | 119 – 155 mg/kg DM [b*] | | |
| Abdouli & Schaefer (1986)[13] | 27% lucerne hay; 73% barley | 64 | 868 | 0·48 mg/l fluid + 2·32 mg NAD/l [a] | NA and NAM † | Rumen fluid |
| | 29% lucerne hay 69% oats | 19 | 166 | 0·32 mg/l fluid + 1·51 mg NAD/l [b] | | |
| Campbell *et al.* (1994)[12] | 60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal) | | | | | Rumen fluid |
| | | | | 0 mg/l fluid [a] | NA | |
| | without niacin | n. d. | - | 0 mg/l fluid | NAM | |
| | with 12 g NA | n. d. | + 12000 NA | 14 mg/l fluid [b] | NA | |
| | | | | 0 mg/l fluid | NAM | |
| | with 12 g NAM | n. d. | + 12000 NAM | 14 mg/l fluid [b] | NA | |
| | | | | 0 mg/l fluid | NAM | |
| | with 6 g NA and 6 g NAM | n. d. | + 6000 NA + 6000 NAM | 12 mg/l fluid [b] | NA | |
| | | | | 0 mg/l fluid | NAM | |
| Santschi *et al.* (2005)[8] | 60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal) | 26 | 520 | 143 mg/kg DM | NA | Solid associated bacteria |
| | | | | 77 mg/kg DM [a] | NAM | |
| | 40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal) | 23 | 453 | 137 mg/kg DM | NA | |
| | | | | 94 mg/kg DM [b] | NAM | |
| | 60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal) | 26 | 520 | 173 mg/kg DM | NA | Liquid associated bacteria |
| | | | | 86 mg/kg DM [a] | NAM | |
| | 40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal) | 23 | 453 | 161 mg/kg DM | NA | |
| | | | | 123 mg/kg DM [b] | NAM | |
| | 60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal) | 26 | 520 | 0·08 mg/l fluid | NA | Particle free fluid |
| | | | | 0·53 mg/l fluid | NAM | |
| | 40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal) | 23 | 453 | 0·09 mg/l fluid | NA | |
| | | | | 0·62 mg/l fluid | NAM | |

NA, nicotinic acid; NAM, nicotinamide; n.d., not determined

[a,b] Values with unequal superscripts within a study differ significantly ($p \leq 0{\cdot}05$)

[*] Depending on different sampling times after feeding (0 to 8 hours), means differed significant at 4 and 6 h after feeding

[†] The vitamin content was determined via microbiological assay, where it is not possible to distinguish between the vitamers

is degraded by the bacteria[17]. This might be the reason why in two studies with cows and feedlot calves where 6 or 2 g NA/d were supplemented[11,15] only 2% and 20%, respectively, of the amount added reached the duodenum. Santschi *et al.*[18] reported a ruminal disappearance rate for niacin of 98·5% as well. The fate of niacin that disappeared from the rumen is not clear. Zinn *et al.*[15] suggested either degradation or absorption. It is not completely clarified if absorption of vitamins could take place in the rumen. Erickson *et al.*[19] found free NAM to be absorbed at 0·98 g/h from a dilution in a washed rumen of cows. NA was not absorbed, because it is ionised under a physiological pH. But usually, most of the niacin is bound in the bacterial fraction[8,19,20]. Therefore, under normal circumstances, no absorption should take place from the rumen[18]. Yet it has to be kept in mind that with niacin supplementation, a high amount of usually free niacin reaches the rumen. Thus, some absorption might occur. However, in the work of Campbell *et al.*[12], supplementation of NAM gave significantly higher duodenal values of niacin than NA. If only NAM is absorbed from the rumen at normal ruminal pH values[19], the opposite would be expected. Consequently, ruminal degradation might be the reason for the high disappearance rate of supplemented niacin from the rumen. Another possible explanation could be that niacin is absorbed in the proximal duodenum, before the duodenal cannula. In man, niacin is absorbable from the stomach as well[21]. To our knowledge, no studies concerning absorption from the abomasum are available.

In summary, niacin concentrations and apparent synthesis in the rumen are affected by niacin supplementation and the ration fed. But it is not known which feed component most influences niacin in the rumen. If niacin is supplemented, only a small part reaches the duodenum. Ruminal absorption might occur, but does not seem to make a large contribution. Ruminal degradation or absorption in the abomasum or before the duodenal cannula seems more likely.

*Effect of niacin on rumen metabolism*

In contrast to ruminal bacteria it is assumed that protozoa are not able to synthesize niacin and need to cover their requirements from feed or bacterial synthesis[22]. Doreau and Ottou[22] observed no effect of 6 g NA on bacteria, but an increase of protozoa[22]. This especially concerned *Ophryoscolecidae*, but *Isotrichidae* were not affected. Increasing protozoal numbers, especially *Entodinia* (family *Ophryoscolecidae*), may increase

**Table 2 : Apparent synthesis of niacin in the rumen of cattle and flow at the duodenum**

| Reference | Feeding ration | Niacin suppl. (g/d) | DM intake (kg/d) | Niacin intake with feed (mg/d) | Duodenal niacin flow (mg/d) | Apparent synthesis (mg/d)[†] |
|---|---|---|---|---|---|---|
| Riddel *et al.* (1985)[(11)‡§] | 55% forage (wheat straw) 45% concentrate (corn starch, dextrose, soyabean meal) | 0 | 8·7 | 50 | 85 | 35 |
| | | 6 NA | 8·7 | 6060 | 138* | -5922 |
| Miller *et al.* (1986)[(32)‡§] | 12% lucerne meal, 88% corn grain, urea | 0 | 6·7 | 204[‖] | 589 | 386 |
| | 13% lucerne meal, 87% wheat grain | 0 | 7·0 | 357 [‖] | 785 | 428 |
| | 13% lucerne meal, 87% oat grain, urea | 0 | 7·4 | 163 [‖] | 750 | 586 |
| | 13% lucerne meal, 87% barley grain, urea | 0 | 6·5 | 485 [‖] | 664 | 179 |
| | 13% lucerne meal, 87% sorghum grain, urea | 0 | 7·3 | 295 [‖] | 813 | 518 |
| | 11% lucerne meal, 89% corn grain | 0 | 6·2 | 93 [‖] | 557 | 485 |
| | 70% lucerne meal, 30% corn grain | 0 | 6·3 | 314 [‖] | 753 | 439 |
| Zinn *et al.* (1986)[(15)‡§¶] | 45% forage (lucerne hay, Sudan grass) 55% concentrates (corn, molasses, fat) | 0 | 3·4 | 67 | 277 | 210 |
| | | 0·2 | 3·4 | 267 | 207 | -60 |
| | | 2 | 3·4 | 2067 | 401 | -1666 |
| Campbell *et al.* (1994)[(12)††] | 60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal) | 0 | 19·9 | n. d. | 1716 NA<br>0 NAM | |
| | | 12 NA | 19·9 | > 12000 NA | 3187 NA[‡‡]<br>0 NAM | |
| | | 12 NAM | 19·9 | > 12000 NAM | 4902 NA[‡‡]<br>0 NAM | |
| | | 6 NA + 6 NAM | 19·9 | > 6000 NA + 6000 NAM | 3922 NA[‡‡]<br>0 NAM | |
| Santschi *et al.* (2005)[(18)] | 58% forage (grass-legume silage, corn silage) 42% concentrate (corn, soyabean meal, protein supplement) | 0 | 19·8 | 465 NA<br>0 NAM | 1334 NA<br>1242 NAM | 912 NA<br>1259 NAM |
| | B-vitamin blend infused post-ruminally | 1·17 NAM | 19·8 | 465 NA<br>1173 NAM | 1815 NA<br>1140 NAM | |

**Table 2 continued**

| Reference | Feeding ration | Niacin suppl. (g/d) | DM intake (kg/d) | Niacin intake with feed (mg/d) | Duodenal niacin flow (mg/d) | Apparent synthesis (mg/d)† |
|---|---|---|---|---|---|---|
| Schwab *et al.* (2006)[16] | 35% forage (corn silage, lucerne and grass hay) 65% concentrate (soyabean hulls and meal, beet pulp) in total 30% NFC | 0 | 21·3 | 620 NA §§‖‖<br>1399 NAM §§‖‖ | 1209 NA<br>1256 NAM§§ | 589 NA<br>-143 NAM‖‖ |
| | 35% forage (corn silage, lucerne and grass hay) 65% concentrate (corn, barley, soyabean hulls and meal, beet pulp) total 40% NFC | 0 | 22·2 | 489 NA §§‖‖<br>838 NAM §§‖‖ | 1504 NA<br>1370 NAM§§ | 1015 NA<br>532 NAM‖‖ |
| | 60% forage (corn silage lucerne and grass hay) 40% concentrate (soyabean hulls and meal, beet pulp, blood meal, fat) total 30% NFC | 0 | 18·1 | 462 NA §§‖‖<br>727 NAM §§‖‖ | 1016 NA<br>892 NAM§§ | 555 NA<br>165 NAM‖‖ |
| | 60% forage (corn silage lucerne and grass hay) 40% concentrate (corn, barley, soyabean hulls and meal, beet pulp, blood meal, fat) total 40% NFC | 0 | 19·8 | 363 NA §§‖‖<br>221 NAM §§‖‖ | 1134 NA<br>837 NAM§§ | 771 NA<br>615 NAM‖‖ |

DMI, dry matter intake; NA, nicotinic acid; NAM, nicotinamide; n.d., not determined; NFC, non fibre carbohydrates

* Significant differences ($p \leq 0{\cdot}05$) between control and niacin groups. In the paper of Santschi *et al.*[18], level of significance was not declared, furthermore Zinn *et al* [15] and Miller *et al.*[32] did not calculate the apparent synthesis, therefore it was not possible to characterise significances in these studies.

† Apparent synthesis = Duodenal flow minus intake

‡ In these studies apparent ruminal synthesis was not calculated by the authors, but daily intake and duodenal flows were given, therefore apparent synthesis was calculated by us.

§ The vitamin content was determined via microbiological assay, where it is not possible to distinguish between the vitamers.

‖ Level of niacin intake differed significantly ($p \leq 0{\cdot}05$).

¶ In this study, the vitamer applied was not named. It was just stated that niacin was supplemented. But since the term niacin is occasionally also used as synonym for NA[45], it is assumed that NA was fed in this survey.

†† In this study, concentrations per litre duodenal digesta were given, but the authors stated that on average duodenal content had a DM content of 6·65% and a daily DM flow of 16·3 kg. Based on this, values presented here are calculated.

‡‡ Significant differences between control versus niacin and NA versus NAM ($p \leq 0{\cdot}05$).

§§ Significant effects of forage ($p \leq 0{\cdot}05$).

‖‖ Significant effects of NFC ($p \leq 0{\cdot}05$).

bacterial numbers as well, because *Entodinia* are able to regulate the ruminal environment by consuming starch[19]. Others also found a significant increase in total protozoa in the rumen fluid due to niacin feeding[23-25], which was once primarily attributable to increases in numbers of *Entodinia*[25]. Therefore, an effect of niacin on the microbial population is likely, but might be mainly on protozoa.

As a result of this probable effect of niacin on microbial population, ruminal N-metabolism could also be affected. A stimulating effect of niacin on microbial protein synthesis has been observed *in vitro*[26] and *in vivo*[23,24]. In contrast, in some *in vivo* studies no influence was seen on microbial protein production, either on the total amount or on the efficiency[12,15].

Whereas some *in vivo* trials[22,27,28] showed no niacin effect on ammonia concentration in the rumen, other *in vitro*[26] and *in vivo*[23,24] experiments showed a decreasing effect of niacin on rumen $NH_3$ – N. An interaction of fat and niacin towards increasing ammonia concentrations in the high fat, and decreasing values in the low fat, diet after niacin feeding was also found *in vivo*[27]. It is known that ammonia fixation of the rumen bacteria and fungi occurs largely via NADP- or NAD-linked glutamic dehydrogenase, and possible assimilation of ammonia via $NAD^+$-dependent glutamic dehydrogenase was also shown for protozoa[29]. This might be favoured by a niacin supplementation.

The fermentation pattern of carbohydrates might also be altered due to a possible niacin effect on microbial population, resulting in a change in volatile fatty acid (VFA) production in the rumen. Results for *in vivo* experiments are presented in Table 3. Butyrate was the VFA which was mostly but inconsistently affected, but there were also influences on acetic and propionic acid; in some surveys, no effect was seen at all. The effect of niacin on butyrate might be induced by the effect on rumen protozoa, since the presence of some protozoa species led to more butyrate produced[30]. This would match with the work of Doreau and Ottou[22], who observed higher protozoal counts and an increase in molar proportion of butyrate. But it is contrary to Samanta *et al.*[24], who observed higher protozoal counts and a decrease in molar proportion of butyrate. Thus, the effect of niacin on protozoa might not be the main reason for its effect on VFA.

In total, the responses of ruminal parameters to niacin feeding vary greatly. Ottou and Doreau[31] concluded that response differences could be due to the level of niacin supplementation, but this was not obvious here, since niacin concentrations varied in an equal range in all studies. Furthermore, Ottou and Doreau[31] listed dietary conditions, diurnal variations in the concentration of rumen protozoa, micronutrients and other

growth factors as an explanation. It must also be kept in mind that measuring ruminal concentrations is dependent on time after feeding, which was not equal for all studies cited. This might explain some of the differences obtained and it cannot be excluded that some of the observed niacin effects are rather due to high diurnal variations in the rumen than a response to niacin.

**Table 3: Effect of niacin on ruminal total VFA concentrations and molar proportions of individual VFA in cattle**

| Reference | Control ration | Niacin / day | Niacin effect |
|---|---|---|---|
| Campbell *et al.* (1994)[12] | 60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn) | 12 g NA | No effect |
| | 60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn) | 12 g NAM | No effect |
| | 60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn) | 6 g NA 6 g NAM | No effect |
| Christen-sen *et al.* (1996)[27] | 40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean hulls and meal) total 2·8% fatty acids | 12 g NA | $C_2$ (↓) $C_4$ ↑ Inter-action with fat |
| | 40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean meal, whole raw soyabeans, tallow) total 5·9 % fatty acids | 12 g NA | $C_2$ (↓) |
| Doreau & Ottou (1996)[22] | 60% forage (corn silage, grass hay) 40% concentrate (soyabean meal, rapeseed meal, urea) | 6 g NA | $C_4$ ↑ |
| Madison-Anderson *et al.* (1997)[28] | 50% forage (lucerne hay, corn silage), 50% concentrate (corn, barley, soyabean meal) | 12 g NA | No effect |
| | 50% forage (lucerne hay, corn silage), 50% concentrate (corn, barley, extruded soyabeans) 3% of DM as unsaturated fat | 12 g NA | No effect |
| Samanta *et al.* (2000)[24] | Corn, ground nut-cake, wheat bran and straw as forage, amounts were not specified | 400 mg NA/kg concentrate | total VFA ↑ $C_3$ ↑ $C_4$ ↓ |
| Kumar & Dass (2005)[23] | 50% forage (wheat straw) 50% concentrate (soyabean cake, wheat bran, corn) | 100 mg NA/kg feed | total VFA ↑ |
| | 50% forage (wheat straw) 50% concentrate (soyabean cake, wheat bran, corn) | 200 mg NA/kg feed | total VFA ↑ |

VFA, volatile fatty acids; BW, body weight; NA, nicotinic acid; NAM, nicotinamide; $C_2$ , acetic acid; $C_3$ , propionic acid; $C_4$ , butyric acid; $C_5$ , valeric acid; iso-$C_5$ , iso-valeric acid
(↓) tendency,

## Duodenum

The amount of niacin reaching the duodenum varies less than does the concentration in the rumen. Duodenal flow values for niacin are given in Table 2. From these data it can be concluded that a niacin supplementation led to higher niacin values reaching the duodenum[11,12,15,18]. But the extent to which this occurs varies and is low. A loss of nia-

cin occurs even when the vitamin is infused into the abomasum[18] but to a lower extent. This indicates abomasal or duodenal absorption before the duodenal cannula. Niacin flow at the duodenum was higher than daily niacin intake after postruminal niacin supplementation, even if the total amount given did not reach the duodenum[18]. This was not the case when niacin was added to the ration[11,12,15]. Therefore, it is likely that an oral niacin supplementation is highly degraded in the rumen and might also suppress niacin synthesis. A higher amount seems to reach the duodenum when it is infused post-ruminally.

The type of feed might modify the amount of niacin reaching the duodenum. Schwab *et al.*[16] found an effect of the F:C ratio. The high-forage ration decreased NAM content in duodenal fluid significantly, and tended to decrease NA content. The NFC content had no effect. Apparent synthesis of niacin in the rumen was affected by NFC, but not by the F:C ratio. This further indicates that the NFC effect on apparent synthesis might be due to different niacin intake, and that the F:C ratio could be important. But more information is lacking.

Even if given post-ruminally, NAM seems to convert to NA. After NAM supplementation only the amount of NA was enhanced at the duodenum, while NAM was even lower than in the control group[18]. The authors concluded that this was due to the acidic environment in the abomasum which may transform NAM to NA. Additionally, supplementation of NAM in feed enhanced the amount of niacin arriving at the duodenum to a higher extent than did NA[12].

Apparent absorption of niacin in the duodenum was not influenced by the type of feed[32] and accounted for 67%[32], 79%[15] and 84% (73% of the NA and 94% of the NAM)[18] of the amount reaching the duodenum. When supplemental niacin was fed, Riddell *et al.*[11] observed a higher amount of niacin reaching the duodenum, but excretion with faeces was equal. Therefore, the authors concluded that absorption in the duodenum must have been higher in the supplemented group. But no measurements were taken in the large intestine, thus results could also be due to a higher degradation or absorption in the large intestine. In other studies, a B-vitamin blend was supplemented, either in the feed or post-ruminally, but did not influence absorption in the duodenum[18].

Little knowledge is available concerning the mechanism of absorption. New research in human subjects suggests that the mechanism for NA absorptions in physiological amounts is dependent on an acidic pH and a specialized $Na^+$-independent carrier-

mediated system[33]. In higher concentrations, diffusion was observed to be the main mechanism in rats[34]. For NAM, absorption was suggested to occur via diffusion at twice the rate of NA[35], but new research on NAM absorption is not available. Furthermore, it is not known if the same mechanisms take place in ruminants.

Briefly, niacin feeding enhances the amount reaching the duodenum. But not the whole quantity supplemented reaches the duodenum, even after post-ruminal infusion. This provides evidence for abomasal or duodenal absorption before the duodenal cannula. Furthermore, there might be influences of the type of feed and vitamer given. Apparent absorption in the duodenum seems to be high, but the mechanism of absorption has not yet been studied in ruminants.

## Blood

*Niacin in blood*

Data concerning blood niacin concentrations are given in Table 4. Obviously, concentrations vary in a wide range. A reason for this might lie in difficulties of vitamin analysis and / or in different blood fractions examined.

There is disagreement about the existence of NA in blood. Whereas Campbell *et al.*[12] found both vitamers, Kollenkirchen *et al.*[36] stated that only NAM was present in blood of sheep. In two studies, only values for NAM were named[37,38]. It was not stated whether only NAM was found, or if only NAM was analysed. The metabolism of niacin in the body might provide an explanation for this discrepancy. There appears to be no direct conversion of NA to NAM. NA is first converted to NAD, and NAM is then produced from hydrolysis of excess NAD[39]. Part of the NAM formed is reutilised to NAD, but NAM is produced in excess to supply extra-hepatic organs with niacin[40]. Therefore, NAM seems to be the main transport form of niacin in blood[4], although the NA that escaped liver metabolism is also transported to various cell types in the body[41].

The difference in niacin content of the analysed blood fractions between control and niacin-supplemented groups was significant in three studies[37,38,42], but not in the others[12,43,44]. Campbell *et al.*[12] found a significant difference between the vitamers. Addition of NA enhanced both NA and NAM, while feeding NAM had a decreasing impact on blood NA and NAM concentrations. This was not expected, since the NAM-supplemented group had the highest duodenal values of niacin; at this point it is not explainable, why this should result in the lowest niacin content of plasma. For rats, it

**Table 4: Niacin concentrations in blood of cattle**

| Reference | Feeding ration | Niacin supplement (g/d) | DMI (kg / d) | Niacin intake (g/d) | Niacin concentration of blood (µg/ml) | Blood fraction |
|---|---|---|---|---|---|---|
| Driver *et al.* (1990)[38]† | 45% forage (lucerne hay and silage), 55% concentrate (ground corn and oats, heat-treated soyabean meal) | 0 | 21·4 | | 0·7 NAM | Plasma |
| | | 6 | 20·1 | | 1·0 NAM* | |
| | 45% forage (lucerne hay and silage), 55% concentrate (ground corn and oats, heat-treated whole soyabeans) | 0 | 19·3 | | 0·6 NAM | |
| | | 6 | 20·4 | | 1·0 NAM* | |
| Martinez *et al.* (1991)[43]†‡ | 40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses) total 2% fat | 0 | 23·8 | | 14·3 | Whole blood |
| | | 12 | 23·3 | | 17·3 | |
| | 40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses, fat) total 4% fat | 0 | 23·6 | | 8·1 | |
| | | 12 | 23·2 | | 9·7 | |
| Lanham *et al.* (1992)[44]†‡ | 40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal) | 0 | 19·8 | 0·69 | 1·1 | Plasma |
| | | approx. 6 ‖ | 16·7 | 5·14 | 1·3 | |
| | 40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal, whole cottonseed) | 0 | 17·4 | 0·56 | 1·3 | |
| | | approx. 6 ‖ | 17·2 | 5·23 | 1·3 | |
| Campbell *et al.* (1994)[12] | 60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal) | 0 | 19·9 | | 0·9 NA + 1·2 NAM | Plasma |
| | | 12 NA | 19·9 | | 1·3 NA¶ + 1·3 NAM | |
| | | 12 NAM | 19·9 | | 0·6 NA ¶ + 0·9 NAM | |
| | | 6 NA + 6 NAM | 19·9 | | 1·0 NA + 1·0 NAM | |
| Ottou *et al.* (1995)[42]‡ | 79% forage (corn silage, hay) 21% concentrate (beet pulp, wheat, barley, rapeseed meal, soyabean meal, molasses), with niacin infused into the proximal duodenum | 0 | 18·4 | | 0·6 | Plasma |
| | | 6 NA | 19·3 | | 2·5 * | |
| | 77% forage (corn silage, hay) 19% concentrate (rapeseed meal, soyabean meal), 3·5% rapeseed oil infused into the proximal duodenum | 0 | 17·9 | | 0·4 | |
| | 3·5% rapeseed oil and niacin infused into the proximal duodenum | 6 NA | 17·7 | | 2·4 * | |

**Table 4 continued**

| Reference | Feeding ration | Niacin supplement (g/d) | DMI (kg / d) | Niacin intake (g/d) | Niacin concentration of blood (µg/ml) | Blood fraction |
|---|---|---|---|---|---|---|
| Cervantes *et al.* (1996)[37] | 8 different forage to concentrate ratios; lucerne hay or haylage and corn silage were used as forage, corn and soyabean meal as concentrate | 0 | 20·3 | | 1·6 NAM | Whole blood |
| | with 12 g NAM | 12 NAM | 24·0 | | 1·9 NAM* | |
| | with 400 g Ca salts of fatty acids and 12 g NAM | 12 NAM | 21·1 | | 1·9 NAM* | |

DMI, dry matter intake; NA, nicotinic acid; NAM, nicotinamide; approx., approximately

* Significant differences ($p \leq 0{\cdot}05$) between control and niacin group have been observed for these parameters.

† In these studies, the vitamer applied was not named. It was just stated that niacin was supplemented. But since the term niacin is occasionally also used as synonym for NA[45], it is assumed that NA was fed in these surveys.

‡ The vitamin content was determined via different assays whithout possibility to distinguish between the vitamers.

‖ Niacin was mixed in the concentrate, the goal was to reach 6 g niacin intake / cow / day.

¶ There was no difference between control and treatment, but between NA and NAM for the NA concentration in blood ($p \leq 0{\cdot}05$).

was demonstrated that NAM is also able to pass from bloodstream back to the lumen[34]. This could explain the previously mentioned results in the NAM group[12], should it occur in ruminants as well. But the reasons for and physiological role of such a process remain unclear[34].

In sheep, the NAM concentration of whole blood was not influenced by NA or NAM supplementation[36]. Hence the conclusion was drawn that concentrations in blood appeared to be unaffected by a supplementation, even though the amount reaching the duodenum was increased. In contrast, Ottou *et al.*[42] infused 6 g niacin into the proximal duodenum and observed an increase in the niacin content of whole blood. The results of this study also lead to the conclusion that ruminal absorption could be excluded as a reason for observed differences in blood niacin content, because changes occurred after post-ruminal infusion. In other studies as well there was no obvious relationship between ruminal and blood niacin concentrations[12,36].

In humans, there seems to be a kind of homeostasis of niacin in blood[45]. Excess niacin gets converted into a storage form of NAD in the liver. Pires and Grummer[46] conducted an experiment with different amounts of NA infused in the abomasum and concluded from effects on blood metabolites that some build-up of NA in blood or adipose tissue might have occurred. If some homeostasis system exists also in ruminants, it would explain studies without an effect on blood niacin, but would fail to elucidate observed differences in the others.

*Effect on blood metabolites*

The effect of niacin on several blood parameters (glucose, NEFA and ß-hydroxybutyrate (BHBA) as main ketone body) has been studied extensively in dairy cattle (Table 5). Only surveys including glucose, NEFA and BHBA are incorporated in this Table. One study mentioned separate results for several lactation weeks[47], and so values for week 2 were included in Table 5 as the earliest sampling time.

*Non-esterified fatty acids*

In Table 5, the only significant effect of a niacin supplementation was an increase of NEFA in the niacin group[43]. This was not expected, since niacin is thought to be anti-lipolytic, which would result in a lower NEFA concentration. The authors proposed that this was due to increased lipoprotein lipase activity, which is stimulated by NA, thus resulting in decreased plasma triglyceride content and increases in NEFA. Apart from

**Table 5: Impact of niacin on several blood metabolites**

| Reference | Feeding ration | Niacin suppl. (g/d) | NEFA (µmol/l) | BHBA (mg/l) | Glucose (mg/l) | Blood fraction | Lactation week † |
|---|---|---|---|---|---|---|---|
| Driver *et al.* (1990)[38]‡ | 45% forage (lucerne hay and silage), 55% concentrate (ground corn, ground oats, heat-treated soyabean meal) | 0 | 69 mg / l | 106 | 495 | Plasma | -1 until 15 |
| | | 6 | 82 mg / l | 97 | 481 | | |
| | 45% forage (lucerne hay and silage), 55% concentrate (ground corn, ground oats, heat-treated whole soyabeans | 0 | 97 mg / l | 116 | 443 | | |
| | | 6 | 74 mg / l | 108 | 457 | | |
| Jaster & Ward (1990)[47] | 50% corn silage, 50% concentrate (ground shelled corn, soyabean meal) | 0 | 250 | 29 | 556 | Plasma | 2 |
| | | 6 NA | 202 | 33 | 518 | | |
| | | 6 NAM | 223 | 22 | 560 | | |
| Martinez *et al.* (1991)[43]‡ | 40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses) total 2% fat | 0 | 367 | n.d. | 711 | Plasma | Average 84 DIM |
| | | 12 | 490 * | | 727 | | |
| | 40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses, fat) total 4% fat | 0 | 468 | | 721 | | |
| | | 12 | 546 * | | 739 | | |
| Erickson *et al.* (1992)[55] | 45% forage (lucerne grass haylage, corn silage), 55% concentrate (high moisture shelled corn, soyabean meal) | 0 | 265 | 65 | 554 | Plasma | 2 until 14 |
| | | 12 NA | 238 | 52* | 553 | | |
| | with 3% calcium salts of long chain fatty acids | 0 | 303 | 78 | 532 | | |
| | with 3% calcium salts of long chain fatty acids and niacin | 12 NA | 352 | 65* | 521 | | |
| Chiliard & Ottou (1995)[53] | 79% forage (corn silage, hay) 20% concentrate (beet pulp, wheat, barley, rapeseed meal, soyabean meal, molasses) | 0 | 130 | 46 | 725 | Plasma | Average 110 DIM |
| | with niacin infused into the proximal duodenum | 6 NA | 93 | 40 | 733 | | |
| | 77% forage (corn silage, hay) 18% concentrate (rapeseed meal, soyabean meal) | | | | | | |
| | 3·5% rapeseed oil infused into the proximal duodenum | 0 | 118 | 49 | 665 | | |
| | 3·5% rapeseed oil and niacin infused into the proximal duodenum | 6 NA | 150 | 47 | 683 | | |
| Cervantes *et al.* (1996)[37] | 8 different forage to concentrate ratios; lucerne hay or haylage and corn silage were used as forage, corn and soyabean meal as concentrate | 0 | 120 | 39 | 588 | Plasma | Average 112 DIM |
| | | 12 NAM | 126 | 38 | 589 | | |
| | with 400 g Ca salts of fatty acids | 0 | 157 | 40 | 600 | | |
| | with 400 g Ca salts of fatty acids and nicotinamide | 12 NAM | 151 | 33 | 592 | | |

**Table 5 continued**

| Reference | Feeding ration | Niacin suppl. (g/d) | NEFA (µmol/l) | BHBA (mg/l) | Glucose (mg/l) | Blood fraction | Lactation week † |
|---|---|---|---|---|---|---|---|
| Christensen *et al.* (1996)[27] | 40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean hulls and meal) total 2·8% fatty acids | 0 | 157 | 64 | 607 | Plasma | Average 30 DIM |
| | | 12 NA | 174 | 58 | 681 | | |
| | 40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean meal, whole raw soyabeans, tallow) total 5·9% fatty acids | 0 | 159 | 50 | 681 | | |
| | | 12 NA | 189 | 54 | 736 | | |
| Minor *et al.* (1998)[54]‡§¶ | 49 - 60% forage (lucerne and corn silage), 51 - 40% concentrate (cracked corn, soyabean meal, roasted soyabeans, whole cottonseeds) | 0 | 378 | 114 | 594 | Plasma | -19 d until 40 |
| | | 12 | 389 | 110 | 610 | | |
| | 40 – 50% forage (lucerne and corn silage), 60 - 40% concentrate (ground corn, starch, soyabean meal, roasted soyabeans, whole cottonseed) | 0 | 293 | 80 | 622 | | |
| | | 12 | 225 | 78 | 640 | | |
| Drackley *et al.* (1998)[48]§¶ | 40 – 50% forage (lucerne haylage, corn silage) 60 – 50% concentrate (soyabean meal and hulls, shelled corn) | 0 | 98 | 50 | 692 | Plasma | 4 until 43 |
| | | 12 NA | 117 | 48 | 693 | | |
| | 40 – 50% forage (lucerne haylage, corn silage) 60 – 50% concentrate (soyabean meal and hulls, whole raw soyabeans, shelled corn, fat) | 0 | 134 | 46 | 718 | | |
| | | 12 NA | 122 | 53 | 694 | | |

BHBA, ß-hydroxybutyrate; NA, nicotinic acid; NAM, nicotinamide; DIM, days in milk; n.d., not determined

* Significant differences ($p \leq 0{\cdot}05$) between control and niacin group have been observed for these parameters.

† Blood values given in this Table derive from that lactation week or are a mean of the given time span, where 0 is calving, therefore negative numbers are weeks prepartum and positive values postpartum.

‡ In these studies, the vitamer applied was not named. It was just stated that niacin was supplemented. But since the term niacin is occasionally also used as synonym for NA[45], it is assumed that NA was fed in these surveys.

§ Several diets postpartum were given, therefore, the forage-to-concentrate ratio differs.

this effect, significant interactions between niacin and fat supplementation were observed, resulting in an increase in NEFA when niacin was supplemented, while NEFA decreased when niacin and fat were given[48]. If only studies are considered where niacin was given to periparturient cows (treatment started 2 weeks before or within 2 weeks after calving), there was no effect of a niacin supplementation (Table 5) as was described by Chamberlain and French[49] as well. Jaster and Ward[47] also analysed influences in other lactation weeks (not included in Table 5), where a decreasing effect of niacin on NEFA in week 4 was observed. Therefore, if given orally, it is not clear that niacin acts more on NEFA in periparturient than other cows.

NA was used as a lipid-lowering agent in humans for decades, but, until recently, cellular mechanisms have not been well understood[50]. In 2003, the receptor HM74A was identified in adipose tissue, to which NA is a high-affinity ligand[51]. Activation of the receptor starts an inhibitory G-Protein signal that reduces adipocytes cAMP concentrations by repressing adenyl cyclase activity, which inhibits lipolysis. The endogenous ligand of HM74A is not known[50]. But NAM acted only as a very weak agonist on HM74A and seems therefore not to affect plasma lipid profiles[51]. For humans it was concluded that the endogenous level of NA is too low to impact receptor activity[52], but supplementation might enhance this level.

It must be kept in mind that after supplementation in the usual range for dairy cows, NAM seems to be the dominating form of niacin in blood. Apart from relatively low NEFA values in some surveys, this could also explain the absence of a niacin effect on NEFA in most studies, even in those where an effect on blood niacin concentrations was shown[37,38,42]. In two of those studies, the increase in blood niacin was an increase of NAM[37,38], which would not be expected to act on lipolysis. Reduction of plasma NEFA was achieved in fasting cows after one single abomasal infusion of 6 mg NA/kg body weight (approx. 5 g/cow)[46], but not after continuous duodenal infusion of 6 g NA/cow per d[53]. Maybe if higher amounts of NA were to reach the duodenum, concentrations of NA in blood would be enhanced, possibly due to an increase in absorption via passive diffusion of NA at higher concentrations. Therefore, lipolysis would be affected, while physiological amounts due to an oral supplementation are converted in the liver into NAM and have therefore no effect.

In human subjects, it was often observed that after the effect of NA decays there was a major rebound of NEFA plasma concentrations[50]. The same result was achieved in dairy cows as well[46]. Pires and Grummer[46] concluded that the magnitude of the re-

bound depends on the dose of NA or duration of time with decreased NEFA. Karpe and Frayn[50] suggested that NA interferes with the ability of adipose tissue to normally regulate its lipolysis, but mechanisms are not known. Pires and Grummer[46] state that if NA is continuously delivered in sufficient quantities, it will limit lipolysis in adipose tissue and therefore reduce plasma NEFA.

This phenomenon might also be an explanation for the increase in NEFA in the work of Martinez *et al.*[43], if blood measurements were done in the rebound phase, but authors only named the day of blood sampling, not time after feeding. The time of measurement might be another explanation for studies without a niacin effect on NEFA. NEFA returned to starting values 4 - 6 h after one abomasal infusion of 6 mg NA/kg body weight (approx. 5 g / cow)[46]. This might take longer with an oral supplementation, since niacin has to pass the reticulo-rumen, but in some studies blood concentration of NEFA was measured before morning feeding[48,53,54], where the effect might already have disappeared.

In conclusion, NEFA have been shown to be lowered by NA under certain conditions, but not by NAM. After the effect of NA disappears, a rebound above basal values occurs, which afterwards returns to normal. Apparently, to induce these effects, the amounts of niacin arriving at the duodenum have to be high, which might not be the case in feeding trials with an oral, not rumen-protected supplementation. However, there were effects after oral supplementation as well. Based on data available, it is not possible to conclude if the presence or absence of an effect after oral supplementation is based on sampling time or the amount of NA arriving in blood.

*ß-Hydroxybutyrate*

The only significant effect of niacin on BHBA in Table 5 was found in the work of Erickson *et al.*[55], where BHBA was lowered due to niacin feeding. Even in studies where niacin concentrations in blood have been enhanced[37,38,42] no effect was found. But an interaction between niacin, fat and week of lactation was detected once[48], since niacin feeding enhanced ketones during fat supplementation and decreased ketones when no fat was added throughout the study. But in lactation weeks 1 to 3, almost the opposite was seen. Jaster and Ward[47] also observed a time effect towards a significant reduction of BHBA in both NA- and NAM-supplemented groups in week 4, but not in lactation weeks 2 and 6 to 12.

An absence of an effect of niacin on BHBA was attributed to the low level of BHBA[27], because supplementation was started later in lactation, after the period with the highest incidence of ketonaemia[12,48,53]. Driver *et al.*[38] found more NAM in the blood of treatment groups, but assumed this is only beneficial if the cows are in state of abnormal carbohydrate or lipid metabolism. As was discussed above, the absence of an effect even if niacin concentrations in blood were enhanced, might as well be due to the fact that NAM has almost no impact on lipolysis.

If an effect was seen, the mode of action of niacin on ketones was not clearly explained. Erickson *et al.*[25] postulated that changes in blood ketone-body levels following administration of NA are mainly and perhaps entirely due to changes in plasma NEFA levels, which was also observed in other surveys[56]. But this is not obvious in several studies in Table 5. BHBA concentrations in the niacin-supplemented group were significantly lower in the work of Erickson *et al.*[55]. This could not be seen in the NEFA level, at least not in the fat-supplemented rations. Others also observed differences in responses of NEFA and BHBA concentrations in blood to a niacin supplementation[48]. Erickson *et al.*[55] concluded that NA impeded ketogenesis, but had no influence on lipolysis. As another mechanism they mentioned that mobilised fatty acids are stored in the liver of niacin-supplemented cows. However, in general it was deduced that the mechanism by which niacin reduces ketones is not known[55].

It was recently discovered that BHBA is an endogenous ligand of HM74A in humans[52]. The authors suggested that BHBA is therefore itself anti-lipolytic and regulates its own production with a negative feedback by decreasing serum level of fatty acid precursors for hepatic ketogenesis. If this also happens in ruminants, it seems to support the theory that an impact of NA on NEFA is responsible for the effect of niacin on BHBA. The lack of responses in most studies might be traced back to either the amount of NA reaching the blood or to a time effect.

*Glucose*

In the studies cited in Table 5, no significant effect of niacin on blood glucose can be seen, even in studies with enhanced blood niacin concentrations. An impact of time after parturition is possible, since Jaster and Ward[47] found no effect in lactation weeks 2 and 8 to 12; however, in lactation weeks 4 and 6, the NAM group exhibited enhanced glucose concentrations, while the NA group was not different from control.

In other studies not included in Table 5, glucose concentrations were equal in control and treatment groups[28,44,57,58] or there was an increase[59] in the niacin supplemented group.

For dairy cows it was assumed that increased glucose and insulin concentrations occured in blood after niacin supplementation due to greater gluconeogenic activity[59]. Others concluded, that it is not clear if this is due to increased gluconeogenesis or decreased removal of glucose[47]. Chilliard and Ottou[53] observed a decreased slope of glucose elimination after an intravenous injection of glucose when niacin was infused into the duodenum of cows in mid-lactation. Furthermore, the decrease in plasma glucose following an insulin challenge was less in the niacin group. In humans, NA was assumed to lower insulin sensitivity, but this was not observed in 20% of subjects studied[60]. Enhanced glucose elimination after intravenous glucose tolerance test was found in cows in negative energy balance, despite lower insulin concentration, which suggests an increased response to endogenous insulin[61]. It was proposed that the decreasing impact of sufficient amounts of NA on NEFA is the cause for observed results, rather than a direct effect of NA, since high NEFA concentrations have been shown to induce insulin resistance[61]. But results seem to be contradictory, which may in part be explained by different levels of energy supply and thus lipolysis. Other explanations can not be given; it can only be concluded that insulin is involved in reactions of blood glucose to niacin.

## Milk

To our knowledge, only two research groups measured the niacin content of milk of dairy cows[14,62]. Values ranged from 0·46 mg/l to 0·87 mg/l[14,62]. Wagner *et al.*[62] found only NAM, while Nilson *et al.*[14] did not distinguish between vitamers. NAM content of milk was enhanced after NA supplementation[62], but the highest niacin intake resulted in lowest milk niacin content in the other study[14]. Ruminal niacin concentrations have also been measured, and no relation was apparent between ruminal and milk niacin concentrations[14]. But other information is lacking; therefore no statement for the carryover of niacin into milk can be made.

The influence of a niacin supplementation on other milk parameters is shown in Table 6, where only studies measuring at least milk yield, fat and protein content are included.

**Table 6: Impact of niacin on several milk parameters**

| Reference | Feeding ration | Niacin supplement (g/d) | Milk (kg/d) | Protein, % (kg/d) | Fat, % (kg/d) | Lact. Week[†] |
|---|---|---|---|---|---|---|
| Driver *et al.* (1990)[(38)‡] | 45% forage (lucerne hay and silage), 55% concentrate (ground corn and oats, heat-treated soyabean meal) | 0 | 38·5 | 2·84 (1·09) | 3·53 (1·34) | |
| | | 6 | 37·8 | 2·83 (1·07) | 3·44 (1·28) | -1 till |
| | 45% forage (lucerne hay and silage), 55% concentrate (ground corn, and oats, heat-treated whole soyabeans) | 0 | 38·5 | 2·66 (1·01) | 3·38 (1·29) | +15 |
| | | 6 | 36·8 | 2·81 (1·03) | 3·45 (1·25) | |
| Erickson *et al.* (1990)[(25)] | 60% forage (corn silage, lucerne-grass silage), 40% concentrate (shelled corn, soyabean meal) | 0 | 24·2 | 3·16 (0·77) | 3·19 | Mid-lactation |
| | | 12 NA | 24·6 | 3·18 (0·78) | 3·22 | |
| | | 12 NAM | 24·5 | 3·15 (0·77) | 3·18 | |
| Martinez *et al.* (1991)[(43)‡] | 40% chopped lucerne hay, 60 % concentrate (beet pulp, whole cottonseed and -meal, corn, wheat, molasses) total 2% fat | 0 | 30·8 | 2·99 | 3·29 (1·01) | On average 84 DIM |
| | | 12 | 30·8 | 2·97 | 3·28 (1·01) | |
| | 40% chopped lucerne hay, 60 % concentrate (beet pulp, whole cottonseed and -meal, corn, wheat, molasses, fat) total 4% fat | 0 | 31·7 | 2·94 | 3·43 (1·09) | |
| | | 12 | 31·2 | 2·94 | 3·41 (1·06) | |
| Lanham *et al.* (1992)[(44)‡] | 40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal) | 0 | 19·3 | 3·61 (0·70) | 4·08 (0·80) | On average 256 DIM |
| | | approx. 6[§] | 19·1 | 3·52 (0·67) | 3·88 (0·74) | |
| | 40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal, whole cottonseed) | 0 | 19·8 | 3·50 (0·67) | 4·02 (0·76) | |
| | | approx. 6 [§] | 18·1 | 3·55 (0·63) | 3·81 (0·69) | |
| Erickson *et al.* (1992)[(55)] | 45% forage (lucerne grass haylage, corn silage), 55% concentrate (high moisture shelled corn, soyabean meal) | 0 | 36·2 | 2·71 (0·97) | 3·32 (1·20) | 2 till 14 |
| | | 12 NA | 36·4 | 2·84[*] (1·03) | 3·32 (1·21) | |
| | with 3 % calcium salts of long chain fatty acids | 0 | 38·2 | 2·55 (0·98) | 3·36 (1·27) | |
| | | 12 NA | 39·3 | 2·68[*] (1·06) | 3·35 (1·31) | |
| Campbell *et al.* (1993)[(12)] | 60% forage (lucerne haylage, corn silage), 40% concentrate (corn, soyabean hulls and meal) | 0 | | 3·22 | 3·79 | On average 200 DIM |
| | | 12 NA | | 3·19 | 3·77 | |
| | | 12 NAM | 26·0[‖] | 3·22 | 3·74 | |
| | | 6 NA+6 NAM | | 3·22 | 3·82 | |
| Bernard *et al.* (1995)[(64)¶] | 54% forage (corn silage, lucerne hay), 46% concentrate (whole soyabeans, soyabean meal and hulls, corn, wheat middlings) | | | | | |
| | Untreated soyabeans | 0 | 25·5 | 4·90 | 3·78 | Whole lactation |
| | Heat-treated soyabeans | 0 | 25·9 | 4·69 | 3·72 | |
| | Niacin | 0 | 25·9 | 4·77 | 3·75 | |
| | | 6 NA | 25·5 | 4·82 | 3·75 | |

**Table 6 continued**

| Reference | Feeding ration | Niacin supplement (g/d) | Milk (kg/d) | Protein, % (kg/d) | Fat, % (kg/d) | Lact. Week[†] |
|---|---|---|---|---|---|---|
| Ottou *et al.* (1995)[(42)] | 79% forage (corn silage, hay), 21% concentrate (beet pulp, wheat, barley, rapeseed meal, soyabean meal, molasses) | 0 | 22·5 | 3·11 (0·70) | 4·34 (0·98) | On average 110 DIM |
| | with niacin infused into the proximal duodenum | 6 NA | 24·1 | 3·15 (0·76) | 4·26 (1·03) | |
| | 77% forage (corn silage, hay) 19% concentrate (rapeseed meal, soyabean meal) | | | | | |
| | 3·5% rapeseed oil infused into the proximal duodenum | 0 | 23·7 | 2·93 (0·70) | 4·23 (1·00) | |
| | 3·5% rapeseed oil and niacin infused into the proximal duodenum | 6 NA | 23·8 | 2·96 (0·70) | 4·22 (1·01) | |
| Cervantes *et al.* (1996)[(37)] | 8 different forage to concentrate ratios; lucerne hay or haylage and corn silage were used as forage, corn and soyabean meal as concentrate | 0 | 30·7 | 3·21 (0·98) | 3·45 (1·07) | On average 112 DIM |
| | | 12 NAM | 33·5* | 3·31 (1·11)* | 3·26 (1·09) | |
| | | 0 | 31·8 | 3·17 (1·00) | 3·57 (1·14) | |
| | with 400 g Ca salts of fatty acids | 12 NAM | 33·2* | 3·14 (1·04)* | 3·46 (1·15) | |
| Christensen *et al.* (1996)[(27)] | 40% forage (lucerne haylage, corn silage), 60% concentrate (corn, soyabean hulls and meal) total 2·8% fatty acids | 0 | 36·1 | 3·04 (1·09) | 3·89 (1·39) | On average 30 DIM |
| | | 12 NA | 36·3 | 3·04 (1·09) | 3·67 (1·32) | |
| | 40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean meal, whole raw soyabeans, tallow) total 5·9% fatty acids | 0 | 37·4 | 3·02 (1·10) | 3·50 (1·27) | |
| | | 12 NA | 36·9 | 2·95 (1·08) | 3·64 (1·34) | |
| Belibasakis & Tsirgogianni (1996)[(57)‡] | 50% forage (corn silage), 50% concentrate (corn, soyabean meal, wheat bran) | 0 | 23·3 | 3·23 (0·75) | 3·46 (0·81) | On average 90 DIM |
| | | 10 | 24·4 | 3·24 (0·79) | 3·89* (0·95*) | |
| DiCostanzo *et al.* (1997)[(59)††] | 50% forage (lucerne haylage, corn silage, earlage), 50% concentrate (cracked corn, whole cottonseed and meal, soyabean meal and hulls, blood meal, wheat midds) | 0 | 28·0 | 2·90 | 3·40 | On average 90 DIM |
| | | 12 NA | 29·0 | 2·91 | 3·33 | |
| | | 24 NA | 25·9 | 2·91 | 3·38 | |
| | | 36 NA | 28·7 | 3·17 | 3·35 | |
| Madison-Anderson *et al.* (1997)[(28)] | 50% forage (lucerne hay, corn silage), 50% concentrate (rolled corn and barley, soyabean meal, molasses) | 0 | 31·9 | 3·03 (0·96) | 3·11 (0·99) | On average 53 DIM |
| | | 12 NA | 32·2 | 3·11 (1·00) | 3·32 (1·05) | |
| | 50% forage (lucerne hay, corn silage), 50% concentrate (rolled corn and barley, extruded soyabeans, molasses) | 0 | 35·1 | 2·96 (1·04) | 3·33 (1·15) | |
| | | 12 NA | 35·5 | 2·92 (1·04) | 3·22 (1·14) | |

**Table 6 continued**

| Reference | Feeding ration | Niacin supplement (g/d) | Milk (kg/d) | Protein, % (kg/d) | Fat, % (kg/d) | Lact. Week[†] |
|---|---|---|---|---|---|---|
| Minor *et al.* (1998)[(54)‡‡‡] | 49 – 60% forage (lucerne and corn silage), 51 - 40% concentrate (cracked corn, soyabean meal, roasted soyabeans, whole cottonseeds) | 0 | 32·0 | 3·01 (0·94) | 3·65 (1·13) | 0 till 40 |
| | | 12 | 31·3 | 3·01 (0·95) | 3·73 (1·18) | |
| | 40 – 50% forage (lucerne and corn silage), 60 - 40% concentrate (ground corn, starch, soyabean meal, roasted soyabeans, whole cottonseeds) | 0 | 34·8 | 3·17 (1·06) | 3·43 (1·14) | |
| | | 12 | 33·1 | 3·19 (1·03) | 3·55 (1·20) | |
| Drackley *et al.* (1998)[(48)‡‡] | 40 – 50% forage (lucerne haylage, corn silage), 60 – 50% concentrate (soyabean meal and hulls, shelled corn) | 0 | 30·5 | 3·29 (0·99) | 3·56 (1·06) | 4 till 43 |
| | | 12 NA | 33·2* | 3·16* (1·04) | 3·50 (1·15) | |
| | 40 – 50% forage (lucerne haylage, corn silage), 60 – 50% concentrate (soyabean meal and hulls, whole raw soyabeans, shelled corn, fat) | 0 | 31·8 | 3·16 (0·98) | 3·68 (1·16) | |
| | | 12 NA | 33·6* | 3·13* (1·05) | 3·60 (1·21) | |

Lact. week, lactation week; n.a., not analysed; NA, nicotinic acid; NAM, nicotinamide; DIM, days in milk; approx., approximately

* Significant differences ($p \leq 0{\cdot}05$) between control and niacin group have been observed for these parameters.

† Values given in this Table derive from that lactation week or are a mean of the given time span, where 0 is calving; therefore negative numbers are weeks pre-partum and positive values post-partum.

‡ In these studies, the vitamer applied was not named. It was just stated that niacin was supplemented. But since the term niacin is occasionally also used as synonym for NA[(45)], it is assumed that NA was fed in these surveys.

§ Niacin was mixed in the concentrate, the goal was to reach 6 g niacin intake / cow / day.

‖ In this study, there was no influence of a niacin supplementation on milk yield; therefore, the authors gave only average milk yield for all groups.

¶ In this study, two years were analysed, mean of both years was taken; furthermore values for each group have not been given, only for the main effects (processing of soyabeans, niacin supplementation), which are presented here.

†† This study was also designed to test the effect of different heat-stress-exposure; therefore different lines not only represent different niacin levels, but also different climatic conditions. Each niacin level had its own control group, but only values for the first one are presented here.

‡‡ Several diets postpartum were given, therefore the forage-to-concentrate ratio differs.

*Milk yield*

In two studies, milk yield was increased after niacin supplementation[37,48], while it was not influenced in the others mentioned in Table 6. The absence of a niacin effect was explained in that cows were too far into lactation and thus not in a negative energy balance[42]. But this would not match with the work of Cervantes *et al.*[37] where an effect was seen even though cows were in mid-lactation and probably not in a negative energy balance. In other studies not presented in Table 6, milk yield was either not affected[62] or was increased due to niacin feeding[47]. But these authors did not observe differences until lactation week 9. In addition, values in the NA group did not differ from control; only the NAM group did[47].

The increase in microbial protein production after niacin feeding was made responsible for enhanced milk production[47]. Furthermore, these authors suggested that the function of niacin in lipid and energy metabolism might play a role. Even if the niacin content of plasma was enhanced after niacin supplementation, this had no impact on milk yield[38,42]. But in one study NAM in plasma and milk yield were enhanced in supplemented animals[37]. Therefore, exact mechanisms remain unclear.

*Milk protein*

In contrast to most studies in Table 6, Erickson *et al.*[55] observed a significant increase, and Drackley *et al.*[48] a significant decrease in milk protein concentration, after niacin supplementation. Furthermore, an interaction between niacin and type of soyabean processing[38], or niacin and fat supplementation[28], was demonstrated. For protein yield, tendencies for an increase due to niacin supplementation have been detected[37,48,55]. There were also tendencies for interactions between niacin, fat and week of lactation[48]. In the other studies in Table 6, no effect of a niacin supplementation was seen. Even in surveys where niacin concentration in blood was significantly enhanced in the supplemented group, differences in the response of milk protein to niacin supplementation occurred[37,38,42]. In one study, no effect was observed[42], while an increase in protein yield was found in another[37]. Furthermore, an interaction between niacin and type of soyabean processing was also observed for protein concentration of milk[38].

Erickson *et al.*[55] assumed that amino acid uptake of the mammary gland might be enhanced due to the effect of niacin on insulin. Intravenous insulin has been shown to increase milk protein and percent of casein in milk[63]. Several studies also measured casein concentrations in milk. No effects of niacin on casein content or yield in milk were

observed[43]; there even was a tendency for lowered casein content and yield after niacin supplementation[48]. However, in another study[44], the decrease in percentage casein-N of total N due to niacin feeding was significant for only one of two rations. It is therefore not possible to conclude if niacin acts via insulin on casein and/or protein synthesis. Especially in the case of protein yield, changes in milk yield might also play a role or were probably the reason for observed differences[48]. A theory for occasionally observed effects of niacin on milk protein content was an increased microbial protein synthesis in the rumen[55]. Other authors stated that mechanisms of niacin to increase protein content of milk still need to be clarified[38]. Thus, it cannot be concluded if effects are rather systemic or ruminal.

*Milk fat*

Except for Belibasakis and Tsirgogianni[57], who observed increased milk fat concentrations and yield after niacin was given, there were no significant effects of niacin on milk fat in studies in Table 6. Cervantes *et al.*[37] observed a tendency for decreasing milk fat content in NAM groups. Nevertheless, there have been several interactions. Interactions were found between niacin and fat[28] as well as between niacin, fat and week of lactation[48]. Bernard *et al.*[64] showed an interaction for niacin and processing of soyabeans. In surveys not mentioned in Table 6 no effect was seen [62], whereas other authors found increased milk fat content in lactation weeks 1 and 4 after NAM but not after NA supplementation[47].

If only studies are considered where niacin supplementation had an impact on blood niacin content, then there was no effect on milk fat[38,42] or a trend towards lower milk fat contents in the niacin-supplemented groups[37]. Therefore, changes following niacin supplementation might rather lie at the ruminal level. But since most research on the effects of niacin in the rumen was focussed only on the rumen, and no milk measurements were done, it is difficult to accept or to reject this thesis. Three studies measured ruminal and milk parameters in the same trial[12,27,28] and all came to different results. One observed no effect of niacin on ruminal VFA concentration, but an interaction between niacin and fat on milk fat content[28]. Another detected a tendency toward decreased molar proportion of acetate and an interaction between fat and niacin for molar proportion of butyrate, which did not lead to changes in milk fat content or yield[27]. Campbell *et al.*[12] found no effect on ruminal VFA concentrations or molar proportions, or on milk fat. Hence, other mechanisms might as well be involved.

## Future research directions

Considering the number of metabolic reactions where NAD(H) and NADP(H) are involved, the importance of niacin is obvious. However, animal trials with niacin supplementation did not lead to consistent results; therefore it is still not possible to determine exact conditions or doses for niacin supplementation. But there are several gaps of knowledge, which could, once resolved, answer this question. First, cognition of the effect of feeding on ruminal fermentation, niacin degradation and synthesis is insufficient. Furthermore, ruminal samples were taken at varying times after feeding and after niacin supplementation, which surely has an impact on the observed results. In addition, it is not known if absorption can occur in the abomasum or before the duodenal cannula and the mechanism of absorption is unspecified for ruminants. Niacin concentrations in blood also vary, which might be due to the different blood fractions analysed or vitamers examined. Different methods for niacin determination may lead to different results as well. It is also unknown whether some type of homeostatic system exists as was suggested for man. NEFA concentrations in blood seem to be lowered by NA, but not by NAM, and it is uncertain if NA acts on ketone bodies via this effect on NEFA or if other mechanisms are involved. Furthermore, the effect on NEFA might also have an impact on glucose metabolism, which is mediated through insulin, even though mechanisms are not clear. The vitamin's mode of action on milk parameters is uncertain and might be systemic or ruminal or a combination of both. If effects are rather systemic, feeding trials with oral, not rumen-protected supplementations will have limits. This seems to be at least the case for blood parameters, since disappearance before the duodenum is high.
Considering these points, we would suggest the following directions for future research:

(1) Different feeding regimes should be compared to characterise the impact of feed on niacin metabolism. Niacin content of the feed should be determined, as well as tryptophan, aspartate and quinolinate contents, since these are precursors of niacin synthesis.

(2) Simultaneous determination of ruminal, duodenal, blood and milk parameters would be useful to detect potential conjunctions.

(3) The time of sampling to investigate ruminal, duodenal and blood parameters should be standardised in relation to time of niacin feeding to avoid confusion between niacin and time effects.

(4) Experiments should be conducted with niacin infused in the abomasum and simultaneous duodenal and blood niacin measurements to study absorption site and extent.
(5) Studies on the mechanism of absorption for both vitamers would be useful.
(6) Surveys on possible metabolic storage, for example, liver or tissues (such as ruminal or duodenal walls) seem to be favourable, where NAD(H) and NADP(H) concentrations are measured as well.
(7) In general, research concerning niacin flow in the body is advisable.
(8) To investigate if effects of niacin on milk parameters are rather systemic or ruminal, surveys with or without post-ruminal niacin infusion are desirable.
(9) Studies on the influence of niacin on insulin in ruminants should be performed.
(10) Distinctions should be made between both vitamers. In addition, the conditions and locations of conversion of one vitamer to another should be better investigated.

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## PAPER II

# The effect of a niacin supplementation to three diets differing in forage-to-concentrate ratio on ruminal fermentation and flow of nutrients to the duodenum of dairy cows

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Prepared for submission

**ABSTRACT**

The objective of this study was to investigate the influence of a niacin supplementation to three diets with different forage-to-concentrate ratios (F:C ratio) on ruminal metabolism. The rations consisted of either 1/3 concentrate and 2/3 forage, 1/2 concentrate and 1/2 forage or 2/3 concentrate and 1/3 forage on dry matter basis. Each diet was fed in one period without and in the following with a supplementation of 6 g niacin (nicotinic acid, NA) per cow and day. Three dry and seven mid-lactation (102 ± 18 days in milk) Holstein - Friesian cows, equipped with cannulas in the dorsal sac of the rumen and proximal duodenum, were used. Ruminal fluid was obtained before and six times after the morning feeding, while duodenal chyme was collected every two hours for five days. $Cr_2O_3$ was used as flow marker.

NA supplementation increased rumen ammonia concentration, whereas it decreased short-chain fatty acid concentration. The amount of organic matter reaching the duodenum was enhanced if niacin was added to the rations. NA supplementation also led to higher flows of microbial protein and undegraded feed protein to the duodenum. Efficiency of microbial protein synthesis was enhanced. The effect of NA on microbial protein synthesis was highest in high concentrate ration and only low with the medium concentrate diet, which resulted in a trend for an interaction of NA and F:C ratio for this variable.

The amounts of niacin and NA reaching the duodenum rose with increasing concentrate proportion and also with NA supplementation, whereas amounts of nicotinamide were only influenced by NA feeding and not by the F:C ratio. Concentrate proportion in the diet affected only apparent synthesis of NA and thus total niacin, but not nicotinamide.

**Keywords:** niacin, forage-to-concentrate ratio, dairy cows, nicotinic acid

## INTRODUCTION

Niacin is of great importance in the energy metabolism because it is incorporated in the two electron-carrying coenzymes NAD(H) and NADP(H). In general, $NAD^+$ is involved in energy yielding metabolism, whereas the major coenzyme for reductive synthetic reactions is NADPH (Bender, 1992). But to date, no recommendations for a general supplementation of niacin to dairy cow rations are given, because ruminal synthesis seems to cover the requirements (NRC, 2001). However, supplemental niacin had several impacts on metabolism and performance in some cases, whereas also studies without an effect can be found, as reviewed by Niehoff et al. (2009).

If niacin was supplemented to cattle, it has been shown that only a part of the supplement arrived at the duodenum (Riddell et al., 1985; Zinn et al., 1987). Santschi et al. (2005a) stated that higher concentrate levels would probably influence bacterial population and rumen passage time, which could affect ruminal synthesis and use of B-vitamins. Hence, diets differing in forage - to - concentrate ratio might cause different amounts of niacin metabolised in the rumen. Therefore, the objective of this study was to investigate the effect of a niacin supplementation to three diets differing in forage-to-concentrate ratio on several measurements of ruminal fermentation and amounts of nutrients, especially niacin, arriving at the duodenum of dairy cows.

## MATERIALS AND METHODS

### *Experimental Design and Animals*

The experiment was conducted according to the European Community regulations concerning the protection of experimental animals and the guidelines of the Regional Council of Braunschweig, Lower Saxony, Germany (File Number 33.11.42502-04-057/07). A total of 10 Holstein-Friesian cows was used. The cows were equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter: 10 cm) and in the proximal duodenum, close to the pylorus (inner diameter: 2 cm). At the beginning of the experiment, animals had an average weight of 599.5 ± 79.0 kg. Seven cows were in lactation (102 ± 18.3 days in milk at the beginning), but three were dry. None of them was primiparous, lactation numbers ranged from second to fifth lactation. Lactating cows were milked at 5:00 and 16:00 h.

The cows were kept in a tethered stall with neck straps and with an individual trough for each cow. They had free access to water and to a salt block containing sodium chloride. Forage was offered at 5:30 and 15:30 h, concentrate was given at 5:30, 7:30, 15:30 and 17:30 h and hand mixed with roughage in the trough. Forage consisted of 60% maize silage and 40% grass silage on DM basis. Composition of concentrate is given in Table 1. Except for the dry cows, amounts offered were adjusted to the expected intake of each cow in order to reach nearly ad libitum intake but avoid refusals. To prevent excessive fattening of dry cows, they were restricted to a feed amount covering their maintenance.

**Table 1: Composition of concentrate in g/kg**

| Components | g/kg |
|---|---|
| Wheat | 250 |
| Corn | 250 |
| Soybean meal | 170 |
| Peas | 150 |
| Dried sugar beet pulp | 150 |
| Mineral and vitamin premix [1] | 20 |
| Calcium carbonate | 7 |
| Urea | 3 |

[1] Composition per kg: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6 g Zn, 5.4 g Mn, 1 g Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 1,000,000 IU vitamin $D_3$ ,1,500 mg alpha tocopherol acetate

The cows were assigned randomly to one of the three experimental diets. The diets applied were the following: low concentrate **(LC)** which consisted of 1/3 concentrate and 2/3 forage on DM basis, medium concentrate **(MC)** with 1/2 concentrate and 1/2 forage and high concentrate **(HC)** which contained 2/3 concentrate and 1/3 forage. The DM content of forage was determined twice weekly and amounts offered were adapted, to maintain the appropriate forage - to - concentrate ratio (**F:C ratio**). Each diet was fed in one period without supplemental niacin and in the following with a supplementation of 6 g niacin per cow and day as nicotinic acid (**NA**). The NA used was powdered NA, with a content of at least 99.5% NA (Lonza Ltd., Basel, Switzerland). NA was mixed in an extra 100 g of mineral and vitamin premix and one half was top dressed on the concentrate during the morning feeding, the other half in the evening. In periods without supplemental NA, 100 g of extra mineral and vitamin premix only were given in the same way. The last period was used to fill gaps in animal number per group.

The experimental design applied (Table 2) is unbalanced, due to different calving dates of the cows.

**Table 2: Animal numbers per diet with or without niacin supplementation**

| | LC[1] | MC[2] | HC[3] |
|---|---|---|---|
| Control | 8 | 7 | 9 |
| With NA[4] supplementation | 9 | 7 | 10 |

[1]LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2]MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3]HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4]NA = nicotinic acid

### *Sample Collection*

Cows were given three weeks to reach the respective concentrate level. Afterwards each period consisted of two weeks of adaptation to the diet, followed by one week of ruminal sampling and a second week of duodenal sampling.

Ruminal samples were taken on one day in the third week of each period. Approximately 100 mL of ruminal fluid were withdrawn from the ventral sac through the rumen fistula using a hand vacuum pump. Fluid was taken before first feeding at 5:30 h in the morning, and 30, 60, 90, 120, 180 and 360 minutes afterwards. For duodenal chyme collection, four 100 mL samples were taken through the duodenal cannula at two hour intervals for five consecutive days. Immediately after withdrawal, pH was measured using a glass electrode (pH525, WTW, Weilheim, Germany). The sample with the lowest pH within the four samples of a cow was added to the daily pooled sample of that cow and stored at -18 °C as described by Rohr et al. (1984). For duodenal flow measurements $Cr_2O_3$ was used as a marker. It was mixed in the rumen every 12 hours starting 10 days before the duodenal sampling period, and every 6 hours one day before and during the sampling week.

Feed samples and possibly occurring feed refusals were collected daily during the duodenal sampling week and pooled. Part of this was freeze-dried (Christ Epsilon 1-15, Martin Christ GmbH, Osterode, Germany) for niacin analysis, the rest was dried at 60 °C for nutrient analysis. Daily duodenal digesta samples were freeze-dried as well. Afterwards, all dried samples were ground through a 1-mm screen.

*Analyses*

Except for dried feedstuffs and refusals for nutrient analysis, samples that could not be analysed immediately were kept frozen at -18°C until analysis. Feeds and refusals were analysed for dry matter (**DM**), crude protein (**CP**), crude ash (**ash**), ether extract (**EE**), crude fibre (**CF**) and starch according to methods of the VDLUFA (Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten; Naumann and Bassler, 1976). The analysis of ADF and NDF was done following Goering and Van Soest (1970). The niacin content in feedstuffs was determined microbiologically with *Lactobacillus plantarum* (VDLUFA method). For the calculation of energy content, digestibility of the forage was estimated in a balance experiment with 4 adult wethers (GfE, 1991).

The pH of rumen fluid was measured immediately after withdrawal (pH525, WTW, Weilheim, Germany). $NH_3$-N in the fluid was determined according to DIN 38406-E5-2 (1998). Short-chain fatty acids (**SCFA**) were analysed using a gas chromatograph (Hewlett Packard 5580, Avondale, PA, USA) equipped with a flame ionization detector as described by Geissler et al. (1976).

In thawed duodenal chyme, nitrogen concentration was quantified by the Kjeldahl method. The content of DM and ash were determined in the freeze-dried and ground duodenal chyme with the same methods as for the feed analysis for each day of the sampling week. The proportion of microbial-N of non-ammonia-N (**NAN**) was determined via near infrared spectroscopy (Lebzien and Paul, 1997). $Cr_2O_3$ was analysed by atomic absorption spectrophotometry according to Williams et al. (1962) and used to calculate duodenal DM flow. Daily duodenal DM flows were utilized to generate one pooled sample per cow per week, in which concentrations of CF, NDF, ADF and starch were quantified with the methods named above for feedstuffs. Niacin (NA and nicotinamide) was determined in the pooled samples by HPLC. Sample preparation was carried out according to the method of Santschi et al. (2005a). 0.5 g freeze-dried digesta sample and 35 mL of HCl (0.1 M) were mixed in a 50 mL brown glass flask, autoclaved for 50 min at 121 °C and after cooling, the mixture was diluted to a final volume of 50 mL with ultrapure water. 10 mL fluid was centrifuged for 30 min at 14000 x g and 4 °C and 20 µL of the supernatant were injected into a Shimadzu HPLC system (model SCL-10A controller, model LC-10AS pump, model SIL-10AC autosampler, model CTO-10AC oven; Shimadzu, Kyoto, Japan) equipped with a multi wavelength detector

(model SPD-M10A VP; Shimadzu, Kyoto, Japan). Samples were run through a C18 column (Inertsil ODS, 150 mm x 3 mm i.d., 5µ) and were eluted using a mobile phase, consisting of 94% of sodium 1-hexanesulfonate monohydrate (5 mM) and sodium 1-pentanesulfonate monohydrate (3.8 mM) in ultrapure water (adjusted to pH 2.55 with 2*M* phosphoric acid) and 6% acetonitrile at a flow rate of 0.5 mL/min. The detection wavelength was 260 nm.

***Calculations and Statistics***

The ME (MJ) content was calculated according to GfE (2001):

$$\text{ME (MJ)} = 0.0312 \text{ g DEE} + 0.0136 \text{ g DCF} + 0.0147 \text{ g (DOM} - \text{DEE} - \text{DCF)} + 0.00234 \text{ g CP}$$

Digestibility values for forage were obtained from the wether balance trial mentioned before, whereas for concentrates, tabular values were used (DLG, 1997).

Daily duodenal dry matter flow (**DMF**) was calculated as follows:

$$\text{DMF (kg/day)} = \frac{\text{chromium application (mg/d)}}{\text{duodenal chromium concentration (mg/g DM)}} / 1000$$

For the calculation of duodenal flow of nutrients, niacin and OM, the DMF at the duodenum was multiplied with their respective concentrations in duodenal chyme. Apparent niacin synthesis in the reticulo - rumen was calculated by subtracting the niacin intake from the amount arriving at the duodenum. Even though niacin analysis in feedstuffs was done microbiologically, it was assumed that the measured concentrations represent only NA (personal communication VDLUFA). This is consistent with data from literature, where also no nicotinamide (**NAM**) was present in feed (Santschi et al., 2005b).

The mean ammonia proportion of total N in duodenal chyme was assumed to be 4.9% (Riemeier, 2004). Thus, the daily flow of NAN was estimated by subtracting 4.9% of the N flow at the duodenum. Following Lebzien and Voigt (1999), utilizable crude protein (**uCP**) at the duodenum was estimated to be:

uCP (g/d) = crude protein flow at the duodenum – endogenous crude protein (**EP**)

Following Brandt and Rohr (1981) EP was calculated using DMF at the duodenum:

EP (g/d) = (3.6 * kg DMF) * 6.25

Rumen-degradable crude protein (**RDP**) and rumen-undegradable crude protein (**RUP**) and fermented organic matter (**FOM)** were calculated with the equations:

RDP (g/d) = CP intake - RUP

RUP (g/d) = 6.25 * [g NAN at the duodenum – (g microbial N + (g EP / 6.25))]

FOM (kg/d) = OM intake – (duodenal OM flow – microbial OM)

Microbial OM was estimated according to Schafft (1983):

Microbial OM = 11.8 * microbial N

The statistical analysis was performed using the statistical software package SAS (Version 9.1, procedure mixed, SAS Institute Inc., Cary, USA). The procedure "MIXED" was applied. Concentrate level ("CONC") and niacin ("NIA") were considered as fixed effects. Additionally, to analyse rumen variables, also the time after feeding in minutes ("MINUTES") was included. OM intake ("OMI") was considered as fixed regressive component. The fact that a cow had to be used in several periods for different treatments was taken into account by using the "RANDOM" statement for the individual "COW" effect. Variances were evaluated with the restricted maximum likelihood method (**REML**) and degrees of freedom were calculated according to the Kenward-Roger method. The "PDIFF" option was applied to test differences between least square means, using a Tukey-Kramer test for post-hoc analysis. Thus, the SAS code for rumen variables was as follows:

**PROC MIXED METHOD** = **REML**;

**CLASS** COW CONC NIA MINUTES;

**MODEL** Y = CONC NIA CONC*NIA OMI MINUTES MINUTES*CONC*NIA

/ **DDFM** = **KENWARDROGER;**

**RANDOM** COW;

**LSMEANS** CONC NIA CONC*NIA MINUTES*CONC*NIA

/ **PDIFF** e ADJUST = TUKEY;

The SAS code applied for duodenal variables was basically the same, except for all "MINUTES" related effects and interactions, which were not of interest in duodenal measurements and therefore deleted in that model. Main effects of NA supplementation, level of concentrate or their interaction were considered as significant if F-statistics revealed $P \leq 0.05$, a trend was announced if $P \leq 0.10$. All values presented are least square means (**LS MEANS**), except for chemical composition of feedstuffs, OM and niacin intakes, where arithmetic means are given.

## RESULTS

### *Feeding*

Due to overnight drying of samples for DM determination, amounts fed could only be adapted at the following feeding. This resulted in differences between the F:C ratios planned and fed. For the LC ration, the real F:C ratio fed was 68.3% forage and 31.7% concentrate; for MC it was 49.8% and 50.2% and for the HC ration it was 35.2% and 64.8% on a DM basis.

Diets applied in the present study were not formulated to be isonitrogenous and –caloric between different F:C ratios. The nutrient composition of each ration is given in Table 3, feed analyses were pooled over the course of the study for this calculation.

The native niacin concentration of the rations seemed to be nearly the same (Table 3). But especially for forage, there was a high variation in native niacin content between different batches (minimum = 21.5 mg/kg; maximum = 77.6 mg/kg). This is also indicated by the large standard deviation, especially in LC ration. Niacin content of concentrates was less variable (minimum = 27.3 mg/kg; maximum = 43.4 mg/kg).

**Table 3: Mean values (n=7) and standard deviation (SD) of nutrient composition and energy content of the different rations fed**

| | LC[1] | | MC[2] | | HC[3] | |
|---|---|---|---|---|---|---|
| Nutrients, g/kg DM | Mean | SD | Mean | SD | Mean | SD |
| OM | 938 | 3 | 940 | 2 | 941 | 2 |
| CP | 132 | 5 | 149 | 6 | 163 | 6 |
| EE [4] | 28.4 | 4.7 | 27.5 | 3.7 | 26.7 | 3.0 |
| CF [5] | 184 | 5 | 151 | 4 | 124 | 3 |
| ADF | 202 | 8 | 168 | 6 | 141 | 5 |
| NDF | 380 | 18 | 323 | 14 | 279 | 12 |
| Starch | 281 | 57 | 335 | 42 | 378 | 31 |
| MJ ME / kg DM | 11.20 | 0.04 | 11.67 | 0.04 | 12.04 | 0.03 |
| Niacin mg/kg DM | 35.0 | 16.9 | 34.6 | 14.0 | 34.4 | 11.7 |

[1] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4] EE = ether extract
[5] CF = crude fibre

The arithmetic mean of OMI per day was almost equal for all rations and is shown in Table 4 together with mean, minimum (**MIN**) and maximum (**MAX**) niacin intakes in the respective diets.

**Table 4: Arithmetic mean, MIN and MAX of mg niacin and arithmetic mean of kg OM intake (OMI) per cow and day in different experimental groups**

| Experimental group | Mean | MIN | MAX | OMI |
|---|---|---|---|---|
| LC [1] | 553 | 222 | 1133 | 12.1 |
| LC + NA [2] | 6449 | 6233 | 6839 | 12.4 |
| MC [3] | 325 | 177 | 412 | 12.3 |
| MC + NA | 6337 | 6178 | 6426 | 12.6 |
| HC [4] | 476 | 233 | 838 | 12.2 |
| HC + NA | 6370 | 6210 | 6509 | 12.4 |

[1] LC= low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2] NA = nicotinic acid
[3] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[4] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis

### *Rumen Fermentation Measurements*

In general, most of the variables analysed showed significant effects of OMI and time after feeding. But since these relationships are well known, they will not be presented.

The results of ruminal measurements over the whole sampling time are shown in Table 5. As expected, the F:C ratio influenced almost all analysed variables. NA had a significant effect on ruminal ammonia concentration ($P < 0.001$), the molar proportions of iso-butyric acid ($P < 0.001$), iso-valeric acid ($P < 0.01$), valeric acid ($P < 0.01$) and the concentration of total SCFA in ruminal fluid ($P < 0.001$). Ammonia concentration

**Table 5: Least square means and (standard error) of analysed ruminal variables over the whole sampling period**

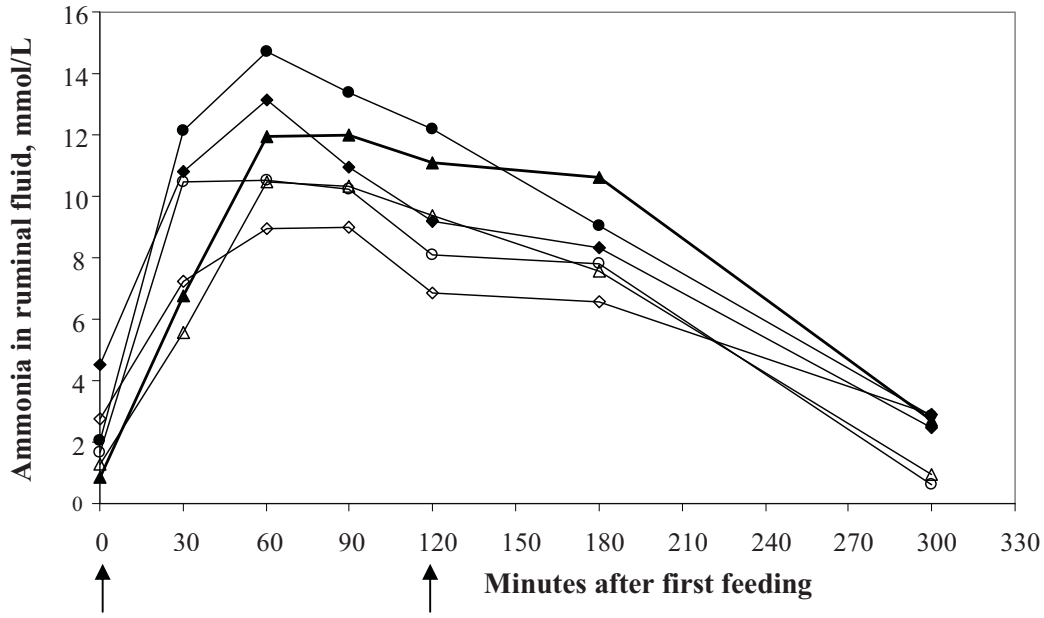
| | LC [1] | | MC [2] | | HC [3] | | P | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | - NA[4] (n = 8) | + NA (n = 9) | - NA (n = 7) | + NA (n = 7) | - NA (n = 9) | + NA (n = 10) | CONC [5] | NA | CONC x NA | MIN [6] x CONC x NA |
| pH | 6.36 (0.06) | 6.37 (0.06) | 6.33 (0.06) | 6.35 (0.06) | 6.25 (0.06) | 6.35 (0.06) | 0.16 | 0.12 | 0.37 | 0.97 |
| Ammonia mmol/L | 6.51 (0.68) | 8.01 (0.66) | 7.06 (0.70) | 9.49 (0.70) | 6.33 (0.66) | 8.48 (0.65) | 0.04 | <0.001 | 0.49 | <0.01 |
| Acetic acid Mol% | 62.7 (0.80) | 63.1 (0.78) | 64.1 (0.83) | 64.3 (0.83) | 58.1 (0.78) | 59.0 (0.76) | <0.001 | 0.24 | 0.76 | 1.00 |
| Propionic acid Mol% | 18.1 (0.80) | 18.4 (0.79) | 18.1 (0.82) | 17.8 (0.83) | 21.8 (0.79) | 20.4 (0.77) | <0.001 | 0.20 | 0.10 | 0.99 |
| Iso-butyric acid Mol% | 0.88 (0.04) | 0.92 (0.04) | 0.91 (0.04) | 1.04 (0.04) | 0.86 (0.04) | 1.03 (0.04) | 0.01 | <0.001 | 0.01 | 0.06 |
| Butyric acid Mol% | 14.7 (0.45) | 14.1 (0.43) | 13.5 (0.47) | 13.7 (0.47) | 14.5 (0.43) | 14.9 (0.42) | <0.01 | 0.80 | 0.24 | 1.00 |
| Iso-valeric acid Mol% | 1.73 (0.12) | 1.67 (0.12) | 1.63 (0.13) | 1.63 (0.13) | 1.45 (0.12) | 1.93 (0.12) | 0.57 | <0.01 | <0.001 | 0.50 |
| Valeric acid Mol% | 1.98 (0.27) | 1.86 (0.26) | 1.86 (0.27) | 1.47 (0.27) | 3.30 (0.26) | 2.77 (0.26) | <0.001 | <0.01 | 0.20 | 1.00 |
| SCFA[7] total mmol/L | 113.6 (3.61) | 99.1 (3.47) | 105.8 (3.77) | 99.2 (3.78) | 114.9 (3.47) | 108.9 (3.35) | <0.01 | <0.001 | 0.20 | 0.97 |

[1] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis,
[2] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4] NA = nicotinic acid
[5] CONC = level of concentrate
[6] MIN = minutes after first feeding
[7] SCFA = short - chain fatty acids

increased, whereas valeric acid and SCFA decreased with NA feeding. Interactions with F:C ratio were not significant for these variables.

For ammonia concentration, the interaction between minutes after feeding, level of concentrate and NA supplementation was also significant, because the fermentation pattern over time differed for the respective concentrate level with or without NA (Figure 1).

Iso-valeric acid decreased with the LC, but it increased with the HC ration, which resulted in a significant interaction between NA and concentrate level ($P < 0.001$). Another interaction was observed for molar proportion of iso-butyric acid ($P = 0.01$), because differences were very small with LC ration, but a distinct increase after NA supplementation was found with the MC and HC ration. A trend for an interaction ($P = 0.10$) was detected for propionic acid, because the molar proportion showed a numerical increase in LC ration due to NA feeding, but it decreased in the other two feeding strategies.




↑ feeding; at 0 minutes, the whole morning feeding amount of forage and half of concentrate was given, the other half at 120 minutes

low concentrate — low concentrate with nicotinic acid — medium concentrate — medium concentrate with nicotinic acid — high concentrate — high concentrate with nicotinic acid

**Figure 1: Ammonia concentration in ruminal fluid at different time points after feeding**

### *Nutrient Flow at the Duodenum*

Nutrient flows at the duodenum are presented in Tables 6 and 7. As was the case for rumen fermentation variables, OMI also influenced most duodenal values, but this will as well not be presented.

The F:C ratio had influences on the digestibility of fibre fractions and most measurements of protein metabolism. As shown in Table 6, the addition of NA to the respective ration led to an increased amount of OM arriving at the duodenum (P = 0.02). As a result, apparent ruminal OM digestibility decreased (P = 0.03) over all three rations fed. No other significant influences of niacin supplementation were observed for the nutrients mentioned in Table 6.

**Table 6: Nutrient flow at the duodenum and apparent ruminal digestibilities as well as the amount of fermented organic matter; LS MEANS and (standard error)**

| | LC [1] | | MC [2] | | HC [3] | | P | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | - NA [4] (n=8) | + NA (n=9) | - NA (n=7) | + NA (n=7) | - NA (n=9) | + NA (n=9) [5] | CONC[6] | NA | CONC x NA |
| OM, kg/d | 6.77 (0.28) | 7.11 (0.27) | 6.88 (0.29) | 7.22 (0.29) | 7.02 (0.27) | 7.55 (0.27) | 0.21 | 0.02 | 0.85 |
| ARD [7] OM, % | 46.9 (2.14) | 43.4 (2.01) | 44.6 (2.23) | 43.3 (2.22) | 45.2 (2.06) | 41.5 (2.01) | 0.48 | 0.03 | 0.70 |
| NDF, kg/d | 2.76 (0.19) | 2.90 (0.18) | 2.41 (0.20) | 2.33 (0.20) | 2.38 (0.18) | 2.54 (0.18) | 0.01 | 0.59 | 0.73 |
| ARD NDF, % | 46.6 (3.73) | 45.0 (3.60) | 44.1 (3.90) | 47.0 (3.89) | 39.0 (3.59) | 37.8 (3.59) | 0.01 | 0.99 | 0.69 |
| ADF, kg/d | 1.38 (0.12) | 1.47 (0.11) | 1.30 (0.12) | 1.19 (0.12) | 1.23 (0.11) | 1.34 (0.11) | 0.13 | 0.68 | 0.45 |
| ARD ADF, % | 49.2 (4.65) | 47.5 (4.49) | 42.8 (4.85) | 48.1 (4.84) | 37.8 (4.48) | 36.4 (4.48) | <0.01 | 0.79 | 0.53 |
| Starch, kg/d | 0.65 (0.09) | 0.66 (0.09) | 0.61 (0.10) | 0.86 (0.10) | 0.87 (0.09) | 0.81 (0.09) | 0.07 | 0.32 | 0.17 |
| ARD Starch, % | 84.6 (1.84) | 83.2 (1.75) | 86.9 (1.95) | 83.0 (1.94) | 83.0 (1.75) | 83.8 (1.74) | 0.63 | 0.24 | 0.35 |
| FOM [8], kg/d | 7.34 (0.19) | 7.21 (0.18) | 7.60 (0.20) | 7.30 (0.20) | 7.51 (0.18) | 7.44 (0.18) | 0.36 | 0.20 | 0.76 |
| FOM of OMI [9], % | 61.0 (1.43) | 59.6 (1.37) | 62.0 (1.51) | 61.0 (1.51) | 62.6 (1.37) | 62.4 (1.37) | 0.15 | 0.35 | 0.86 |

[1] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4] NA = nicotinic acid
[5] Values of one cow were excluded from this group, because she refused feed intake in duodenal sampling week
[6] CONC = level of concentrate
[7] ARD = apparent ruminal digestibility
[8] FOM = fermented organic matter
[9] OMI = organic matter intake

Several effects of niacin supplementation can be observed on protein metabolism (Table 7). Even though the proportion of microbial-N in NAN was not influenced by NA, the amount of N and NAN at the duodenum increased significantly during vitamin feeding ($P < 0.01$). Also, the amount of microbial protein increased ($P < 0.01$). As this increase was most pronounced with the HC ration (1166 g MP without and 1412 g MP with NA), a trend for an interaction between NA and concentrate level was found ($P = 0.10$).

Furthermore, the amount of RUP arriving at the duodenum increased as well ($P = 0.01$) due to NA feeding, as was thus also the case if it is expressed in percent of crude protein intake ($P < 0.01$). As uCP includes RUP and MP, uCP also rose after NA supplementation ($P < 0.01$).

**Table 7: N flow at the duodenum and efficiency of microbial protein synthesis; LS MEANS and (standard error)**

| | LC [1] | | MC [2] | | HC [3] | | P | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | - NA [4] (n=8) | + NA (n=9) | - NA (n=7) | + NA (n=7) | - NA (n=9) | + NA (n=9)[5] | CONC[6] | NA | CONC x NA |
| N, g/d | 242 (16.3) | 275 (15.6) | 291 (17.2) | 298 (17.2) | 301 (15.6) | 365 (15.5) | <0.001 | <0.01 | 0.13 |
| NAN, g/d | 230 (15.5) | 262 (14.8) | 277 (16.4) | 283 (16.4) | 286 (14.8) | 347 (14.8) | <0.001 | <0.01 | 0.13 |
| Microbial-N, % of NAN | 65.7 (0.77) | 64.8 (0.75) | 66.2 (0.79) | 66.2 (0.79) | 65.4 (0.75) | 65.5 (0.75) | 0.15 | 0.45 | 0.46 |
| MP [7], g/d | 946 (66.8) | 1053 (64.3) | 1139 (70.0) | 1163 (69.8) | 1166 (64.2) | 1412 (64.1) | <0.001 | <0.01 | 0.10 |
| MP per FOM[8], g/kg | 125 (11.7) | 146 (11.3) | 151 (12.2) | 159 (12.1) | 150 (11.3) | 183 (11.3) | <0.01 | <0.01 | 0.31 |
| MP per MJ ME, g/MJ | 6.28 (0.41) | 7.22 (0.40) | 7.40 (0.43) | 7.55 (0.43) | 7.19 (0.40) | 8.68 (0.40) | <0.001 | <0.01 | 0.10 |
| MP per RDP, g/g | 0.65 (0.06) | 0.83 (0.06) | 0.71 (0.06) | 0.72 (0.06) | 0.67 (0.06) | 0.92 (0.06) | 0.31 | <0.01 | 0.10 |
| RUP, g/d | 306 (34.4) | 382 (32.8) | 403 (36.4) | 407 (36.4) | 433 (32.7) | 550 (32.7) | <0.001 | 0.01 | 0.18 |
| RUP, % of feed CP | 17.1 (1.66) | 22.7 (1.57) | 19.3 (1.77) | 19.6 (1.77) | 19.5 (1.57) | 25.3 (1.57) | 0.11 | <0.01 | 0.15 |
| uCP [9], g/d | 1253 (92.6) | 1435 (88.2) | 1539 (97.8) | 1571 (97.6) | 1599 (88.1) | 1964 (88.0) | <0.001 | <0.01 | 0.12 |

[1] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4] NA = nicotinic acid
[5] Values of one cow were excluded from this group, because she refused feed intake in duodenal sampling week
[6] CONC = level of concentrate
[7] MP = microbial crude protein
[8] FOM = fermented organic matter
[9] uCP = utilizable crude protein

Variables analysing the effectiveness of microbial protein synthesis were also influenced by the addition of NA. The amount of MP synthesized increased for all concentrate levels, either expressed per kg FOM ($P < 0.01$) or per MJ ME intake ($P < 0.01$). However, for MP per MJ ME also a trend for an interaction with F:C ratio was observed ($P = 0.10$), because differences were most pronounced with the HC ration (7.19 g/ MJ ME without NA versus 8.68 g/ MJ ME with NA) and low with MC diet. Also, the amount of microbial protein per g RDP was increased after NA feeding ($P < 0.01$). A trend for an interaction with level of concentrate can be seen for this variable as well, as again the greatest difference was found with the HC ration (0.67 g/g without NA; 0.92 g/g with NA supplementation), whereas values for MC diet differed only marginally.

***Niacin Flow at the Duodenum***

The measured niacin flow and calculated apparent synthesis are presented in Table 8. As no NAM was found in feed, the amount of NAM at the duodenum represents the apparent synthesis of NAM. The F:C ratio influenced all measurements except for NAM flow. The daily amount of NA arriving at the duodenum rose with increasing concentrate level ($P < 0.001$), but differences were small between MC and HC. Even though there was no effect of F:C ratio on NAM, this increasing NA flow led to a significant rise in total niacin ($P < 0.01$). Apparent synthesis was also influenced by the proportion of concentrate, as it was considerably lower with the LC ration than with MC or HC.

Addition of NA to the diet also influenced the niacin flow. The amount of NA reaching the duodenum was enhanced in all three rations after NA supplementation ($P < 0.001$). Even though NA was added, the NAM flow at the duodenum also rose ($P = 0.05$) after NA feeding, as was thus seen for total niacin flow ($P < 0.001$) as well. There was a large effect of niacin addition on apparent niacin synthesis. For all supplemented groups, apparent synthesis of either total niacin or NA was below zero, indicating a substantial disappearance of the 6 g NA given. Concentrate level also had an effect, disappearance of supplemental niacin was least with HC ration. Calculated from the LS MEANS given in Table 8, 88% of the 6 g NA supplemented did not reach the duodenum in HC ration, whereas it was 94% in MC and 93% in LC diet.

**Table 8: Duodenal flow and apparent synthesis of niacin; LS MEANS and (standard error)**

| Item | LC [1] | | MC [2] | | HC [3] | | P | | |
|---|---|---|---|---|---|---|---|---|---|
| | - NA [4] (n=8) | + NA (n=9) | - NA (n=7) | + NA (n=7) | - NA (n=9) | + NA (n=9) [5] | CONC[6] | NA | CONC x NA |
| NA, mg/d | 880 (128.1) | 1242 (123.9) | 1114 (133.3) | 1395 (132.9) | 1188 (123.6) | 1762 (123.5) | <0.001 | <0.001 | 0.25 |
| NAM [7], mg/d | 724 (58.5) | 779 (55.1) | 780 (62.5) | 831 (62.6) | 707 (55.1) | 871 (55.0) | 0.61 | 0.05 | 0.49 |
| Niacin total, mg/d | 1602 (164.8) | 2021 (158.3) | 1886 (172.9) | 2221 (172.5) | 1895 (158.0) | 2630 (157.8) | <0.01 | <0.001 | 0.25 |
| AS [8] NA mg/d | 335 (139.4) | -5199 (133.5) | 804 (146.6) | -4915 (146.3) | 713 (133.3) | -4608 (133.1) | <0.001 | <0.001 | 0.22 |
| AS niacin mg/d | 1057 (171.4) | -4419 (163.3) | 1575 (181.1) | -4089 (180.9) | 1421 (163.1) | -3738 (162.8) | <0.01 | <0.001 | 0.22 |

[1] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[2] MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis
[3] HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis
[4] NA = nicotinic acid
[5] Values of one cow were excluded from this group, because she refused feed intake in duodenal sampling week
[6] CONC = level of concentrate
[7] NAM = nicotinamide
[8] AS = apparent synthesis, calculated as difference of amount arriving at the duodenum and intake

## DISCUSSION

The effects of the F:C ratio on ruminal fermentation measurements have already been intensively investigated elsewhere (e.g., Yang et al., 2001; Moorby et al., 2006). Thus, they will only be discussed for duodenal niacin flows or if significant interactions with supplemental niacin occurred.

### *Rumen*

Descriptions of the effects of a niacin supplementation on ruminal ammonia concentration in the literature are miscellaneous. As in this trial (Table 5), an increase in ammonia concentration after niacin supplementation was found in one study (F:C ratio 50:50; Riddell et al., 1980). Applying the same concentrate level, Madison-Anderson et al. (1997) detected only a trend for enhanced concentrations in niacin supplemented cows. Furthermore, Christensen et al. (1996) observed an interaction between content of fat in the diet and NA. NA feeding enhanced ammonia concentration in the rumen in high fat diets, but resulted in a decrease in low fat rations (F:C ratio 50:50). This decrease in ruminal ammonia concentration after niacin supplementation was also observed in other

studies, in vivo (Samanta et al., 2000a) as well as in vitro (Shields et al., 1983; Samanta et al., 2000b). However, other in vivo experiments did not show an effect of a niacin supplementation on ruminal ammonia concentration (Arambel et al., 1986; Doreau and Ottou, 1996). No particular F:C ratio was obvious in these studies, where an effect in either direction was always seen. This is in accordance with our results, because the augmentation of ammonia concentration occurred at all three concentrate levels.

Riddell et al. (1980) attributed the observed increase in ruminal ammonia concentration to an apparent stimulation of ureolytic activity in the rumen of niacin fed cows, because urea nitrogen contents were lowered in those animals.

Another explanation could be an effect on rumen protozoa. It is often assumed that niacin is beneficial for protozoa in the rumen, because they are not able to synthesize the vitamin and significant increases of protozoa in rumen fluid have been observed after niacin feeding (Horner et al., 1988; Erickson et al., 1990; Doreau and Ottou, 1996). Faunation typically increases ruminal $NH_3$ – N concentration (Firkins et al., 2007). Thus, niacin might have been advantageous for the protozoan population, resulting in higher ammonia concentrations in the rumen.

The difference between the results reported here and those of other studies might be in part explainable with different sampling times and diurnal variation of ruminal ammonia concentration. In this study, trends or significant differences between treatments were found at 30 and 60 minutes after feeding (Figure 1). Numerically, differences existed until 3 h after feeding, but concentrations were almost equal for all treatments at the last sampling time (5 h after feeding). Most studies cited above named two hours after feeding as their earliest sampling time, where differences might have already disappeared or been less pronounced.

Supplementation of 6 g NA did not lead to significant changes in molar proportions of major SCFA (acetic, propionic and butyric acid; Table 5), which is in accordance with several other studies (Riddell et al., 1980; Campbell et al., 1994; Madison-Anderson et al., 1997). However, trends or significant differences in molar proportions of acetate (Christensen et al., 1996), butyrate (Arambel et al., 1986; Doreau and Ottou, 1996; Christensen et al., 1996) or propionate (Arambel et al., 1986; Samanta et al., 2000a) have been found after niacin supplementation as well. No particular F:C ratio could be identified from these trials, where effects were always or never present.

But a trend for an interaction of niacin and concentrate on the molar proportion of propionic acid was observed in the present experiment (Table 5). Molar proportions

increased with LC, but decreased with MC and HC ration after niacin supplementation. Influences on propionate reported in the literature have been inconsistent. Samanta et al. (2000a), without specifying the F:C ratio, observed increased molar percentages of propionate after niacin feeding. NA supplementation increased molar percentage of propionic acid also in a ration containing toasted soybean meal, but decreased it, when untoasted soybean meal was used (Arambel et al., 1986; F:C ratio 55:45). This finding was not explained by the authors.

The already mentioned stimulating effect of niacin on rumen protozoa may be a reason for the observed trend for an interaction in the present trial. Faunation has been shown to decrease molar proportions of propionate and increase acetate and butyrate (Eugene et al., 2004), because protozoa produce only very little amounts of propionate. As higher concentrate levels are detrimental for protozoa (Eugene et al., 2004), a stimulatory effect of niacin on protozoa might have been more important under these conditions.

Another explanation is a change in the ratio of oxidized and reduced coenzymes. The presence of electron donors and acceptors influences the pathway of pyruvate conversion to SCFA (Van Houtert, 1993). If supplemental NA is incorporated into the coenzymes, it is converted to $NAD^+$ (Michal, 1999), which is an electron acceptor and is hence needed in electron - donating pathways. Thus, the ratio of $NAD^+$ and its reduced form NADH might be enhanced after niacin feeding. A high $NAD^+$/NADH ratio reduces the flow of carbon through electron-accepting pathways like propionate production (Van Houtert, 1993). This might be more pronounced if more carbohydrates reach the rumen in high concentrate rations. However, Shields et al. (1983) observed a numerical increase in the $NAD^+$/NADH ratio after 6 h of incubation with 100 ppm NA only in fermenters with $NH_4Cl$ as nitrogen source, but those with amino acids showed a decrease. Yet total nucleotides were enhanced in both groups following NA supplementation in this study. But NAD failed to increase in another survey and it was concluded that microbial cell levels of NAD seem to be independent of free extra - cellular niacin concentration (Abdouli and Schaefer, 1986). Therefore, this interpretation of the results has to be treated with care and further research is needed concerning the extent of microbial NAD(H) and NADP(H) synthesis and the ratio of oxidized and reduced coenzymes.

The decrease in total SCFA concentration in niacin supplemented groups was not expected (Table 5). Others observed a significant increase (Samanta et al., 2000a; Kumar

and Dass, 2005) or no effects (Campbell et al., 1994; Christensen et al., 1996; Madison-Anderson et al., 1997) after NA or NAM feeding. However, Arambel et al. (1986) fed 55% forage and found an interaction between niacin and type of soybean processing. Total SCFA concentration increased after niacin feeding in the ration containing toasted soybean meal, whereas it decreased significantly in the rumen of cattle fed untoasted soybean meal. Riddell et al. (1980) observed a significant reduction of SCFA in the NA supplemented groups 6 h after feeding, when a ration containing 50% forage was fed. But this effect was not significant if the whole measured time span of 12 h is considered. No F:C ratio was apparent which either always or never caused effects in these studies. This is also consistent with our observations, because no interaction with F:C ratio was observed in the present trial.

The present results concerning SCFA concentration do not seem to be explainable with a probable effect of niacin on protozoa, as a decrease in ruminal SCFA concentration is usually observed in defaunated animals (Eugene et al., 2004). However in sheep, faunated animals also were found to have lower SCFA concentrations (Santra et al., 2007), therefore it might be possible. But another explanation may also be that niacin supplementation influenced digesta kinetics. Duodenal liquid dilution rates have been shown to be higher in niacin supplemented cattle (Arambel et al., 1986). Furthermore, although not significant, the turnover of rumen fluid in mL/min was higher in niacin fed sheep and cows (Schussler et al., 1978). Schaetzel and Johnson (1981) also assumed that niacin might alter ingesta kinetics. However, in another study, no influence was found (Christensen et al., 1996).

### *Nutrient Digestibility*

Apparent ruminal digestibilities were not significantly influenced by niacin supplementation, except for OM (Table 6). In other experiments, apparent total tract digestibility was calculated and showed as well no effects of niacin feeding on NDF or ADF digestibility (Arambel et al., 1986; Erickson et al., 1992; Ottou et al., 1995). Also no effect of niacin on apparent ruminal OM digestibility was observed in other studies (Doreau and Ottou, 1996; Christensen et al., 1996; 40 - 60% concentrate). This is in contrast to the results of this trial, because apparent ruminal OM digestibility was decreased in niacin fed groups, even though differences were small in MC ration. But the observed decline matches well with lowered concentration of SCFA in the rumen during NA supplemen-

tation. This might also indicate faster passage of ruminal contents to the duodenum, thus leaving less time for degradation in the rumen. Furthermore, microbial OM is also included in OM at the duodenum. Hence, the increase in microbial growth due to niacin supplementation might also contribute to the increase in OM arriving at the duodenum in supplemented groups. Differences between groups are no longer significant if the amount of FOM at the duodenum is considered, thus supporting this hypothesis.

***Duodenal N flow and Microbial Protein Synthesis***

N, NAN and microbial protein flow at the duodenum were higher in niacin supplemented groups at all F:C ratios (Table 7). But a trend for an interaction was observed for microbial protein, because differences were most pronounced with the HC, and negligible with the MC ration. In the HC diet, approximately 250 g more microbial protein was synthesized per day in niacin fed animals. An increase in microbial protein synthesis has been observed in other studies after niacin supplementation (Riddell et al., 1980; Samanta et al., 2000a; Kumar and Dass, 2005). However, no effect was detected in other experiments (Campbell et al., 1994; Doreau and Ottou, 1996; Christensen et al., 1996). From the studies cited, no F:C ratio is obvious in leading always to an effect after niacin supplementation. This does not match with our results, because we observed a trend to an interaction between niacin and concentrate level. But diets applied in the studies had F:C ratios between 40:60 and 60:40, which is less than the HC ration in the present trial.

The observed higher microbial protein synthesis despite higher ruminal ammonia concentrations seems to be controversial in our experiment. But Riddell et al. (1980) also observed increases in ammonia and bacterial protein concentrations in the rumen after niacin supplementation. As already mentioned, they attributed this to a stimulation of ureolytic activity.

It is stated several times in the literature that an increase in protozoal numbers is responsible for an increase in microbial protein production after niacin supplementation (Dennis et al., 1982; Samanta et al., 2000a). But usually faunation results in a lower microbial protein flow to the duodenum due to predation of bacteria by protozoa and thus greater N-recycling inside the rumen (Eugène et al., 2004). Yet more recent research suggests that the relative amount of bacterial N consumed by protozoa could be less than previously thought, especially in vivo in dairy cows (Firkins et al., 2006). For ex-

ample starch grains are quickly engulfed, fill protozoa and therefore limit bacterial predation (Hristov and Jouany, 2005). This might explain the interaction of concentrate level and niacin in our trial, because effects were most pronounced in HC ration (Table 7). Furthermore, as we hypothesized an increase in digesta passage after niacin supplementation in the present trial, this may reduce the protozoal predation of bacteria as well. But it was also suggested that an increase in the passage rate directly influences microbial protein synthesis instead of a mediated effect through less predation of bacteria (Firkins et al., 2007). Apart from protozoa, other ruminal micro - organisms have niacin requirements as well. Ford et al. (1958) showed this for several *Lactobacillus* and *Streptococcus* strains isolated from the rumen of sheep. Thus, the NA supplementation might have directly influenced the bacterial population of the rumen as well, with highest benefits in the HC ration.

NA supplementation increased the amount of RUP at the duodenum, although differences were only small with the MC ration (Table 7). This increase observed in niacin fed cows seems to be contradictory to increased ruminal ammonia concentration and increased microbial protein synthesis in the same animals. However, a trend for an increase in dietary N flow in niacin fed animals was also observed by Doreau and Ottou (1996), feeding a diet with 55% forage. It seems that even though less dietary protein was degraded in the present trial, it was used more efficiently for microbial protein synthesis, as may also be concluded from results of Schaetzel and Johnson (1981). Even though they observed no direct effect of NA addition to fermenters, TCA precipitable N was 25% higher in fermenters, where the inoculum came from a niacin adapted animal compared to those inoculated from a non - adapted donor. Furthermore, this augmentation occurred despite less substrate disappearance. This might indicate a shift in microbial population due to niacin towards micro - organisms, which utilise nitrogen more efficiently. If it occurs together with an assumed enhancement in ingesta passage and a stimulation of ureolytic activity, an increase in undegraded feed protein, ruminal ammonia concentration and microbial protein seems possible.

As can be seen from all measurements for microbial fermentation, niacin supplementation increases the efficiency of microbial protein production (Table 7). This was also concluded by others (Shields et al., 1983). Furthermore, an interaction between niacin supplementation and level of concentrate might exist as well. A trend for this interaction was found if the efficiency was expressed on a MJ ME or g RDP basis, because differences were most distinct with the HC ration and only marginal with the MC ration.

These results seem to be suggestive of the same direction as if microbial protein synthesis is considered alone, namely a shift in microbial population, perhaps associated with a change in ingesta flow. Additionally, information is lacking on how degraded vitamins are used by rumen microbes (Schwab et al., 2006). As is discussed in the next paragraph, substantial amounts of the NA supplement do not reach the duodenum. Hence, there may as well be other advantages for rumen microbes in the niacin supplemented groups. But influences appear to be highest in the HC ration, while only minor in the MC diet.

***Niacin Flow at the Duodenum***

In discussing the effects on vitamin flow at the duodenum it has to be kept in mind that different methods for niacin analysis can lead to different results. Santschi et al. (2005c) compared different sampling sites in the rumen and different sample preparation treatments for ruminal fluid, and found an effect of both factors on the niacin concentration. Thus, different studies might not be completely comparable.

In the present study, total niacin flows at the duodenum ranged from 1602 mg/d with LC – NA to 2630 mg/d with HC + NA (Table 8). Some older surveys were carried out with calves (Zinn et al., 1987), steers (Miller et al., 1986) or cattle (Riddell et al., 1985) and resulted in lower values for duodenal niacin flow, ranging from 85 mg/d (Riddell et al., 1985) to 813 mg/d (Miller et al., 1986). More recent studies undertaken with lactating dairy cattle, as in our experiment, came to higher values (Campbell et al., 1994; Santschi et al., 2005a; Schwab et al., 2006). In these studies, total niacin flow lay between 1908 mg/d (Schwab et al., 2006) and 2946 mg/d (Santschi et al., 2005a). Campbell et al. (1994) measured niacin concentrations in duodenal fluid, but named average daily duodenal DM flow and DM content. If niacin flows are calculated from these values, they varied between 1716 mg/d and 4902 mg/d in that trial. Hence our values are low to middle range, compared with those previously found in dairy cattle.

In all measurements and calculations, influences of F:C ratio and niacin supplementation were observed in the present experiment, except for NAM flow and apparent NAM synthesis (Table 8). Total niacin and NA flow to the duodenum increased with an increasing proportion of concentrate, but differences between MC and HC ration were small in unsupplemented groups. For total niacin, this increase is consistent with results of Schwab et al. (2006). If the ration contained 35% instead of 60% forage, these au-

thors observed an increased flow of niacin to the duodenum. In contrast, Miller et al. (1986) observed no differences in duodenal niacin flow between a diet containing either 30% or 89% corn. Santschi et al. (2005b) found only numerical increases in niacin concentration of solid- and liquid-associated bacteria in a diet containing 60% concentrate, compared to a high forage ration (F:C ratio 60:40). But Nilson et al. (1967) observed the highest ruminal niacin values in a ration consisting of 100% grain - concentrate mixture, compared to an all - corn - silage or all - alfalfa - hay diet. This is consistent with results of Hayes et al. (1966), where also the ration containing 100% corn caused higher ruminal niacin concentrations than a diet with long or ground hay and corn. Even though 100% grain or corn rations can not be considered as adequate for ruminants, and ruminal vitamin concentrations might not reflect the total output (Zinn et al., 1987), these latter studies may also suggest that niacin supply to the duodenum is higher with higher concentrate proportions.

But the vitamer influenced seems to vary. In our experiment, NA flow increased with higher concentrate proportions, whereas NAM was not influenced (Table 8). However, in the experiment of Schwab et al. (2006), also NAM flow to the duodenum increased, whereas there was only a trend for enhanced NA flow in the low-forage ration. Higher NAM concentrations with a low forage (F:C ratio 40:60) compared to a high forage ration (F:C ratio 60:40) were also observed in solid- and liquid-associated bacteria by Santschi et al. (2005b).

Amounts of NA, NAM and thus total niacin at the duodenum rose with vitamin supplementation in all three levels of concentrate (Table 8). Numeric increases after vitamin supplementation were also observed in the work of Zinn et al. (1987). In other studies, the augmentations were significant (Riddell et al., 1985; Campbell et al., 1994). These results were confirmed in sheep as well (Kollenkirchen et al., 1992). But findings concerning NAM are controversial in the few studies differentiating between the vitamers, as Campbell et al. (1994) did not detect NAM in duodenal fluid, which disagrees with our results and those of Kollenkirchen et al. (1992). Santschi et al. (2005a) also stated, that NAM can be found if the whole duodenal content is taken.

Even though no NAM was present in feed in the present trial, it was found in duodenal chyme. Therefore, the amount of NAM reaching the duodenum also represents the apparent NAM synthesis in the rumen. This is consistent with the results of Santschi et al. (2005b), but in the work of Schwab et al. (2006), also NAM was present in feed. In our trial, the amounts of NAM reaching the duodenum were enhanced when supplemental

NA was fed. Kollenkirchen et al. (1992) also observed an increase in duodenal NAM concentration after an intraruminal infusion of 2 mmol NA in sheep. Additionally, these authors described that 2 mmol supplemental NAM disappeared rapidly from the rumen. This finding was interpreted to result from hydrolysis of NAM to NA, which was also found elsewhere (Campbell et al., 1994). Furthermore, NAM also seems to disappear or be converted to NA if it is given postruminally. In the work of Santschi et al. (2005a), abomasal infusion of NAM did not change the duodenal flow of NAM, but an increase was observed in the amount of NA reaching the small intestine. These authors concluded that the acidic environment in the abomasum leads to the conversion of NAM to NA. Thus, the amounts of NAM arriving at the duodenum obviously do not represent the total amount of NAM synthesized, but are enhanced in NA supplemented cows in the present trial.

The apparent synthesis was calculated by subtracting the amount reaching the duodenum from the intake. To our knowledge, no surveys were conducted with a niacin supplementation to diets with different F:C ratios and calculation of apparent synthesis. But several studies exist where niacin was added to one ration. As was found in the present trial, apparent niacin synthesis always became negative in these cases. Riddell et al. (1985) fed 6 g NA. Apparent synthesis was calculated by us, and resulted in -5922 mg/d. Furthermore, if these calculations were also done for results of Zinn et al. (1987), where either 0, 200 or 2000 mg niacin per d were supplemented for feedlot calves, apparent synthesis was 210 mg/d, - 60 mg/d or – 1666 mg/d. Thus, substantial amounts of a supplementation disappeared before the duodenal cannula. It was stated that there seems to be an optimal niacin concentration in the rumen. Below this level, synthesis will occur and above it, niacin is degraded by bacteria (Hannah and Stern, 1985; Riddell et al., 1985). This is supported by the fact that even in vitro, net niacin synthesis was found to be negative if niacin was supplemented (Hannah and Stern, 1985). But disappearance of supplemental niacin might also be due to absorption from the reticulo - rumen, abomasum or duodenum before the duodenal cannula. Free NAM was found to be absorbed at 0.98 g/h in a washed rumen of cows. NA was not absorbed, probably because it is ionized at physiological pH values in the rumen (Erickson et al., 1991). Thus, ruminal absorption did not seem to happen with the NA supplementation given in our experiment. Furthermore, it was found that most of the niacin is bound in the bacterial fraction (Erickson et al., 1991; Santschi et al., 2005b), which probably limits absorption from the rumen. Yet for the abomasum and the small part of the duodenum before the

duodenal cannula, no results for ruminants are available. But even with post-ruminal infusion, losses of niacin occurred before the duodenal cannula (Santschi et al., 2005a). All these results together seem to indicate that reasons for the disappearance of niacin might be both, ruminal degradation and absorption before the duodenal cannula. Additionally, these losses appear to be less in rations containing higher proportions of concentrate.

Compared to LC ration, apparent synthesis of total niacin as well as of NA increases with higher proportions of concentrate in the diet in unsupplemented groups (Table 8). However, apparent synthesis was higher with the MC than with the HC diet, due to a little lower niacin intake with the MC ration and an intermediate flow at the duodenum. For cows receiving a supplementation, this was not observed since the HC ration had the least negative values. This may indicate that degradation of supplemental niacin is reduced at higher concentrate level. As was discussed in the previous section, apparent synthesis of NAM is identical with NAM amounts reaching the duodenum, because no NAM was found in feed. Thus, the apparent synthesis of this vitamer was not increased due to an augmentation of concentrate proportion.

The increase in apparent synthesis of total niacin and NA is not concordant with the work of Schwab et al. (2006) who observed an influence of the non-fibre carbohydrates (**NFC**) content in the ration, whereas the F:C ratio only had an effect on the amount reaching the duodenum, and not on apparent synthesis. But niacin intake differed largely between different NFC contents in that study, which obviously had an impact on apparent synthesis. However, higher concentrate proportions are also associated with higher NFC content in our trial, as diets were not formulated to be equal in NFC content but different in F:C ratio. Thus, a differentiation between NFC and F:C ratio effects is not possible from our study. Miller et al. (1986) compared either 11% alfalfa meal and 89% corn grain or 70% alfalfa meal and 30% corn grain in rations of steers. These authors concluded as well that apparent synthesis was not influenced by level of concentrate. Yet values for the ration containing only 11% alfalfa meal were numerically higher than for the low - concentrate diet (485 mg/d vs. 439 mg/d). But more information is lacking. To our knowledge, no other studies using different F:C ratios and measuring duodenal niacin flow exist. Also the mechanisms behind the stimulatory effects on synthesis or inhibitory effects on degradation are completely unknown, irrespective if the reasons are different NFC contents or F:C ratios.

## CONCLUSIONS

Feeding 6 g supplemental NA caused an increase in ammonia concentration in the rumen, whereas total SCFA concentration decreased. Furthermore, molar proportions of some minor SCFA were influenced as well. It is suggested that this might be due to a positive effect of niacin on protozoa, other shifts in microbial population or changes in ingesta passage. But further research is needed to prove this theory.

There was a distinct effect on N flow at the duodenum towards more microbial protein in niacin supplemented groups, either expressed in g/d, g/MJ ME or g/g RDP. But a trend for an interaction was found between niacin and F:C ratio, because the increase in microbial protein synthesis and efficiency was highest with the HC ration and least with the MC diet. Thus, it seems that a niacin supplementation is more advantageous for microbial populations in rations containing high levels of concentrate.

The vitamin flow at the duodenum was also influenced by F:C ratio and niacin supplementation, towards increasing niacin flows with higher proportions of concentrate and in supplemented groups. However, apparent synthesis of niacin in unsupplemented groups was highest with the MC ration. From the present experiment, it can thus be concluded that synthesis of niacin is less in rations containing a high proportion of forage, but mechanisms are unknown and further research is needed. Furthermore, apparent synthesis of niacin becomes negative if NA is supplemented. Depending on the ration fed, 88% to 94% of the amount added did not reach the duodenum. This indicates degradation in the rumen or absorption before the duodenal cannula. But the contribution of either factor to the disappearance of supplemental niacin is unknown as well. Hence, also in this area further research is needed and it would be interesting to compare the NA used in this study with a rumen - protected NA with different F:C ratios in future studies.

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## PAPER III

# Investigations on the effect of a niacin supplementation to three diets differing in forage-to-concentrate ratio on several blood and milk variables of dairy cows

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## Abstract

The objective of this study was to investigate the influence of a niacin supplementation to three diets with different forage-to-concentrate ratios (F:C ratio) on blood and milk parameters. Seven midlactation (102 ± 18 days in milk) and three dry cows of the Holstein-Friesian breed, equipped with cannulas in the dorsal sac of the rumen and proximal duodenum were used. On a dry matter basis the rations applied consisted of either 1/3 concentrate and 2/3 forage (LC), 1/2 concentrate and 1/2 forage (MC) or 2/3 concentrate and 1/3 forage (HC). They were fed in one period without and in the following with a supplementation of 6 g niacin (nicotinic acid, NA) per cow per day. The basal niacin content was 35.0 mg/kg for LC, 34.6 mg/kg for MC and 34.4 mg/kg for the HC diet on dry matter basis. Blood was sampled before, three and six hours after first morning feeding from the *vena jugularis externa*. Milk samples were obtained on two days. In serum, concentrations of non-esterified fatty acids (NEFA), ß-hydroxybutyrate (BHBA), glucose, urea and niacin were measured, while milk samples were analysed for their content of fat, protein, lactose, urea and niacin as well as for their fatty acid pattern.

NA feeding enhanced concentrations of glucose and urea in serum ($p < 0.05$). No effect of supplemental NA was seen on serum NEFA or BHBA concentrations. Increasing proportion of concentrate and NA supplementation enhanced serum nicotinamide (NAM) concentration ($p < 0.01$). NAM concentrations or amounts excreted via milk were only influenced by F:C ratio ($p < 0.05$) and not by NA supplementation. But milk fat composition showed an effect of additional NA, as the proportion of oleic acid increased in milk of niacin supplemented cows ($p = 0.04$). For all parameters analysed, no significant interaction between F:C ratio and NA supplementation was found.

**Keywords:** niacin, nicotinic acid, forage-to-concentrate ratio, dairy cows

# 1. Introduction

As niacin is incorporated in the coenzymes NAD(H) and NADP(H) it is of great importance for metabolism and performance. NAD(H) and NADP(H) are involved in a large number of oxidation and reduction reactions as proton and electron carriers (Bender 1992). The oxidized forms of the coenzymes, $NAD^+$ and $NADP^+$, are acceptors of reduction equivalents in some reactions of the citric acid cycle, the glycolytic pathway, and the degradation of free fatty acids and proteins. The reduced forms are important for gluconeogenesis, synthesis of free fatty acids, provision of amino nitrogen through aspartate, urea biosynthesis and the pentose phosphate pathway (Harmeyer and Kollenkirchen 1989). Ruminants have different sources of niacin: feed, endogenous synthesis from tryptophan and synthesis by rumen microorganisms (GfE 2001). It is believed that ruminal niacin synthesis covers the requirements of dairy cows (GfE 2001; NRC 2001). But even so, beneficial effects of a niacin supplementation on energy metabolism (Erickson et al. 1992; DiCostanzo et al. 1997) or performance (Cervantes et al. 1996; Drackley et al. 1998) have been observed. However, also several experiments without an effect can be found, as reviewed by Niehoff et al. (2009a). Thus, a niacin supplementation might interact with other factors, which leads to inconsistent responses.

Higher concentrate levels in the diet could affect ruminal synthesis or use of B-vitamins (Santschi et al. 2005). Consequently, the ration fed may alter the niacin flow to the duodenum, which might have also effects on the metabolism. An increase in niacin flow to the duodenum was observed with niacin supplementation and also with higher concentrate proportions of the diet (Niehoff et al. 2009b). Hence, the present study aims to complete the experiment reported by Niehoff et al. (2009b) in examining the effects of a niacin supplementation on several blood and milk parameters and to assess the influence of different forage - to - concentrate ratios (F:C ratios) on responses to supplemental niacin. Special emphasis was placed on niacin concentrations in blood and milk.

# 2. Materials and methods

## *2.1. Experimental design and animals*

The experiment was conducted in observance of the European Community regulations concerning the protection of experimental animals and the guidelines of the Regional Council of Braunschweig, Lower Saxony, Germany (File Number 33.11.42502-04-

057/07). The detailed design is described by Niehoff et al. (2009b). Briefly, 10 cows of the Holstein-Friesian breed, equipped with large rubber cannulas in the dorsal sac of the rumen and simple T-shaped cannulas in the proximal duodenum, close to the pylorus, were used. At the beginning of the experiment, animals had an average weight of 599 ± 79 kg. Seven cows were in lactation (102 ± 18 days in milk at the beginning), while three were dried off. No cow was primiparous and lactation numbers ranged from second to fifth lactation. Lactating cows were milked at 5:00 and 16:00 h.

The cows were kept in a tethered stall with neck straps and one individual trough for each cow. They had free access to water and to a salt block containing sodium chloride. Roughage was offered at 5:30 and 15:30 h, concentrate was given at 5:30, 7:30, 15:30 and 17:30 h. Forage consisted of 60% maize silage and 40% grass silage on dry matter (**DM**) basis. The composition of concentrate is given in Table 1.

**Table 1: Composition of concentrate in g/kg**

| Components | g/kg |
|---|---|
| Wheat | 250 |
| Corn | 250 |
| Soybean meal | 170 |
| Peas | 150 |
| Dried sugar beet pulp | 150 |
| Mineral and vitamin premix [1)] | 20 |
| Calcium carbonate | 7 |
| Urea | 3 |

[1)] Composition per kg: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6 g Zn, 5.4 g Mn, 1 g Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 1,000,000 IU vitamin $D_3$ ,1,500 mg alpha tocopherol acetate

The amounts offered were adjusted to expected intake of each cow in order to reach near *ad libitum* intake but to avoid refusals. To prevent excessive fattening of dry cows, they were restricted to a feed amount covering their maintenance.

The cows were assigned randomly to one of the three experimental diets. The diets applied were the following: low concentrate **(LC)** which consisted of 1/3 concentrate and 2/3 forage on DM basis, medium concentrate **(MC)** with 1/2 concentrate and 1/2 forage and high concentrate **(HC),** which contained 2/3 concentrate and 1/3 forage. Each diet was fed in one period without supplemental niacin and in the following with a supplementation of 6 g niacin per cow and day, provided as nicotinic acid (**NA**). The NA used

was powdered NA, with a content of at least 99.5% NA (Lonza Ltd., Basel, Switzerland). NA was mixed in an extra 100 g of mineral and vitamin premix and one half was top dressed on the concentrate during the morning feeding, the other half in the evening. In periods without supplemental NA, 100 g of extra mineral and vitamin premix only were given in the same way. One last period was used to fill gaps in animal number per group.

### *2.2. Sample collection*

Cows were given three weeks to reach the respective concentrate level. Afterwards each period consisted of two weeks of adaptation to the diet, followed by one week of sampling. During the sampling week, blood was collected on one day at 5:30 h just before the morning feeding and at 8:30 h and 11:30 h in serum tubes from the *vena jugularis externa*. Approximately one hour after sampling, serum was separated by centrifugation at 3000 x g for 30 minutes at 15 °C. The serum obtained was divided into tubes for niacin analysis, these were stored at -80 °C and tubes for analyses of other blood parameters, which were kept at -18 °C.

Milk yield was recorded daily. Two milk samples were collected in each period on two days from the morning and evening milking. One (50 mL) was used for the analysis of milk ingredients, the other (approximately 800 mL) was utilized for the determination of niacin content and milk fat composition. Samples for ingredient analyses were conserved with Bronopol and stored at 8 °C until analysed. Samples for niacin and milk fatty acid analyses were mixed daily aliquot, freeze-dried immediately afterwards (Christ Epsilon 1-15, Martin Christ GmbH, Osterode, Germany) and subsequently stored at -18 °C.

Feed samples were taken each day during one week of each period. They were mixed to one sample per period. Part of this was freeze-dried for niacin analysis, the rest was dried at 60 °C for nutrient analyses. All feed samples were ground to pass a 1 mm screen. Those reserved for niacin determination were kept at -18 °C until analysis.

### *2.3. Analyses*

Feedstuffs were analysed for their content of crude nutrients according to methods of the VDLUFA (Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten; Naumann and Bassler 1976). Analysis of acid and neutral detergent fibre

(**ADF** resp. **NDF**) was conducted following methods of Goering and Van Soest (1970). The niacin content was determined microbiologically by VDLUFA.

Serum was analysed for glucose, non-esterified fatty acids (**NEFA**), ß-Hydroxybutyrate (**BHBA**) and urea concentrations in the laboratory of the Cattle Clinic Hannover, Germany. Glucose analysis was done using Hexokinase (ABX, Art. A 11A22116), NEFA concentrations were determined via enzymatic colour test (Wako, Art. 99475409, Wako Chemicals GmbH, Neuss, Germany). An enzymatic UV-test was applied to measure concentrations of BHBA (Randox, Art. RB 1008, Randox Laboratories GmbH, Krefeld) and urea (ABX, Art. A11A00075).

Contents of milk fat, protein, lactose, urea and somatic cell count were analysed in each sample, using an infrared milk analyser (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). The analysis of milk fat composition was carried out in freeze dried samples after extraction with chloroform/methanol (2:1) and transesterification with trimethylsulfoniumhydroxide. A gas chromatograph (Hewlett Packard 6890, USA) equipped with a flame ionization detector (FID) and a capillary column (model Zebron 7HG-G009-11, nitroterephthalic acid-polyethylene glycol; 30 m x 250 µm, 25 µm film thickness) was used for separation of fatty acids from butyric acid ($C_{4:0}$) up to linolenic acid ($C_{18:3}$). Niacin (NA and nicotinamide) concentrations in serum and milk were determined via HPLC after fat extraction with n-hexane and protein precipitation using cold ethanol. After centrifugation at 14000 rpm, the supernatant was quantitatively transferred into a flask and evaporated in a nitrogen stream at 40 °C. The residue was dissolved in 150 µL of the respective aqueous mobile phase A. After filtration (amcro filter, PVDF, 0.45 µm) 20µL of the filtrate were injected into a Shimadzu HPLC system (model SCL-10A controller, model LC-10AS pump, model SIL-10AC autosampler, model CTO-10AC oven; Shimadzu, Kyoto, Japan) equipped with a multi wavelength detector (model SPD-M10A VP; Shimadzu, Kyoto, Japan). Samples were run through a C18 column (Inertsil ODS, 150 mm x 3 mm i.d., 5µ) at a flow rate of 0.4 mL/min and were eluted using a binary gradient system. For milk analyses mobile phase A consists of sodium 1-hexanesulfonate monohydrate (IPCC6; 2.5*mM*) and sodium 1-pentanesulfonate monohydrate (IPCC5; 2.5*mM*) in ultrapure water (adjusted to pH 2.3 with 2*M* phosphoric acid) and mobile phase B consists of 80% acetonitrile and 20% mobile phase A. Serum analyses were performed with 10 *mM* IPCC6 in ultrapure water at a pH of 2.3 (mobile phase A) and 100% acetonitrile (mobile phase B). The detection wavelength was 260 nm.

### *2.4. Calculations and statistics*

Mean concentrations of milk constituents per period were used for statistics and calculated as a weighted mean according to the milk yield at each milking time. For milk fat composition and niacin concentration, a mean of the two values obtained per period was used.

Fat-corrected milk (**FCM**) was calculated following Gaines (1928):

FCM (kg/d) = ((milk fat * 0.15) + 0.4) * kg milk yield

Data were analysed statistically using the software package SAS (Version 9.1, procedure mixed, SAS Institute Inc., Cary, USA). The procedure "MIXED" was applied. Concentrate level ("CONC") (LC = 1; MC = 2; HC = 3), niacin ("NIA") (without NA = 1; with NA = 2). Organic matter intake ("OMI") was treated as fixed regressive component. For the analyses of blood variables, the time after feeding in minutes ("MINUTES") was included, as well as its interaction with CONC and NIA. The fact that a cow had to be used in several periods for different treatments was taken into account using the "RANDOM" statement for the individual "COW" effect. Thus, for blood parameters, the following SAS code was applied:

**PROC MIXED METHOD** = **REML**;
**CLASS** COW CONC NIA MINUTES;
**MODEL** Y = CONC NIA CONC*NIA OMI MINUTES MINUTES*CONC*NIA / **DDFM** = **KENWARDROGER;**
**RANDOM** COW;
**LSMEANS** CONC NIA CONC*NIA MINUTES*CONC*NIA /
**PDIFF** e ADJUST = TUKEY;

For milk parameters, the stage of lactation was additionally considered ("LAKTSTA" 1 = 1-150 days postpartum; 2 = > 150 days postpartum), while all "MINUTES"-related effects and interactions were deleted from the model.

Variances were evaluated with the restricted maximum likelihood method (REML) and degrees of freedom were calculated according to the Kenward-Roger method. The "PDIFF" option was applied to test differences between least square means, using a Tukey-Kramer test for post-hoc analysis. All values presented are LS MEANS; except

for intakes of the different nutrients and chemical composition of feedstuffs. Significance was declared if F-test statistics revealed $p \leq 0.05$, while a trend was announced if $p \leq 0.10$.

## 3. Results

### *3.1. Feeding*

OM Intake was approximately similar in all experimental groups. For the LC ration without NA, it was 12.1 kg, while it averaged 12.4 kg for LC + NA, 12.3 kg for MC, 12.6 kg for MC + NA, 12.2 kg for HC and 12.4 kg OM for HC + NA. The actual F:C ratio fed was 68.3% forage and 31.7% concentrate for LC, 49.8% and 50.2% for MC and 35.2% and 64.8% for HC ration, respectively. Diets were not formulated to be isonitrogenous and isocaloric between different F:C ratios. The chemical composition of the three diets is given in Table 2.

**Table 2: Arithmetic mean (n=7) ± standard deviation of chemical composition of the three rations**

| Nutrients, g/kg DM | **LC** | **MC** | **HC** |
|---|---|---|---|
| OM | 938 ± 3 | 940 ± 2 | 941 ± 2 |
| CP | 132 ± 5 | 149 ± 6 | 163 ± 6 |
| EE | 28.4 ± 4.7 | 27.5 ± 3.7 | 26.7 ± 3.0 |
| CF | 184 ± 5 | 151 ± 4 | 124 ± 3 |
| ADF | 202 ± 8 | 168 ± 6 | 141 ± 5 |
| NDF | 380 ± 18 | 323 ± 14 | 279 ± 12 |
| Starch | 281 ± 57 | 335 ± 42 | 378 ± 31 |
| MJ ME / kg DM | 11.2 ± 0.04 | 11.7 ± 0.04 | 12.0 ± 0.03 |
| Niacin mg/kg DM | 35.0 ± 16.9 | 34.6 ± 14.0 | 34.4 ± 11.7 |

LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; OM = organic matter; CP = crude protein; EE = ether extract; CF = crude fibre; ADF = acid detergent fibre; NDF = neutral detergent fibre

The mean native niacin content of the three rations was nearly the same. But especially in forages, it differed a lot (minimum = 21.5 mg/kg DM; maximum = 77.6 mg/kg DM), while the niacin content of concentrates was less variable (minimum = 27.3 mg/kg DM; maximum = 43.4 mg/kg DM). Thus, niacin intakes per cow varied greatly, especially in LC diet due to the high variation in niacin content between different forage batches. Mean niacin intake per cow with LC, MC and HC ration without supplementation was 553 mg/d, 325 mg/d and 476 mg/d, respectively, and with 6 g NA supplementation it averaged 6449 mg/d, 6337 mg/d and 6370 mg/d, respectively.

### *3.2. Serum measurements*

Table 3 presents the results of serum analysis over the whole sampling time. Apart from the main effects of NA supplementation, F:C ratio and their interaction, the interaction between niacin, level of concentrate and time after feeding is also included to show possible changes in time pattern due to niacin or different F:C ratios. Solely nicotinamide (**NAM**) was detected in serum, while NA was always below the detection limit, except for one cow. But for this cow, NA was found in five out of the six periods where she was used. Only when she received MC ration with supplemental NA, surprisingly no NA was detected in serum obtained from this cow.

As expected, level of concentrate influenced all analysed parameters, except for glucose concentration in blood. BHBA decreased, while urea and NAM concentrations increased with higher concentrate level. Compared to the LC diet, NEFA concentrations decreased with higher concentrate level, but the lowest values were obtained in the MC ration. The interaction between sampling time, NA and F:C ratio resulted from the fact that in LC and HC groups without NA NEFA decreased after feeding, while they remained constant in NA supplemented groups of these two rations. But this was not observed with the MC ration (Figure 1).

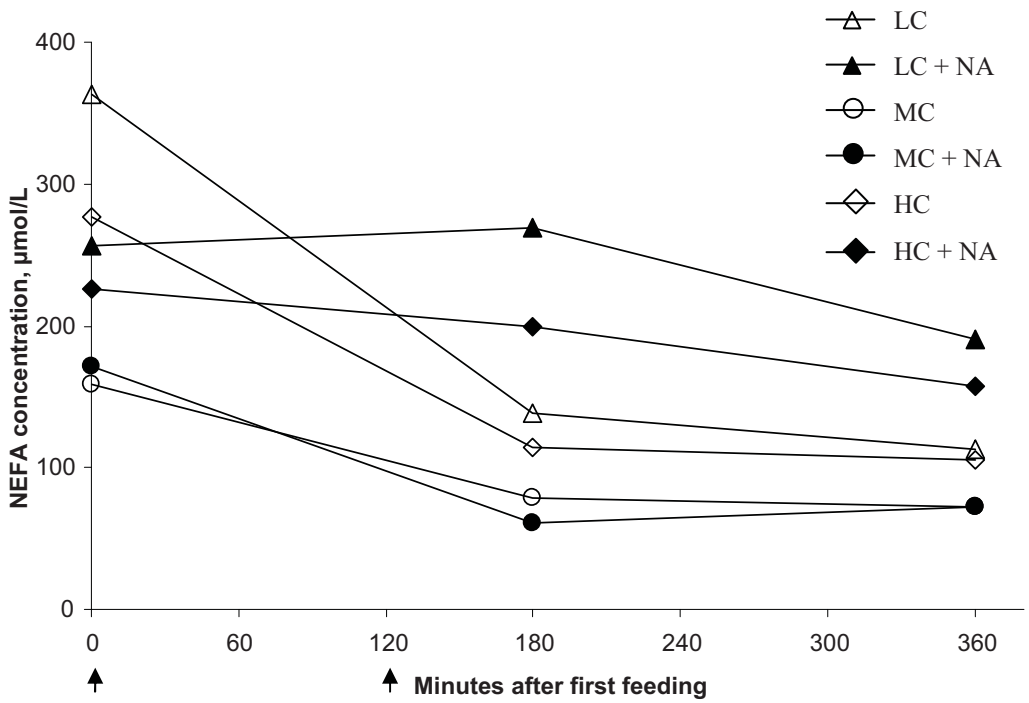


**Figure 1: NEFA concentrations (µmol/L) at different time points after feeding**

↑ feeding; at 0 minutes, the whole morning feeding amount of forage and half of concentrate was given, the other half at 120 minutes; LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; NA = nicotinic acid

However, the difference between control and NA supplemented groups was never significant at each concentrate level, indicating no major effect of NA supplementation. The addition of NA to the diet had several effects on the variables analysed. In niacin supplemented groups, an increase in glucose ($p = 0.02$) as well as in urea ($p < 0.01$) concentrations was observed. NAM ($p < 0.01$) concentrations in serum also increased in supplemented animals, even though augmentations were small with LC ration.

### *3.3. Milk measurements*

Results are presented in Table 4. F:C ratio influenced almost all parameters analysed, except for FCM and lactose content of milk. The addition of niacin to the ration resulted in increasing values for milk urea concentration ($p = 0.05$) with LC and HC, while with the MC diet, concentrations were almost equal. But this did not result in a significant interaction between F:C ratio and NA supplementation. Furthermore, there was a trend for an increase in fat ($p = 0.09$) and protein ($p = 0.07$) content of milk in supplemented groups. In milk, also only NAM was present and NA was below the detection limit, even for the cow where NA was measured in blood. NA supplementation neither influenced the NAM concentration in milk nor the amount of NAM excreted via milk significantly.

**Table 3: Effects of niacin and different forage-to-concentrate ratios on several blood parameter; LS MEANS and (standard error)**

| | LC | | MC | | HC | | p | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | - NA (n = 8) | + NA (n = 9) | - NA (n = 7) | + NA (n = 7) | - NA (n = 9) | + NA (n = 10) | CONC | NA | CONC x NA | MIN x CONC x NA |
| Glucose mmol/L | 3.11 (0.09) | 3.25 (0.09) | 3.09 (0.10) | 3.30 (0.10) | 3.19 (0.09) | 3.30 (0.09) | 0.66 | 0.02 | 0.79 | 0.94 |
| NEFA µmol/L | 205 (20.2) | 239 (19.0) | 103 (21.7) | 101 (21.7) | 165 (19.0) | 194 (18.0) | <0.01 | 0.20 | 0.65 | 0.02 |
| BHBA mmol/L | 0.88 (0.13) | 1.05 (0.12) | 0.96 (0.13) | 0.85 (0.13) | 0.55 (0.12) | 0.66 (0.12) | <0.01 | 0.48 | 0.34 | 0.09 |
| Urea mmol/L | 2.86 (0.18) | 3.44 (0.17) | 3.38 (0.19) | 4.09 (0.19) | 3.49 (0.17) | 4.54 (0.17) | <0.01 | <0.01 | 0.19 | 0.61 |
| NAM mg/L | 0.32 (0.04) | 0.36 (0.04) | 0.35 (0.04) | 0.46 (0.04) | 0.41 (0.04) | 0.52 (0.04) | <0.01 | <0.01 | 0.11 | 0.99 |

LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; CONC = level of concentrate; NA = nicotinic acid; MIN = minutes after feeding; BHBA = ß-Hydroxybutyrate; NEFA = non-esterified fatty acids; NAM = nicotinamide

**Table 4: Effects of niacin and different F:C ratios on several milk parameter; LS MEANS and (standard error)**

| | LC | | MC | | HC | | p | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | - NA (n = 5) | + NA (n = 6) | - NA (n = 5) | + NA (n = 5) | - NA (n = 6) | + NA (n = 7) | CONC | NA | CONC x NA |
| Milk yield kg/d | 24.3 (1.65) | 24.5 (1.59) | 26.9 (1.62) | 26.2 (1.66) | 30.0 (1.55) | 29.5 (1.51) | <0.01 | 0.63 | 0.89 |
| FCM kg/d | 22.2 (1.40) | 23.2 (1.30) | 21.8 (1.36) | 21.9 (1.41) | 21.5 (1.26) | 22.7 (1.19) | 0.78 | 0.35 | 0.85 |
| Fat % | 3.44 (0.20) | 3.74 (0.18) | 2.79 (0.20) | 2.82 (0.20) | 2.10 (0.18) | 2.46 (0.16) | <0.01 | 0.09 | 0.59 |
| Fat yield kg/d | 0.83 (0.06) | 0.90 (0.5) | 0.74 (0.06) | 0.75 (0.06) | 0.63 (0.05) | 0.73 (0.05) | <0.01 | 0.14 | 0.71 |
| Protein % | 2.83 (0.07) | 2.90 (0.06) | 2.88 (0.06) | 2.97 (0.07) | 2.96 (0.06) | 2.98 (0.06) | 0.03 | 0.07 | 0.60 |
| Protein yield kg/d | 0.68 (0.04) | 0.70 (0.04) | 0.76 (0.04) | 0.77 (0.04) | 0.88 (0.03) | 0.88 (0.03) | <0.01 | 0.61 | 0.79 |
| Urea mmol/L | 1.76 (0.22) | 2.04 (0.19) | 2.56 (0.21) | 2.51 (0.21) | 2.55 (0.18) | 3.29 (0.17) | <0.01 | 0.05 | 0.13 |
| NAM mg/L | 0.54 (0.06) | 0.55 (0.06) | 0.66 (0.06) | 0.72 (0.06) | 0.61 (0.06) | 0.64 (0.05) | 0.02 | 0.26 | 0.80 |
| NAM yield mg/d | 12.8 (2.29) | 13.5 (2.19) | 18.0 (2.24) | 18.8 (2.31) | 18.8 (2.13) | 19.1 (2.05) | <0.01 | 0.59 | 0.98 |

LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; CONC = level of concentrate; NA = nicotinic acid; FCM = fat corrected milk; NAM = nicotinamide

The composition of milk fat is shown in Table 5, values are presented in weight percent of total fatty acids measured. The F:C ratio influenced all proportions of fatty acids except for capric acid ($C_{10:0}$). NA addition significantly enhanced the proportion of oleic acid ($C_{18:1}$; $p = 0.04$), while there was a trend for reduced lauric acid ($C_{12:0}$) proportions ($p = 0.10$) in supplemented groups.

**Table 5: Effect of niacin supplementation and F:C ratio on milk fat composition expressed as weight percent of total fatty acids measured; LS MEANS and (standard error)**

| | LC | | MC | | HC | | p | | |
|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | - NA (n=5) | + NA (n=6) | - NA (n=5) | + NA (n=5) | - NA (n=6) | + NA (n=7) | CONC | NA | CONC x NA |
| Butyric $C_{4:0}$ | 6.76 (0.41) | 7.01 (0.38) | 5.84 (0.40) | 5.37 (0.41) | 5.33 (0.36) | 5.49 (0.34) | <0.01 | 0.93 | 0.54 |
| Caproic $C_{6:0}$ | 3.90 (0.21) | 3.90 (0.20) | 3.47 (0.21) | 3.19 (0.22) | 3.16 (0.19) | 3.21 (0.18) | <0.01 | 0.60 | 0.59 |
| Caprylic $C_{8:0}$ | 1.63 (0.10) | 1.58 (0.09) | 1.49 (0.09) | 1.38 (0.10) | 1.44 (0.09) | 1.46 (0.08) | 0.09 | 0.46 | 0.63 |
| Capric $C_{10:0}$ | 3.50 (0.23) | 3.31 (0.22) | 3.38 (0.23) | 3.04 (0.24) | 3.53 (0.22) | 3.51 (0.21) | 0.16 | 0.14 | 0.56 |
| Lauric $C_{12:0}$ | 5.92 (0.39) | 5.34 (0.37) | 5.90 (0.38) | 5.47 (0.39) | 6.50 (0.35) | 6.46 (0.34) | <0.01 | 0.10 | 0.53 |
| Myristic $C_{14:0}$ | 12.6 (0.38) | 12.4 (0.36) | 13.1 (0.37) | 13.0 (0.39) | 14.1 (0.35) | 13.6 (0.34) | <0.01 | 0.16 | 0.70 |
| Myristoleic $C_{14:1}$ | 1.45 (0.27) | 1.36 (0.25) | 1.70 (0.26) | 1.91 (0.27) | 2.47 (0.24) | 2.24 (0.23) | <0.01 | 0.85 | 0.53 |
| Palmitic $C_{16:0}$ | 38.6 (1.51) | 37.7 (1.42) | 34.2 (1.47) | 32.4 (1.52) | 32.1 (1.38) | 31.6 (1.32) | <0.01 | 0.20 | 0.80 |
| Palmitoleic $C_{16:1}$ | 2.12 (0.37) | 2.02 (0.35) | 2.14 (0.36) | 2.29 (0.37) | 3.23 (0.33) | 2.82 (0.32) | <0.01 | 0.56 | 0.49 |
| Stearic $C_{18:0}$ | 6.08 (0.42) | 6.60 (0.39) | 5.61 (0.41) | 6.14 (0.43) | 4.73 (0.38) | 4.88 (0.35) | <0.01 | 0.14 | 0.79 |
| Oleic $C_{18:1}$ | 15.2 (1.23) | 16.4 (1.11) | 19.5 (1.21) | 21.9 (1.24) | 17.5 (1.07) | 19.7 (0.99) | <0.01 | 0.04 | 0.84 |
| Linoleic $C_{18:2}$ | 2.18 (0.30) | 2.07 (0.28) | 3.30 (0.29) | 3.43 (0.30) | 5.28 (0.27) | 4.50 (0.25) | <0.01 | 0.18 | 0.11 |
| Linolenic $C_{18:3}$ | 0.28 (0.07) | 0.31 (0.07) | 0.46 (0.07) | 0.56 (0.07) | 0.56 (0.07) | 0.50 (0.06) | <0.01 | 0.65 | 0.47 |

LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; CONC = level of concentrate; NA = nicotinic acid,

## 4. Discussion

As the focus of the present study was laid on the impacts of a niacin supplementation, these effects will be predominantly discussed. For all variables analysed, no significant interaction between F:C ratio and NA supplementation was detected. Hence, responses to supplemental niacin were not altered by different F:C ratios.

### *4.1. Serum measurements*

Serum glucose concentration increased significantly in niacin supplemented groups in the present trial (Table 3). DiCostanzo et al. (1997) observed enhanced plasma glucose only after feeding of 36 g NA in a diet containing 55% forage, while supplementation of 12 g or 24 g per cow and day had no effect. In some older work, a trend toward an increase in blood glucose concentration of ketotic cows after NA treatment was also found (Fronk and Schultz 1979). Several other workers did not observe changes in serum or plasma glucose concentration in niacin supplemented cows (Martinez et al. 1991; Erickson et al. 1992; Lanham et al. 1992; Belibasakis and Tsirgogianni 1996; Christensen et al. 1996; Madison-Anderson et al. 1997; Minor et al. 1998; Drackley et al. 1998). The F:C ratios applied in these studies varied from 40:60 to 60:40 and are thus in between the range of the present experiment.

DiCostanzo et al. (1997) assumed that the increase in blood glucose might be an indication of greater gluconeogenesis promoted through the suppression of lipolysis by NA. But Jaster and Ward (1990) stated that it is unknown whether niacin increases blood glucose by an increase in gluconeogenesis in the liver or by reducing the rate of glucose removal from blood or via both mechanisms. Alterations in glucose removal have occasionally been observed. As a result of glucose and insulin challenges, it was assumed that duodenal infusion of NA causes resistance to insulin in mid-lactation cows receiving a diet containing 77 – 79% forage (Chilliard and Ottou 1995). In contrast, increased glucose clearance, despite lower insulin concentrations, was observed during an intravenous glucose tolerance test in the plasma of feed-restricted cows with abomasal NA infusion (Pires et al. 2007). This suggests an augmentation in the response to insulin. The authors assumed that the effect of NA on insulin and glucose is mediated through the influence of NA on NEFA, rather than a direct effect of NA on glucose. But this was not obvious in the current study and in the work of Chilliard and Ottou (1995), where NEFA were also not affected by NA supplementation. This might be explained partly by the fact that cows in the present experiment and in the one of Chilliard and Ottou (1995) were in a positive energy balance, whereas animals in the study of Pires et al. (2007) were feed-restricted. The amount of NA given also differed largely. While in the present investigation and the trial of Chilliard and Ottou (1995) only 6 g NA were fed or infused, Pires et al. (2007) infused a total of 51.9 g per cow per day into the abomasum. Hence, higher serum glucose concentration in the present trial could have been due to a

decrease in insulin sensitivity as in the trial of Chilliard and Ottou (1995), but this was not measured.

However, other factors might have contributed to the increase observed in the current experiment as well. Fronk and Schultz (1979) concluded that an effect of niacin on rumen microorganisms can not be ruled out, leading to positive effects on carbohydrate metabolism. Microbial protein synthesis was enhanced in niacin supplemented groups of the present trial (Niehoff et al. 2009b). But as especially glucose concentration in blood is regulated by a large variety of factors, other mechanisms might also be important.

The fact that cows in MC ration had the lowest NEFA concentrations is surprising (Table 3). But as this was not reflected in BHBA concentrations, it is assumed to be random or unknown reasons may exist.

No significant effect of niacin was observed on serum NEFA or BHBA concentrations. This was seen as well in plasma values in other studies (Chilliard and Ottou 1995; Cervantes et al. 1996; Christensen et al. 1996; Minor et al. 1998; Drackley et al. 1998), feeding 35 – 79% forage. But also increases (Martinez et al. 1991; F:C ratio 40:60) and decreases (Pires and Grummer 2007; Pires et al. 2007; feed-restricted cows, abomasal NA infusion) in plasma concentrations of NEFA after niacin supplementation have been described. Cows in the present study might have been too far into lactation and hence not in a tense metabolic situation to show effects. Another explanation might be the fact that only NAM was enhanced in niacin supplemented animals. NAM does not seem to share the distinct antilipolytic effect of NA observed in humans (Carlson 2005). NA is a high affinity ligand to the responsible receptor, while NAM acted only as a very weak agonist (Wise et al. 2003). If mechanisms are similar in ruminants this would explain the absence of an effect on NEFA or BHBA in the present trial, since except for one cow NA was not detected in serum. The reason for this might have been the comparatively small amount of NA supplemented. Pires et al. (2007) and Pires and Grummer (2007) infused 4.8 g to 74.7 g into the abomasum, which must have resulted in much more NA reaching the duodenum than the 6 g fed in the present experiment.

The present results concerning urea concentrations in the serum as shown in Table 3 are not concordant with most former studies, since usually no effect of niacin on serum or plasma urea concentrations was seen (Jaster et al. 1983; Martinez et al. 1991; Erickson et al. 1992; Christensen et al. 1996). Diets applied contained 40 – 60% concentrate. But also a decreasing effect was found (Belibasakis and Tsirgogianni 1996; F:C ratio

50:50). Furthermore, an interaction between duodenal infusion of niacin and rapeseed oil occurred. Infusion of niacin alone decreased plasma urea concentration, while it was increased with simultaneous infusion of niacin and rapeseed oil (Chilliard and Ottou 1995). A fact that might contribute to differences is the time of blood sampling. In the present experiment, blood samples were drawn just before, and 180 and 360 minutes after first feeding. In the other studies mentioned, usually one sample was taken and time points varied largely from before feeding till 5.5 h after feeding. It has been shown that especially for serum and milk urea concentrations, diurnal variations should be considered and time of sampling versus time of feeding is very important (Gustafsson and Palmquist 1993).

In the current experiment, urea concentration in blood was consistent with measurements in the rumen, where an increase in ammonia concentration was found after niacin feeding (Niehoff et al. 2009b). Hence it is assumed that effects on serum urea are a consequence of changes in rumen ammonia concentration, since a relation between rumen ammonia and plasma urea exists (Gustafsson and Palmquist 1993; Rodriguez et al. 1997).

The values for niacin concentration in serum (Table 3) are relatively low compared to what was found in former studies. In five experiments, niacin concentrations of plasma and whole blood ranged from 0.4 mg/L till 2.5 mg/L (Driver et al. 1990; Lanham et al. 1992; Campbell et al. 1994; Ottou et al. 1995; Cervantes et al. 1996). In three other studies, concentrations between 8.1 mg/L and 17.3 mg/L were measured in whole blood, plasma or red blood cells (Dufva et al. 1983; Jaster et al. 1983; Martinez et al. 1991). The reasons for the wide margin of concentrations might lie in the different analytical methods applied. Some researchers used microbiological, others spectrophotometric or HPLC techniques. Furthermore, the different blood fractions examined may also play a role. The values obtained in the present study were in the lower range of the first five studies mentioned. Serum concentrations of the six feeding groups ranged from 0.32 mg NAM/L up to 0.52 mg NAM/L. Values for individual cows lay between 0.07 and 0.97 mg NAM/L.

In accordance with some studies (Driver et al. 1990; Ottou et al. 1995; Cervantes et al. 1996) an increase in niacin concentration after niacin supplementation was found in this experiment. However, no effect was observed in other trials (Dufva et al. 1983; Martinez et al. 1991; Lanham et al. 1992). But in the study of Dufva et al. (1983), cows in the control group exhibited a significant decrease in red blood cell niacin content after par-

turition, while concentrations pre- and postpartum were equal in niacin supplemented cows. No F:C ratio was apparent which either always or never had an effect.

In our experiment, NA supplementation and a higher level of concentrate enhanced the amount of niacin reaching the duodenum (Niehoff et al. 2009b). Thus it is assumed that an increase in serum NAM concentration reflects this enhanced delivery of the vitamin to the duodenum. To our knowledge, only Campbell et al. (1994) measured duodenal and blood niacin concentrations in dairy cattle, but their results do not support this thesis. Campbell et al. (1994) fed a ration containing 40% concentrate and observed increases in duodenal NA flow due to niacin supplementation compared to control groups. Furthermore, duodenal NA flow was also higher when 12 g NAM was fed compared to 12 g NA. In blood, they found no difference between control and supplemented animals. But the NA and NAM groups differed. NA feeding increased the niacin concentrations in blood, while supplementation of NAM decreased them. This is the opposite of duodenal results from that study, because NAM feeding had caused the highest concentrations at the duodenal level. In sheep, it was found that NAM concentrations in blood were neither influenced by an intraruminal supplementation of 2 mmol NA or NAM, nor correlated with amounts arriving at the duodenum (Kollenkirchen et al. 1992). It was concluded by these authors, that the niacin supply of animals was adequate with control diet and thus a supplementation was not necessary. Maybe if site and mechanism of absorption are clarified for ruminants, this will provide an explanation for the different results obtained.

In this experiment, more NA than NAM was present in duodenal chyme (Niehoff et al. 2009b), while in blood serum only NAM was detected. Thus, some conversion of NA must have occurred during, or shortly after, absorption. It can not be excluded that NA might have been found if whole blood would have been analysed instead of serum. But also some conversion of NA during or shortly after absorption seems likely. In the wall of the rat jejunum, it has been shown *in vivo* and *in vitro* that labelled NA incubated was rapidly, and also almost completely, converted to NAM (Stein et al. 1994). It was concluded that the purpose of this mechanism might be to maintain a gradient to enhance absorption or the maintenance and regulation of intestinal NAD level (Stein et al. 1994). Maybe the same happens in the ruminant.

### *4.2. Milk measurements*

Neither milk yield nor FCM yield was affected by niacin supplementation (Table 4). In discussing effects on milk parameters it has to be kept in mind that this study was not specifically designed to measure impacts of niacin on performance. Mid-lactation and double fistulated cows were used since the focus of the trial was laid on metabolic processes in the rumen and duodenum (as described in Niehoff et al. 2009b), as well as in blood and niacin flows and concentrations in various body fluids. Thus, results may differ from what could be obtained in other lactation stages, especially early lactation. However, trends or effects observed will also be discussed.

Two trends were found in the present trial after NA supplementation: an increase in milk fat and an increase in the protein content in supplemented groups ($p < 0.10$; Table 4). Increases in milk fat content after niacin feeding have been observed as well by Belibasakis and Tsirgogianni (1996; F:C ratio 50:50), while also a trend for a decreasing effect of 12 g NAM was observed (Cervantes et al. 1996; 40-65% concentrate). No effect was seen in other experiments (Martinez et al. 1991; Ottou et al. 1995; Christensen et al. 1996; Minor et al. 1998; 19 – 60 % concentrate). It was concluded that the reasons for an effect of niacin on milk fat are unknown, but may be related to an effect on rumen fermentation (Belibasakis and Tsirgogianni 1996). These authors named niacin-induced increases in protozoal numbers, NDF digestion or molar proportion of acetic acid in the rumen as possible mechanisms. Rumen fermentation was influenced in the present experiment, but molar proportion of acetic acid increased only slightly and not significantly (Niehoff et al. 2009b). Additionally, NAD(H) or NADP(H) are required in a large number of reactions in mammary secretory cells. Synthesis of fatty acids requires NADPH as source of reducing equivalents (Bauman and Davis 1974). Thus, increases in blood NAM concentrations might have been beneficial on this level. However, no effect on milk fat content was observed in other studies with an increase in blood niacin concentration in niacin supplemented groups (Driver et al. 1990; Ottou et al. 1995). Even a trend towards lower milk fat concentrations in niacin supplemented groups was found (Cervantes et al. 1996). Therefore, other mechanisms might be involved.

In accordance with the present trial, trends and significant increases in protein content or yield have also been described elsewhere (Erickson et al. 1992; Cervantes et al. 1996; Drackley et al. 1998). Concentrate proportions in these studies varied from 19 - 60%. But other experiments showed no influence of a niacin supplementation on either pro-

tein concentration or yield (Martinez et al. 1991; Ottou et al. 1995; Belibasakis and Tsirgogianni 1996; Minor et al. 1998). Erickson et al. (1992) supposed that NA supplementation might spare tryptophan, and this may be beneficial for milk protein since casein contains one to two tryptophan residues. Others concluded that the increase in milk protein occurs due to an observed augmentation in casein synthesis (Horner et al. 1986). These authors suggested that this may be due to an increase in insulin. But because inconsistent reactions of niacin on casein have been observed (Horner et al. 1988; Lanham et al. 1992; Christensen et al. 1996; Drackley et al. 1998), no definite deductions can be made. It was also suspected that due to a possible effect of NA on insulin, amino acid uptake of the mammary gland may be enhanced (Erickson et al. 1992). These statements lead to the conclusion that niacin has a systemic effect on milk protein. But also in studies where niacin supplementation increased blood niacin concentration, results concerning milk protein differed. An increase in protein yield was observed once (Cervantes et al. 1996), while in another study no effect (Ottou et al. 1995) or an interaction between niacin and soybean processing (Driver et al. 1990) existed. Hence, this thesis can neither be rejected nor verified.

Erickson et al. (1992) suggested further, that benefits of NA on milk protein derive from enhanced microbial protein synthesis. Microbial protein synthesis was augmented in the present experiment (Niehoff et al. 2009b). But there was a trend for an interaction with F:C ratio, since the increase following niacin supplementation was highest with HC and negligible with MC diet. For milk protein concentration, the inverse is the case. Thus, also this thesis can not be completely verified from the present experiment and mechanisms need to be further evaluated.

The observed effect of NA supplementation on milk urea concentration (Table 4) may be attributed to higher serum urea concentrations in supplemented cows (Table 3). Broderick and Clayton (1997), Gustafsson and Palmquist (1993), Lebzien et al. (2006) and Roseler et al. (1993) also showed a close relationship between serum and milk urea. However, this was not the case with MC ration. Serum urea concentrations have also been enhanced in niacin-fed animals with this diet, which surprisingly was not found in milk. No explanation is obvious for this result.

To our knowledge, niacin concentrations in milk of dairy cattle were recorded in only two studies (Nilson et al. 1967; Wagner et al. 1997). Wagner et al. (1997) fed a ration containing 40% forage and infused 6 g NA daily with or without stearic acid in the duodenum. Only NAM was analysed in milk, but it was not stated if this was due to an (as-

sumed) absence of NA or to other reasons. In that experiment, concentrations of NAM increased from 0.54 mg/L in unsupplemented to 0.87 mg/L in milk of NA supplemented cows. In contrast, no effect of NA supplementation was observed in the present trial (Table 4). This difference may be caused by the different amounts of NA in the duodenum during duodenal infusion or with oral supplementation as in the present trial. Nilson et al. (1967) tested how rumen and milk niacin concentrations responded when cows where changed abruptly from a conventional diet to diets containing either 100% corn silage, 100% alfalfa hay or 100% of a grain concentrate mixture. No supplementation was fed, and niacin intake was highest with the alfalfa hay ration (673 mg/d) compared to 374 mg/d from corn silage and 350 mg/d from concentrate. But the niacin concentration in milk was lowest in the alfalfa hay ration, while for the grain and corn silage diets it was almost similar and averaged 1.0 mg/kg milk three weeks after cows have been changed to the rations. Niacin was determined microbiologically, where it is not possible to differentiate between NA and NAM.

The observed increase in niacin concentrations at higher concentrate level in the present experiment is not in accordance with effects found by Nilson et al. (1967), since the 100% grain concentrate mixture performed equally with the 100% corn silage. However, it has to be stated that a ration containing 100% grain-concentrate mixture can not be considered as adequate for ruminants. This could have masked other effects. But results of that experiment may also suggest that no relation between niacin intake and concentration in milk exists, as seems to be the case in the present experiment. Research in humans revealed inconsistent results. Powers et al. (1997) postulated that maternal supplements can raise low breast milk levels of niacin in malnourished mothers, while in older human studies it was found that the values for niacin in human breast milk show a relationship to milk volume rather than to niacin intake (Coryell et al. 1947). Also in the current experiment, the effect of F:C ratio on the amount of niacin excreted seems to be due to an increased milk production. However, this was not obvious for the niacin concentration (Table 4). It is surprising that even though niacin concentrations in blood were enhanced in supplemented groups (Table 3), this did not lead to an effect on milk NAM concentrations (Table 4).

In older studies on human, 1 – 22% of niacin intake was excreted via milk, on average 7% (Coryell et al. 1947). This matches with our data, since with LC, MC and HC ration without additional NA these values were 2.6, 4.3 and 3.8% respectively, while with these diets supplemented with NA it was 0.1, 0.4 and 0.3% (data not shown). To our

knowledge, the mechanism of niacin secretion into milk has not yet been described for ruminants, but might provide explanations for these results.

The fatty acid composition of milk showed a trend towards decreasing proportions of lauric acid and a significant increase in oleic acid in NA supplemented cows (Table 5). Effects of niacin on fatty acid composition described in the literature are inconsistent. No effect was observed in one study (Christensen et al. 1998). Interactions with rapeseed oil infusion for butyric and myristoleic acid (Ottou et al. 1995) and with the content of unsaturated fat in the diet for oleic and linoleic acid (Madison-Anderson et al. 1997) were also seen. Furthermore, decreases in short and middle chain fatty acids (Martinez et al. 1991; Klippel et al. 1993; Wagner et al. 1997) and an increase in oleic acid proportion (Klippel et al. 1993; Wagner et al. 1997) have been found as well.

Long chain fatty acids in milk are of dietary or endogenous origin (van Knegsel et al. 2007), hence the increase in milk oleic acid proportion observed in the present trial may be due to a lower extent of hydrogenation of dietary oleic acid in the rumen, an incomplete biohydrogenation of other dietary polyunsaturated fatty acids or to a change in activity of enzyme systems in the udder. Christensen et al. (1998) observed no change in net flow of $C_{18:1}$ to the duodenum in either high or low fat diets due to NA supplementation, though there was a trend towards higher net flows of $C_{18:2}$ and $C_{18:3}$. But in total, biohydrogenation of unsaturated $C_{18}$ was not influenced by NA supplementation (Christensen et al. 1998). However in this study, no effect was seen on milk fatty acid composition either, which is different from observations of the present trial.

Wagner et al. (1997) attributed their observation of an increase in oleic acid to an influence of NA on the activity of mammary enzyme systems. An important enzyme in the generation of unsaturated fatty acids is stearoyl-CoA desaturase ($\Delta^9$ desaturase), which introduces a double bound in the $\Delta^9$ position (Ntambi 1995). NAD(P)H is involved in this reaction (Ntambi 1995), hence an impact of niacin may be possible. But oleic acid is produced in the udder from stearic acid by the action of $\Delta^9$ desaturase enzyme (Moate et al. 2007). Thus, an increase in oleic acid should come along with a reduction in stearic acid proportion. But even though not significant, numerically stearic acid was also enhanced in niacin supplemented cows in the present study (Table 5). $\Delta^9$ desaturase catalyzes the conversion of $C_{14:0}$ to $C_{14:1}$, $C_{16:0}$ to $C_{16:1}$, $C_{18:0}$ to cis-9 $C_{18:1}$ and $C_{18:1}$ to $C_{18:2}$ (Griinari et al. 2000; Mosley and McGuire 2007). Moreover, Mosley and McGuire (2007) stated that the desaturation of stearic acid yielded ~ 43% of the oleic acid in milk fat. This might not be generally valid since $\Delta^9$ desaturase activity is influenced by many

factors among which are also dietary factors (Ntambi and Miyazaki 2004). But it shows that for oleic acid, only approximately one half is influenced by this enzyme. And proportions of other mono-unsaturated fatty acids ($C_{14:1}$ or $C_{16:1}$) were not altered in the present experiment. Hence, from the observed values it can not be concluded that the NA supplementation increased oleic acid proportion in milk fat via a stimulation of $\Delta^9$ desaturase.

## 5. Conclusions

The addition of niacin to the diet caused several changes in metabolism. These responses were not altered by the F:C ratio fed, because no significant interactions occurred. Serum glucose concentration was enhanced in niacin supplemented groups. This may have been due to changes in glucose removal from blood, or an increase in gluconeogenesis. But since especially glucose in blood is regulated by a large variety of factors, it can not be definitively concluded from this trial how NA exerted its influence. NA supplementation, as well as higher level of concentrate led to higher NAM concentrations in serum. This may reflect greater niacin flow to the duodenum. No NA was present in blood, thus conversion of NA to NAM must have occurred. But mechanisms or locations are unknown.

No effect of NA was seen on serum concentrations of NEFA or BHBA. One reason for this might be the lactation state of the cows. It is also possible that this occurred due to the amount of non-rumen protected NA supplemented orally, which only caused an increase in NAM in serum. Serum NAM apparently does not have an antilipolytic action. Urea concentrations in serum were higher in NA fed cows. These changes seem to be a consequence of alterations in ruminal fermentation of niacin fed cows. Milk fatty acid composition was influenced by NA as well, since oleic acid proportion was increased. An impact on mammary enzymes is possible, but seems rather unlikely from results obtained in the present study. However, other mechanisms can not be identified from this experiment.

Contrary to serum values, NAM concentrations in milk or amounts daily excreted via milk were not modified by NA supplementation, but showed impacts of level of concentrate. For these results, no explanation can be given and further research especially on the mechanism of niacin secretion into milk is needed.

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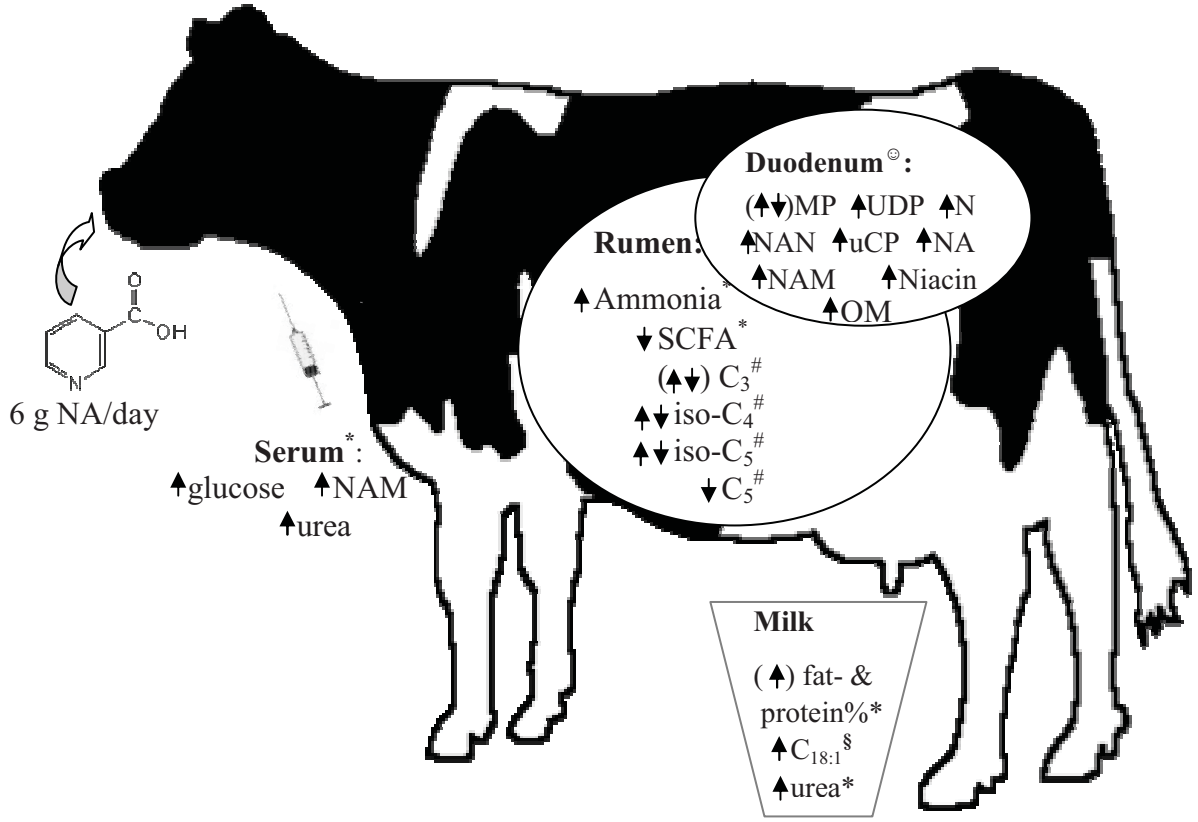
## General discussion

Even though it is assumed in general that ruminal synthesis meets the niacin requirements of dairy cows (Girard, 1998; GfE, 2001) it was also stated that this may not always be the case (Flachowsky, 1993; Girard, 1998; Girard and Matte, 2005; Santschi et al., 2005a). But experiments with niacin supplementation for ruminants came to inconsistent results (**Paper I**). Furthermore, usually impacts only on parts of the metabolism were regarded, e.g. influences only on ruminal fermentation or only on performance. Niacin flow or concentrations in various body fractions are often not determined, as was also shown in **Paper I.** Hence, the present experiment aimed to investigate the effect of supplemental niacin on the whole animal, starting with effects on the rumen and nutrient flow to the duodenum as well as several blood metabolites and performance parameters. Special emphasis was placed on the niacin flow to the duodenum and its concentrations in serum and milk.

It was stated that ruminal B-vitamin synthesis or use might be altered in diets with higher amounts of concentrate (Santschi et al., 2005a). Therefore, the effect of diets differing in forage-to-concentrate ratio (**F:C ratio**) on responses to supplemental niacin was also investigated. For this purpose, three diets differing in F:C ratio were fed to double fistulated, midlaction or dry dairy cows. Diets consisted of either 1/3 concentrate and 2/3 forage (low concentrate, **LC**), 1/2 concentrate and 1/2 forage (medium concentrate, **MC**) or 2/3 concentrate and 1/3 forage (high concentrate, **HC**) on dry matter basis. Each ration was fed once without niacin supplementation, while in the following period, a supplement of 6 g niacin (nicotinic acid, **NA**) per cow and day was given.

The results of the experiment as tested by F-test statistics with significance set at $P \leq 0.05$ and trends at $P \leq 0.10$ are shown in Figure 1. Significant interactions of the F:C ratio fed and NA supplementation were found for molar proportions of iso-valeric acid and iso-butyric acid. Trends for an interaction are also presented, as observed for molar proportion of propionic acid and the amount of microbial protein reaching the duodenum as well as for some measurements for the efficiency of microbial protein synthesis. For all other variables analysed, responses to supplemental niacin were not altered due to the feeding of different diets. The results shown in Figure 1 thus represent the main effects of niacin over all three rations for these parameters.




MP = microbial crude protein; UDP = undegraded feed crude protein; N = nitrogen; NAN = non-ammonia-N; uCP = utilizable crude protein; NA = nicotinic acid; NAM = nicotinamide; OM = organic matter; SCFA = short chain fatty acids; $C_3$ = propionic acid; iso-$C_4$ = iso-butyric acid; iso-$C_5$ = iso-valeric acid; $C_5$ = valeric acid; $C_{18:1}$ proportion of oleic acid in milk fat; ↑ significant increase; ↓ significant decrease; ↑↓ interaction with F:C ratio; (↑) trend, * concentration; # molar proportion; ☺ flow per day; § weight percent

**Figure 1: Effects of a niacin supplementation on various parameters of dairy cow metabolism as observed in the present experiment**

*Effects of supplemental NA on ruminal fermentation and flow of nutrients to the duodenum*

NA supplementation lowered short-chain fatty acids (**SCFA**) concentration in ruminal fluid (**Paper II,** Figure 1), with greatest differences in the LC ration. Valeric acid was decreased in NA supplemented groups, but molar proportions of major SCFA (acetic, butyric and propionic acid) were not altered significantly. As already mentioned, there was a trend for an interaction with F:C ratio for molar proportion of propionic acid, while the interaction was significant for iso-butyric and iso-valeric acid. But due to the difficulties in analysing the small amounts of the latter fatty acids, this may not be over-interpreted.

The decrease in SCFA concentration may have occurred due to a change in ingesta passage, caused by niacin. Schussler et al. (1978) measured a higher turnover of rumen

fluid (mL/min) in niacin fed cows and sheep, even though this increase was not significant. Duodenal liquid dilution rates have also been shown to be faster in niacin supplemented cattle (Arambel et al., 1986). Thus, niacin might have enhanced ingesta passage through the rumen, which consequently reduced the extent of feed degradation and the SCFA production. But also other studies exist, where no effect on ruminal fractional passage rate, turnover rate or ruminal dilution rate was observed (Doreau and Ottou, 1996; Christensen et al., 1996), thus further research is needed to clarify the relationships.

Several effects existed on N and protein metabolism (Figure 1). NA supplementation enhanced ruminal ammonia concentration (**Paper II**). Riddell et al. (1980) observed an increase in ammonia concentration 6 h after feeding as well. They attributed this to an increase in ureolytic activity, since ruminal urea concentrations were lowered in those animals. Additionally, it is often claimed that niacin is beneficial for rumen protozoa and niacin supplementation was found to have a positive impact on protozoal numbers (Horner et al., 1988; Erickson et al., 1990; Doreau and Ottou, 1996; for more details on influenced protozoal families and genera see **Paper I**). Firkins et al. (2007) stated that faunation usually results in higher ruminal ammonia concentrations. Thus, a stimulating effect of niacin on protozoa might have also been a reason for the observed increase in ruminal ammonia concentrations in niacin supplemented groups.

It was also proposed that this increase in protozoa is responsible for increases in microbial protein synthesis in niacin fed animals (Dennis et al., 1982; Samanta et al., 2000). Microbial protein synthesis was enhanced in the present trial in niacin fed animals as well. But usually, faunation results in decreased microbial protein flow at the duodenum due to protozoal predation of bacteria (Jouany, 1996). However, for example starch grains limit protozoal predation of bacteria, since they are quickly engulfed and fill protozoa (Hristov and Jouany, 2005). This could at least in part explain the observed trend for an interaction of NA and F:C ratio, since differences between supplemented and unsupplemented groups are largest with HC diet where starch content was highest, and only minor with the balanced MC ration. But apart from protozoa, niacin requirements have also been shown for several *Lactobacillus* and *Streptococcus* strains isolated from the rumen of sheep (Ford et al., 1958), hence other effects may exist as well.

Furthermore, undegraded feed crude protein (**UDP**) rose with NA addition to the ration, although differences were again only small with MC ration (**Paper II**). The increase in UDP in conjunction with enhanced ruminal ammonia concentration and amounts of

microbial crude protein reaching the duodenum seems to be controversial. But Riddell et al. (1980) also observed higher ammonia concentrations in the rumen and an increase in microbial protein concentration. As already mentioned, they attributed this to an increase in ureolytic activity in the rumen together with enhanced incorporation of ammonia in microbial protein. Schaetzel and Johnson (1981) observed no direct effect of NA addition to fermenters. But they reported that when the inoculum came from an animal which was adapted to NA, TCA precipitable N was significantly higher than if it was taken from a non-adapted donor. The increase occurred despite less substrate disappearance. Hence, results indicate a shift in microbial population towards microorganisms, who utilise N more efficiently. This may also be concluded for the present experiment, since all measurements of microbial efficiency have been enhanced in niacin supplemented animals (**Paper II**). If this shift happens together with a change in ingesta passage and stimulation of ureolytic activity, an increase in microbial crude protein, UDP and ruminal ammonia concentration seems to be possible. But stimulating effects of NA feeding seem to be highest with HC ration, while only negligible with MC diet. Microbial crude protein flow at the duodenum as well as microbial crude protein synthesized per MJ ME and per g ruminally degraded crude protein also showed a trend for an interaction with F:C ratio.

Apart from N flow at the duodenum, OM flow and apparent ruminal OM digestibility were influenced by NA supplementation. Other nutrient fluxes showed no impact (**Paper II**). The observed increase in OM flow and decrease in apparent ruminal OM digestibility may be a consequence of an increased passage rate in the rumen and matches well with the decrease in total ruminal SCFA concentration. However, the augmentations of OM may also be a consequence of increased microbial protein synthesis in niacin fed animals, since microbial OM is also included in amounts of OM arriving at the duodenum. This is supported by the fact that differences between groups are no longer significant if the amount of FOM is considered.

*Effects of NA supplementation on serum metabolites*

Only few influences of NA supplementation were observed on serum variables in the present study and no interaction was found between niacin feeding and F:C ratio (**Paper III**, Figure 1). Niacin enhanced serum glucose concentrations (**Paper III**). It has been concluded that it is unknown if this occurs due to an augmentation of glucose synthesis in the liver or a decrease in glucose removal or via both mechanisms (Jaster and

Ward, 1990), and as especially glucose concentrations in blood are regulated by many factors, no definitive deductions can be made.

Urea concentrations in blood were enhanced in niacin supplemented animals, irrespective of concentrate level (**Paper III**). Since rumen ammonia concentrations were also higher in those animals, the increase in serum urea concentration is assumed to be a consequence of alterations in the rumen.

No significant effect of NA supplementation was seen on mean NEFA and BHBA concentration in the present study, as was also the case in many other experiments (**Paper I**). Cows may have been to far in lactation to show a response to niacin. Furthermore, the amounts supplemented led to an augmentation in serum niacin concentration, but the vitamer increased was nicotinamide (**NAM**; **Paper III** and Figure 1). In humans, it was clearly shown that NAM was only a weak agonist to the receptor which exerted an antilipolytic action after stimulation with NA (Wise et al., 2003). Hence, if only NAM in blood was enhanced after NA supplementation as in the present experiment, this may not lead to a decrease in NEFA, if mechanisms are similar in ruminants.

*Effects of NA supplementation on milk parameters*

No significant effects of niacin supplementation on milk constituents were observed in the present trial, apart from an increase in proportion of oleic acid in milk fat and an enhancement of milk urea concentration. The increase in milk urea concentration was only observed with LC and HC ration. With the MC diet, there even was a minor decrease, although this was not distinct enough to cause an interaction between NA supplementation and F:C ratio. Enhanced milk urea concentrations are assumed to be a consequence of alterations in ruminal fermentation. This seemed to cause increased serum urea concentrations, which are related to milk urea concentrations (Roseler et al., 1993; Lebzien et al., 2006). However, this would not match for MC ration, where serum urea concentrations have also been enhanced in niacin fed animals, which surprisingly was not found in milk.

Furthermore, two trends were observed. NA supplementation increased fat and protein content of milk. But since these increases did not lead to trends or significant changes in fat or protein yield, they may not be overvalued.

It was concluded that reasons for increases in fat percentage in niacin supplemented groups are unknown, but may occur due to alterations on the ruminal level, as for example an increase in molar proportions of acetic acid (Belibasakis and Tsirgogianni,

1996). Even though molar proportion of acetic acid increased numerically in the present trial (**Paper II**), this was not significant. Another explanation may be an effect of enhanced NAM concentrations in blood, since NADPH is required as a reducing equivalent in milk fat synthesis (Bauman and Davis, 1974). However, Driver et al. (1990) observed enhanced NAM concentrations in blood, but milk fat yield was only higher in niacin supplemented groups when heat-treated whole soybeans were fed, while cows receiving heat-treated soybean meal did not show a reaction. Also, in another trial enhanced niacin concentrations in blood were found without an increase in milk fat percentage (Ottou et al., 1995). Thus, other mechanisms might be involved as well.

Increases in milk protein concentrations have been attributed to a tryptophan sparing effect of niacin (Erickson et al., 1992) or to an increase in insulin concentrations, which in turn stimulated casein synthesis (Horner et al., 1986). Erickson et al. (1992) stated that enhanced milk protein contents or yields may also be due to increases in microbial protein synthesis in the rumen. This may provide an explanation for results of the present trial, since microbial crude protein flow at the duodenum was enhanced in niacin supplemented animals (**Paper II**). Lanham et al. (1992) suggested that due to the anti-ketogenic effect of niacin, less amino acids would be used for gluconeogenesis and hence be available for protein synthesis in the udder. No anti-ketogenic effect was observed in the present study, but increases in serum glucose concentration (**Paper III**), which may also have spared amino acids.

Milk fat composition was also altered by NA supplementation (**Paper III**). NA addition to the diets tended to decrease the proportion of lauric acid and enhanced the proportion of oleic acid significantly. Wagner et al. (1997) attributed the increase in oleic acid proportion found in their experiment to an influence on the activity of enzyme systems in the udder parenchyma. An important enzyme for the generation of mono-unsaturated fatty acids in the udder is $\Delta^9$ desaturase (Ntambi, 1995). NAD(P)H is involved in this reaction, hence an influence of higher amounts of NAM in blood seems possible. But the conversion of $C_{14:0}$ to $C_{14:1}$, $C_{16:0}$ to $C_{16:1}$, $C_{18:0}$ to cis-9 $C_{18:1}$ and $C_{18:1}$ to $C_{18:2}$ is also catalysed by this enzyme (Mosley and McGuire, 2007). It was stated that the ratio of $C_{14:0}$ to $C_{14:1}$ is strongly influenced by the $\Delta^9$ desaturase activity, since essentially the only source of $C_{14:1}$ in milk fat is desaturation by this enzyme (Soyeurt et al., 2008). But $C_{14:1}$, as well as all other mono-unsaturated fatty acids, were not affected by NA supplementation in the present experiment (**Paper III**). Hence, other factors seem to be involved, for example incomplete biohydrogenation of oleic or linoleic or linolenic acid

in the rumen. But in the only study measuring fatty acid flows to the duodenum with or without NA supplementation, duodenal $C_{18:1}$ flow was not changed (Christensen et al., 1998). However, in that study no effect was seen on milk $C_{18:1}$ either. Hence, in the present trial this may be different, but definite conclusions cannot be drawn.

*Effects of niacin on metabolism and performance: ruminal or systemic?*

Schwab et al. (2006a) stated that it seems unlikely that production responses to supplemental niacin occur due to alterations in the rumen, and metabolic effects seem more likely. In **Paper I** it was concluded that it is not clear if effects are rather ruminal or systemic. The answer of this question would be useful to decide if supplemental NA should be rumen-protected, semi-rumen protected or without protection as used in this trial.

It was clearly shown in the present experiment that non-rumen-protected NA had effects on the animal. Several of these effects have been on the ruminal level (**Paper II**). But also from this experiment it is difficult to conclude if effects on intermediary metabolism and on performance result from changes on the ruminal level or are a consequence of the observed higher NAM concentrations in blood. For urea, it seems safe to assume that the influence of niacin on milk and serum urea concentrations (**Paper III**) is a consequence of alterations in the rumen. But for impacts on serum glucose, milk fat and protein content and molar proportion of oleic acid in milk fat, results are not clear. Even though some hints are present, definite conclusions on these variables are neither possible from the literature cited in **Paper I** nor from results of the present experiment (**Papers II** and **III**). As no effect of NA supplementation on NEFA or BHBA was observed in the present experiment (**Paper III**), but a remarkable effect on NEFA was found in the trial of Pires and Grummer (2007) with abomasal infusion, it seems that a possible effect on NEFA is systemic. It was not observed in the present experiment either due to the lactation state of the cows or to the substantial amount of NA lost before the duodenum. Further research is required, desirably with abomasal or duodenal infusion of NA to clarify if effects on intermediary metabolism and performance are systemic, as was also demanded in **Paper I**. If this is the case, a rumen-protected supplementation may be more suitable. But a completely rumen-protected supplementation would not cause the observed stimulating effects on microbial protein synthesis. Thus, from the present point of view, a semi-protected NA supplementation may have greatest influences. But to prove this thesis, further examinations are neccessary.

*Niacin flows and concentrations in the body*

As emphasis was placed on niacin flows and concentrations, these are discussed separately. They were determined in feed (**Papers II** and **III**), duodenal chyme (**Paper II**), blood (**Paper III**) and milk (**Paper III**). For the analyses of feed contents, microbiological methods were used, whereas concentrations of free NA and NAM in the other matrices were determined via HPLC. While in feed only NA was present, both vitamers can be found in duodenal chyme. But in milk and serum only NAM was detected. In Figure 1 it was shown that all measured niacin flows or concentrations have been affected by NA supplementation. Additionally, several influences of F:C ratio existed as well, but no interactions occurred. The observed vitamin flows and concentrations are given in Table 1 and are presented in comparison to the only other study measuring duodenal and blood concentrations (Campbell et al., 1994).

Higher concentrate proportions increased the amount of total niacin and NA at the duodenum, but not NAM flow (**Paper II** and Table 1), even though differences were small between MC and HC ration in unsupplemented groups. Schwab et al. (2006b) also observed that rations containing 35% instead of 60% forage led in tendency to higher duodenal flows of NA and a significant increase in total niacin. But also for NAM, these authors found a significant increase. Hence, the observed results for NAM do not match with the present experiment. These differences may in part be explainable by different NAM intakes. In the work of Schwab et al. (2006b) NAM intake differed largely between different F:C ratios (221 mg/d – 1399 mg/d for different groups), while in our study no NAM was present in feed.

NA supplementation also enhanced all flows of niacin at the duodenum (**Paper II** and Table 1). This is in accordance with several experiments with dairy cows (Riddell et al., 1985; Campbell et al., 1994) or sheep (Kollenkirchen et al., 1992) as well as increased niacin concentrations *in vitro* (Shields et al., 1983). But not the total amount supplemented reaches the duodenum (**Paper II**), indicating degradation in the rumen and/or absorption before the duodenal cannula. Both processes may contribute to the disappearance of supplemental niacin, since also *in vitro* net niacin synthesis was found to be negative if either NA or NAM were supplemented (Hannah and Stern, 1985). Additionally, even with abomasal NAM infusion substantial losses occurred before the duodenal cannula (Santschi et al., 2005a).

**Table 1: Niacin flows and concentrations in feed and several body compartments of cows in the present experiment and in the work of Campbell et al. (1994) (LS MEANS; arithmetic means for feed content)**

| Ration | Feed mg/d | RF NA mg/L | DF Nia mg/d | DF NA mg/d | DF NAM mg/d | AS Nia mg/d | B NA mg/L[1] | B NAM mg/L[1] | M NAM mg/L |
|---|---|---|---|---|---|---|---|---|---|
| Present experiment | | | | | | | | | |
| LC | 553 | n.d. | 1602 | 880 | 724 | 1057 | 0 | 0.32 | 0.54 |
| LC + 6 g NA | 6449 | n.d. | 2021 | 1242 | 779 | -4419 | 0 | 0.36 | 0.55 |
| MC | 325 | n.d. | 1886 | 1114 | 780 | 1575 | 0 | 0.35 | 0.66 |
| MC + 6 g NA | 6337 | n.d. | 2221 | 1395 | 831 | -4089 | 0 | 0.46 | 0.72 |
| HC | 476 | n.d. | 1895 | 1188 | 707 | 1421 | 0 | 0.41 | 0.61 |
| HC + 6 g NA | 6370 | n.d. | 2630 | 1762 | 871 | -3738 | 0 | 0.52 | 0.64 |
| Campbell et al. (1994) | | | | | | | | | |
| Control[3] | n.d. | 0 | 1716 [2] | 1716 [2] | 0 | n.d. | 0.92 | 1.16 | n.d. |
| 12 g NA | n.d. | 14 | 3187 [2] | 3187 [2] | 0 | n.d. | 1.27 | 1.26 | n.d. |
| 12 g NAM | n.d. | 14 | 4902 [2] | 4902 [2] | 0 | n.d. | 0.62 | 0.85 | n.d. |
| 6 g NA+ 6 g NAM | n.d. | 12 | 3922 [2] | 3922 [2] | 0 | n.d. | 1.02 | 1.01 | n.d. |

RF = rumen fluid; DF = duodenal flow; NA = nicotinic acid; Nia = niacin total; NAM = nicotinamide; AS = apparent synthesis; B = blood; M = milk; LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis; n.d.= not determined

[1] Niacin analysis was done in serum in the present experiment and in plasma in the work of Campbell et al. (1994)

[2] Duodenal flow values were not given, but concentrations in duodenal chyme and it was stated that duodenal fluid contained 6.65 % DM and average DM flow to the duodenum was 16.3 kg/d, hence, duodenal flow values were calculated by us.

[3] 60 % forage (alfalfa haylage, corn silage) 40 % concentrate (corn, soybean hulls and meal)

In the present experiment, duodenal flows of niacin, NA and NAM as well as apparent niacin synthesis showed strong correlations with OM intake and microbial N flow at the duodenum (Table 2). The relation between duodenal niacin flow or apparent synthesis and microbial protein synthesis (net synthesis as reflected in flow of microbial protein at the duodenum) is also shown in Figure 2.

**Table 2 : Pearson correlation coefficients between apparent synthesis of niacin, flows to the duodenum and OM intake as well as microbial N flow; all values are significant with p < 0.01**

| Parameter | DF Nia, mg/d[1] | DF NA, mg/d[1] | DF NAM, mg/d[1] | AS Nia, mg/d[2] |
|---|---|---|---|---|
| OM intake, kg/d | 0.83 | 0.77 | 0.86 | 0.82 |
| Microbial N, g/d | 0.92 | 0.92 | 0.83 | 0.93 |

DF = duodenal flow; Nia = niacin total; NA = nicotinic acid; NAM = nicotinamide; AS = apparent synthesis; OM= organic matter

[1] For duodenal flows of Nia, NA and NAM, all groups were included

[2] For apparent synthesis of Nia, only groups without NA supplementation were included, since apparent synthesis became always negative when NA was fed

Schwab et al. (2006b) also found a significant positive correlation between apparent niacin synthesis and microbial N flow. Positive correlations between amounts of microbial nitrogen and vitamin flow at the duodenum have been found for thiamine, as another B-group vitamin as well (Breves et al., 1981; Lebzien et al., 1986). It seems from these studies that if microbial growth is enhanced, production of vitamins also rises. Furthermore, degradation of supplemental niacin seems to be reduced if microbial protein synthesis rises. This can be seen from the black symbols in Figure 2, which indicate the supplemented groups of each ration. Cows with very low microbial protein synthesis also had a low total niacin, NA and NAM flow, even in supplemented groups. It can not be excluded that this is influenced by OM intake, since these cows also had a low feed intake. However, the correlation between OM intake and all niacin flows was weaker than the correlation with microbial N flow at the duodenum. Other explanations cannot be given and mechanisms remain to be explored. The same is valid if relations of microbial protein synthesis and apparent niacin synthesis of unsupplemented cows are examined.

Apparent synthesis of niacin was calculated by subtracting the intake from the amount reaching the duodenum. Apparent synthesis of total niacin and NA were influenced by F:C ratio. Since no NAM was present in feed, duodenal NAM flow also represents apparent NAM synthesis and was not affected by F:C ratio (**Paper II** and Table 1). If only unsupplemented rations are considered, apparent niacin synthesis was higher with MC and HC diet than with LC (Table 1 and **Paper II**). In rations fortified with NA, HC diet had the least negative values. This may indicate that degradation of supplemental NA is reduced in rations containing higher proportions of concentrate. However, Schwab et al. (2006b) found a trend towards increased apparent NAM synthesis with higher concentrate proportions, while for NA and total niacin synthesis no impact of F:C ratio was

seen. These were only positively influenced by higher dietary NFC concentrations (30% instead of 40% NFC in each F:C ratio). But there were large differences in niacin intake between different NFC levels in that experiment. This may have contributed to the observed effect of NFC on apparent synthesis. Anyway, diets with higher concentrate proportions were also the diets with higher NFC contents in the present experiment. Hence, no separation can be made between effects of NFC and F:C ratio in our study.

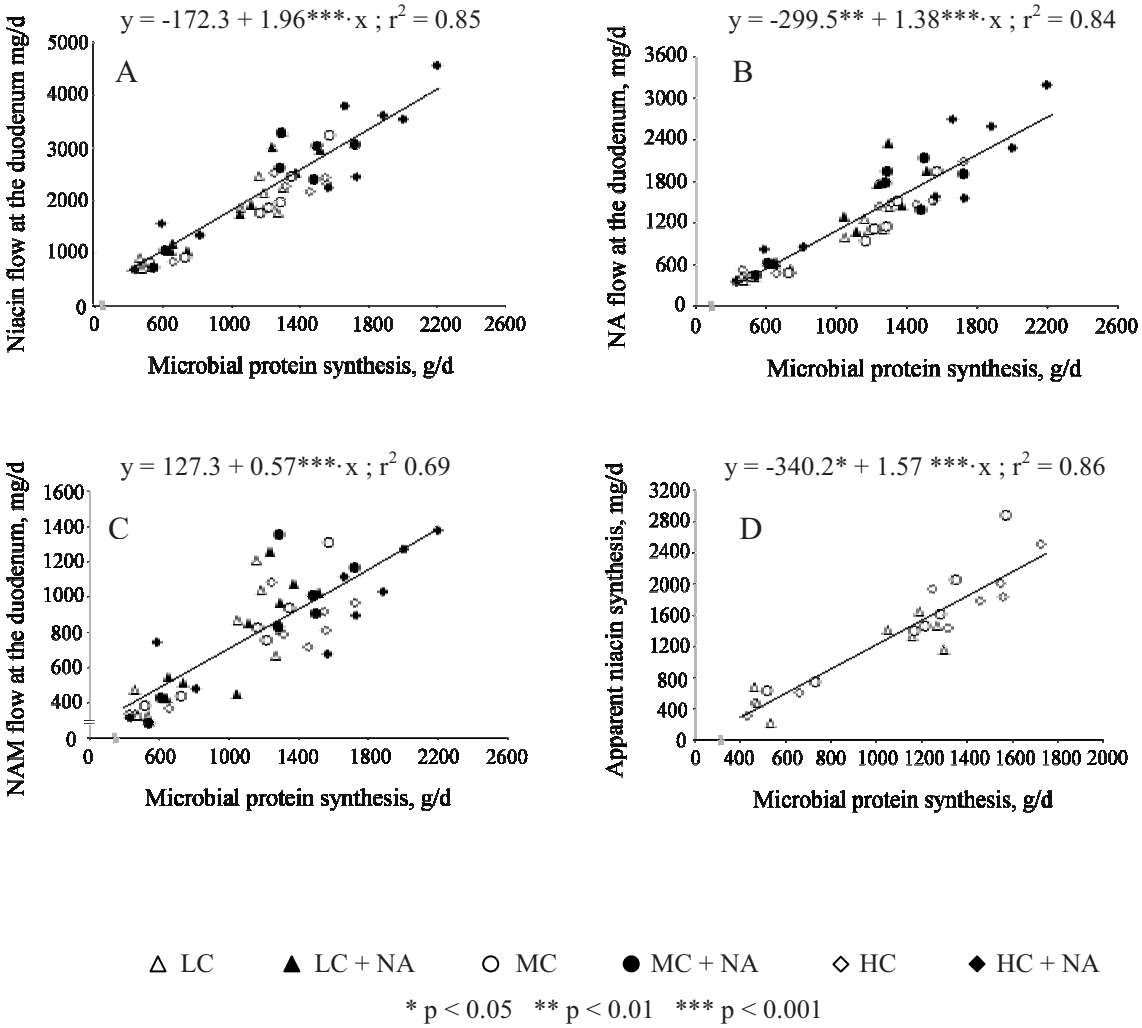



**Figure 2: Relationship between microbial protein synthesis and duodenal flow of niacin (Figure A), nicotinic acid (NA; Figure B), nicotinamide (NAM; Figure C) and apparent niacin synthesis (Figure D)**

If NA was supplemented, apparent synthesis became always negative (Table 1), indicating degradation of NA in the rumen or absorption before the duodenal cannula. Also in other studies the whole amount supplemented did not reach the duodenum (Riddell et al., 1985; Zinn et al., 1987; Campbell et al., 1994), even after postruminal infusion (Santschi et al., 2005a). As was discussed above and stated in **Paper I**, it may be as-

sumed that ruminal degradation as well as absorption before the duodenal cannula seem to contribute to these losses.

Only NAM was detected in serum, except for one cow where also NA was found in five out of six periods. Only when she received MC ration with supplemental NA, surprisingly NA in serum was below the detection limit as for the other animals.

NAM concentrations in blood were influenced by level of concentrate and by NA supplementation towards higher values in niacin supplemented groups and with higher concentrate proportions (**Paper III** and Table 1). Differences between supplemented and unsupplemented groups were small in LC and highest in HC ration. It seems that concentrations in serum reflect the amounts at the duodenal level, since NA supplementation as well as F:C ratio also influenced the amounts of niacin arriving at the duodenum (**Paper II** and Table 1). But differences between unsupplemented and supplemented animals were lowest in MC ration at the duodenal level, while this was LC for differences in serum concentrations. However, correlations between serum NAM concentration and flow of total niacin, NA and NAM at the duodenum are significant and shown in Figure 3.

It has to be remarked that even though only NAM was detected in serum, NAM flow at the duodenum showed the weakest relationship with serum concentrations ($r^2 = 0.23$), while NA flow at the duodenum had the strongest $r^2$ ($r^2 = 0.63$) for serum NAM concentrations. And quantitatively, also more NA than NAM was found at the duodenum (**Paper II** and Table 1). Hence, conversion of NA into NAM must have occurred during or shortly after absorption. Admittedly, it may also be possible that some NA would have been found if whole blood is analysed instead of serum as in the present experiment. In older research it was concluded that most of the NA is located in the erythrocytes (Pearson, 1939). But for rat erythrocytes, it was shown later that NA was taken rapidly from the medium and was enzymatically converted to the coenzymes, which do not readily diffuse from the cell (Leifer et al., 1948; Lan and Henderson, 1968). Thus, the existence of free NA in the erythrocytes is questionable, but may be possible. However, five out of the eight trials where niacin concentrations were determined in blood, also used serum or plasma. Hence, our serum measurements allow the comparison with literature data.

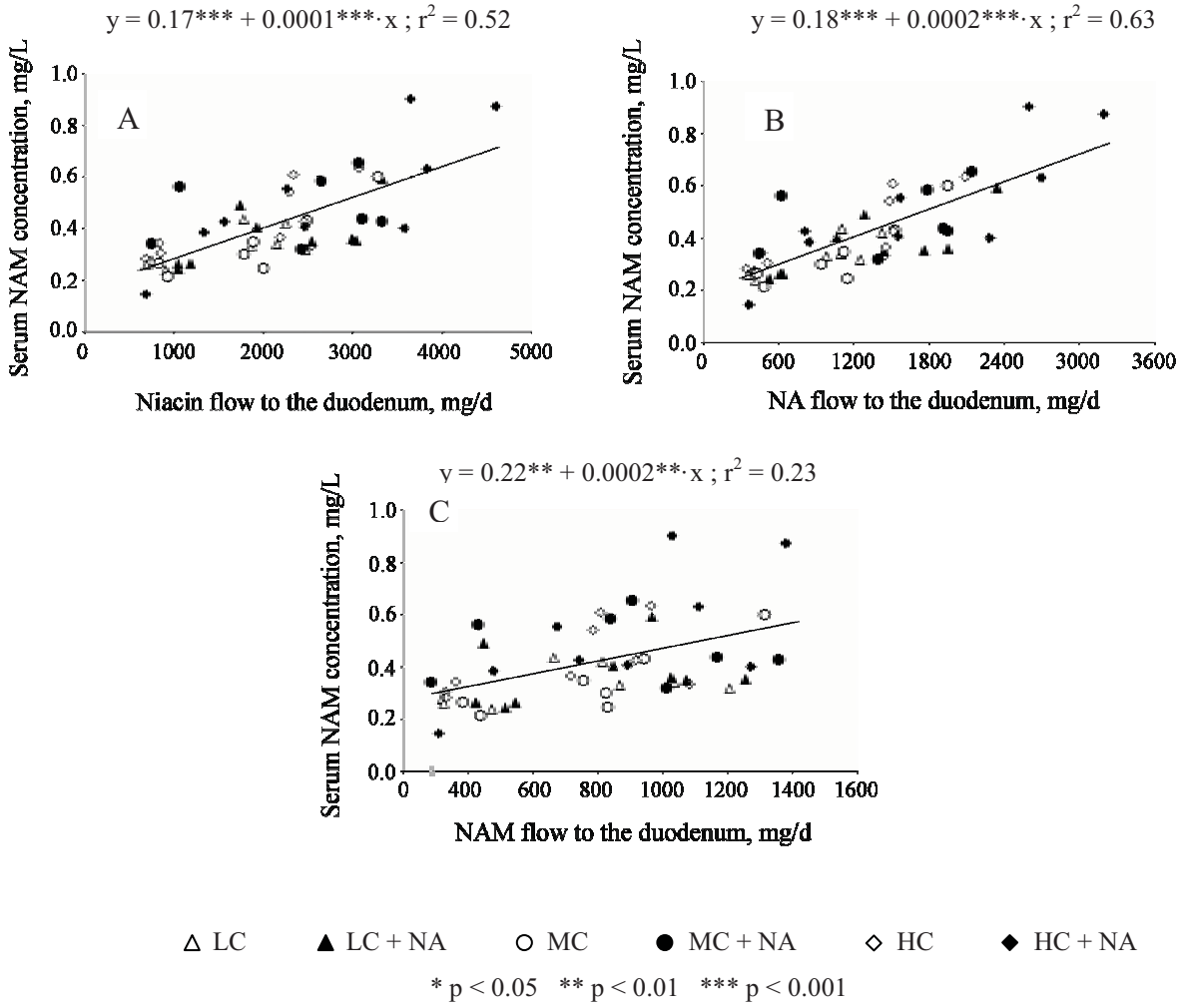



**Figure 3: Relationship between serum nicotinamide (NAM) concentration and niacin (Figure A), nicotinic acid (NA; Figure B) and NAM (Figure C) flow to the duodenum**

Only in one other study duodenal and blood niacin concentrations were measured (Campbell et al., 1994, shown in Table 1). As in the present trial, NA concentrations in duodenal fluid were enhanced if either 12 g NA or NAM were supplemented in that experiment. Cows with NAM supplementation had highest duodenal NA concentrations. In plasma, neither NA nor NAM concentrations were influenced compared to control groups. But cows supplemented with NA had significantly higher plasma NA concentrations than those with NAM supplementation, which is the opposite of duodenal results, and plasma NAM was not affected at all. This may be related to a mechanism discovered in rats, where NAM is able to pass back from bloodstream into the lumen (Stein et al., 1994). But this has not been investigated for ruminants.

In comparing the present trial with the work of Campbell et al. (1994) several differences can be found. First, no NAM was found in duodenal chyme in that study, but in our trial. Kollenkirchen et al. (1992) and Santschi et al. (2005a) stated also, that NAM

can be found if the whole duodenal content is taken. The second difference is the amount of niacin reaching the blood. Even with a relatively similar niacin flow at the duodenum with the control ration of Campbell et al. (1994; 1716 mg/d) and LC or MC diet without NA supplementation of the present experiment (1602 and 1886 mg/d), concentrations in blood were much higher in the trial of Campbell et al. (1994). Additionally, they also detected NA in blood, while this was below the detection limit in the present experiment. Explanations for these differences are not obvious and further research is needed. However, differences on the duodenal level are all reflected in serum concentrations in the present experiment, while in the work of Campbell et al. (1994) plasma concentrations of NA or NAM seemingly are not influenced by duodenal flows.

As was stated in **Paper I**, the existence of NA in blood is discussed. It may be concluded that this seems to be related to the amount reaching the duodenum and hence the amount supplemented or the supplementation technique. In two other studies with 6 g NA or 12 g NAM supplementation, also only NAM was analysed (Driver et al., 1990; Cervantes et al., 1996), even though it was not stated if this was due to a proven absence of NA in blood or other reasons. But this theory would not match with results of Campbell et al. (1994), where NA was always present, even without niacin supplementation. However, other studies cannot be found for dairy cattle. Hence, further research is needed where and to which extent the vitamers are converted into each other, as was also suggested in **Paper I**.

Also in milk, only NAM was detected in the present trial. Concentrations were influenced by F:C ratio, but only marginally by NA supplementation (**Paper III** and Table 1). Since F:C ratio also enhanced concentrations of NAM in blood, the correlation between serum NAM and milk NAM concentration was nevertheless significant. This can also be seen from Figure 4.

To the author's knowledge, niacin in milk was analysed in only two other studies (Nilson et al., 1967; Wagner et al., 1997). In contrast to the present results, Wagner et al. (1997) observed an increase in milk NAM concentration after duodenal infusion of 6 g NA. This difference may be explainable with the different niacin amounts at the duodenum and hence maybe in blood. With duodenal infusion, these amounts must have been higher as with oral supplementation like in the present trial.

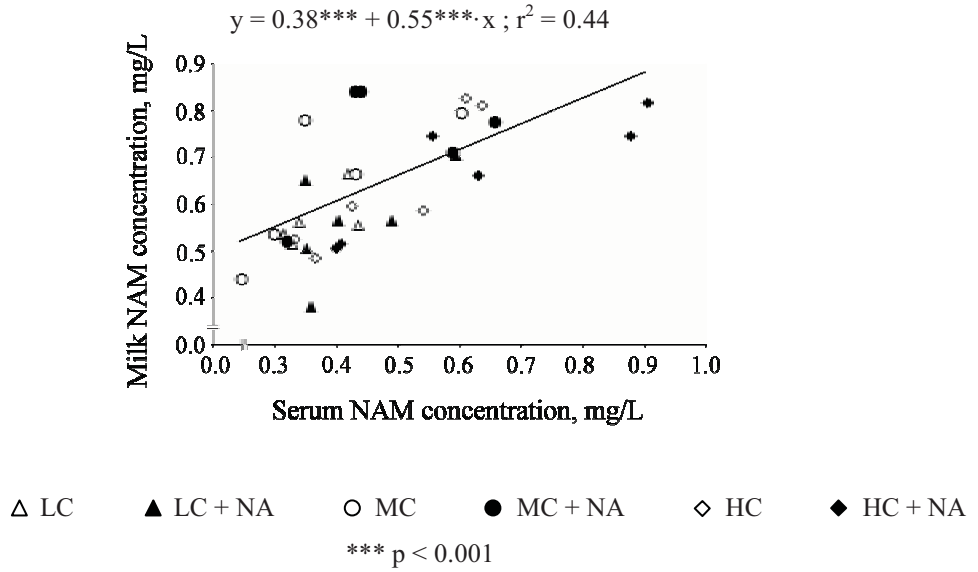



**Figure 4: Relationship between serum nicotinamide (NAM) and milk NAM concentration**

Nilson et al. (1967) changed cows abrupt from a conventional ration to a diet containing either 100% corn silage, 100% alfalfa hay or 100% grain-concentrate mixture. Niacin intake was highest with the alfalfa hay ration, but niacin content of milk was lowest. But grain and corn silage rations caused almost equal milk niacin concentrations of approximately 1.0 mg/kg. Even though this seems to be in accordance with our results that niacin in milk is independent of niacin intake, it does not show the effect of F:C ratio observed in the present experiment, since feeding of corn silage and grain diet led to the same milk niacin concentrations. Thus, no conclusions can be made and further research concerning the mechanism of niacin secretion into milk is required.

*Critical view on the present trial*

Even though some aspects of niacin impacts on several parameters could be clarified in the present experiment, there were also other points which were not optimal. Dry or midlaction and double fistulated cows may not represent the ideal experimental group for NEFA, BHBA and milk measurements. For these parameters, the use of higher numbers of unfistulated high yielding dairy cows in early lactation seems more reasonable. But since the focus was set on processes in the rumen and the amounts of niacin in various body fluids, double fistulated cows had to be used. Due to animal welfare reasons, experimental practice applied at the Institute includes that fistulated cows are allowed to rest at least two month after calving before they are included in trials, but probably these two month are of most interest for changes in blood and milk variables

in response to niacin. Also, the inclusion of dry cows into measurements could be seen critically, since their metabolism may differ from that of lactating cows. But due to limited animal numbers it was necessary, and the same number of dry animals was used with or without NA supplementation at the respective concentrate level. Furthermore, this enabled us to cover a broad spectrum of feed intakes.

After laboratory and statistical analysis it was concluded that the main explanations for observed effects on ruminal metabolism and the amounts of nutrients reaching the duodenum are a shift in microbial population, increases in ureolytic activity and changes in digesta kinetics. Thus, from the present point of view it would have been useful to include these measurements to prove these hypotheses. Furthermore, we neither determined niacin excretion with urine nor with faeces. This surely would be interesting, but is also rarely done in previous research.

Some critical considerations are associated with feed and feeding. First, animals were not fed from the same silo during the whole trial due to the length of the experiment. Thus, there were different silage batches, which differed in niacin content (**Papers II** and **III**) and to a lesser extent also in other nutrients. But differences in niacin content seem to be small (**Papers II** and **III**) compared to the 6 g NA supplemented. Additionally, we neither determined tryptophan nor aspartate or quinolinic acid contents in feed, even though these are precursors of endogenous or microbial niacin synthesis. As we measured duodenal niacin flow and thus the result of microbial niacin synthesis, determination of precursors of microbial synthesis was not necessary. Furthermore, instead of the tryptophan content of feed, the tryptophan determination in duodenal chyme would have been of most interest in the present experiment, as this is the amount available for the animal. But this would have gone beyond the scope of the experiment, as it requires further extensive analyses. Also in previous research, tryptophan determination in duodenal chyme was rarely done, even though other amino acids in duodenal chyme are determined in several experiments (Lebzien, 1997). However, it would be interesting to include these measurements in future research.

N concentrations in duodenal chyme were measured, and hence N flows could be calculated. However, NAN flow was not determined experimentally. The flow was calculated by assuming that always 4.9% of N represents ammonia, and hence 95.1% of N was believed to be NAN (Riemeier, 2004; **Paper II**). Since niacin supplemented groups always had higher ammonia concentrations in the rumen it is possible that also the proportion of ammonia N in total N at the duodenum was different in niacin fed cows.

Thus, the assumption of a constant proportion of 4.9% may not be completely correct for these groups. However, also with an ammonia proportion of 6.5% for niacin supplemented animals, which reflects the increase in ruminal ammonia concentration of approximately 30%, significant increases in microbial crude protein and UDP are still observed.

One last point considers niacin analytics. We determined concentrations of free NA and NAM in all matrices as is the general procedure in newer research, where modern HPLC methods are applied. But from the present point of view it would have been interesting to determine the concentrations of NAD(H) and NADP(H) additionally, especially in blood, since these are the biologically active part. This remains to be evaluated in future research.

*Placement of the present study in current research and future research directions*

Niacin is of importance in feeding practice of high producing dairy herds. Kellog et al. (2001) stated that 42% of the interrogated farms in the USA supplemented niacin. And as was shown in the previous sections and especially in **Paper I**, also a lot of research has been conducted with a niacin supplementation to dairy cow rations. But usually, the animal is not regarded in total, since only influences on rumen fermentation, duodenal flows or on performance are monitored. Furthermore, niacin concentrations in various body compartments are often not determined. To the author's knowledge, there is only one study, where duodenal as well as blood niacin concentrations are determined (Campbell et al., 1994). In this study, niacin intake from the basal ration was not determined, hence statements concerning amounts of niacin apparently synthesised or degraded in the duodenum are not possible. Furthermore, only one ration was used, fortified either with 12 g NA or NAM or 6 g of each. Thus, no conclusions on the transferability of the results obtained on other feeding regimes can be done. Contrary, other studies measuring the influence of different F:C ratios are always undertaken without niacin supplementation. Hence the present study attempted to consider both factors and their possible interaction on the whole animal.

As was mentioned at the beginning, it is assumed that ruminal niacin synthesis covers the requirements of dairy cows. This seems to be confirmed by the present experiment if values are compared with the NRC estimates for requirements of dairy cows (NRC, 2001). But tissue requirements were extrapolated from lactating sows (NRC, 2001) and

not determined experimentally, thus they are afflicted with some uncertainty. Table 3 shows NRC requirement and synthesis data adapted to our cows and measured values.

**Table 3: Daily requirements, duodenal flow and apparent synthesis of niacin (mg/d)**

| | Daily estimated requirement[1] | | | Ration | Duodenal flow[2] | Ruminal synthesis[2] |
|---|---|---|---|---|---|---|
| | Tissue | Milk | Total | | | |
| NRC (650 kg cow, 35 kg FCM) | 256 | 33 | 289 | | | 1804 |
| Present experiment (644 kg cow, 21 kg FCM) | 254 | 21 | 275 (254)[3] | LC | 1602 | 1057 |
| | | | | MC | 1886 | 1575 |
| | | | | HC | 1895 | 1421 |

FCM = 4% fat-corrected milk; LC = low concentrate 1/3 concentrate, 2/3 forage on dry matter (DM) basis; MC = medium concentrate, 1/2 concentrate, 1/2 forage on DM basis; HC = high concentrate, 2/3 concentrate, 1/3 forage on DM basis

[1]NRC requirements were based on several sources (NRC, 2001), while requirements for our study were calculated by dividing the NRC values with the respective weight and milk yield and multiplying this with our cow weight and FCM yield

[2]LS MEANS are given; for our experiment, apparent ruminal niacin synthesis was calculated by subtracting intake from duodenal flow

[3]values in parentheses represent requirement of dry cows, which were also used in the present experiment

But even though requirements seemed to be covered already in unsupplemented cows, effects of NA supplementation have been observed nevertheless. However, the mechanisms of these positive actions were not always clearly identified in the present trial and several questions remain unanswered or new ones are raised. In **Paper I** suggestions for future research directions were given based on literature data. With this trial, we fulfilled the first two suggestions of investigating effects under different feeding conditions and the analysis of the whole animal. Furthermore, we analysed niacin concentrations or flows in several body fluids as was recommended in point No. 7 of **Paper I**.

But the other proposals still remain valid. Additionally, some new suggestions loom from the experimental part of this thesis. Since it is assumed that increases in protozoal counts and ingesta passage are the reason for the observed results during NA feeding, measurements of these parameters may be included in future experiments. Also, degradation and real synthesis of vitamins in the rumen should be investigated in order to determine if influences on apparent synthesis result from impacts on degradation or on *de novo* synthesis. Furthermore, it should be investigated if degradation products of niacin may also exert effects on rumen metabolism. The hypothesis that only enhanced NA and not NAM concentrations in blood exert an influence on NEFA should be examined, as well as the mechanisms which lead to enhanced NA concentrations in blood

and site and extent of conversion of duodenal NA to serum NAM, NAD(H) and NADP(H). The influence of NA on biohydrogenation of dietary fatty acids in the rumen may also be of interest as a reason for observed changes in milk fatty acid composition. Also, the mechanism of NAM secretion into milk should be studied.

# Conclusions

The experimental questions were raised in the scope of the thesis. In order to be as concise as possible, remarks to the last question concerning the influence of the F:C ratio fed on responses to supplemental niacin are not presented separately, but included in each of the other three sections.

*What is the impact of a niacin supplementation on rumen fermentation and duodenal nutrient flow and how is this influenced by the F:C ratio fed?*

Ammonia concentrations in ruminal fluid increased, while total SCFA concentration decreased in NA supplemented groups. It is assumed that these alterations occurred due to changes in ingesta passage, a stimulating effect of niacin on protozoa or other shifts in microbial population. Molar proportions of the minor SCFA (iso-butyric, iso-valeric and valeric acid) were also influenced. A trend for an interaction with F:C ratio was observed, since NA supplementation to LC diet enhanced molar proportion of propionic acid in ruminal fluid, while with MC and HC diet it is decreased in supplemented groups. This result also may be attributed to a positive effect of niacin on protozoa, which is more pronounced under conditions which are suboptimal for protozoa, as is the case in HC ration. But since protozoal counts were not done and ingesta flow was not measured, further research is needed to approve these interpretations.

N flow at the duodenum was affected by NA supplementation as well. More microbial crude protein was found in niacin supplemented groups, either expressed in g/d, g/MJ ME or g/g rumen degraded crude protein. A trend for an interaction was found between niacin supplementation and F:C ratio, since the increase in microbial protein synthesis and efficiency was highest with the HC ration and almost negligible with MC diet. Hence, it seems that a niacin supplementation enhances microbial growth more in rations containing high levels of concentrate, while relatively little changes occur in well balanced diets.

*What is the impact of a niacin supplementation on blood and milk variables and how is this influenced by the F:C ratio fed?*

Also in intermediary metabolism, several changes were observed in NA supplemented animals. No significant interactions occurred for these measurements, hence responses to supplemental NA were not altered by the F:C ratio fed. In niacin supplemented

groups, serum glucose concentration was enhanced. The reason for this can not be definitively identified, since glucose in blood is regulated by a large variety of factors and further measurements concerning influences on glucose metabolism have not been carried out in the present trial.

Concentrations of NEFA or BHBA were not influenced by addition of NA to the diet. An explanation for this might be the lactation state of the cows. But it may also be assumed that this occurred due to the absence of NA in serum, and only an enhancement of serum NAM in niacin supplemented animals. NAM apparently does not act antilipolytic.

Urea concentrations in serum and in milk were higher in cows supplemented with niacin. These changes are assumed to be a consequence of alterations in the rumen, since ruminal ammonia concentrations were also enhanced in these animals.

Addition of NA to the diet had no significant impact on performance, but tended to enhance milk fat and protein content. Furthermore, milk fatty acid composition was altered significantly towards an increase in oleic acid proportion. Even though no mode of action of niacin on this fatty acid can be identified from the present experiment, an impact on mammary $\Delta^9$-desaturase could probably be excluded. But also in this area, further research is needed. No interaction with F:C ratio was observed for performance measurements as well.

*What is the impact of a niacin supplementation on duodenal niacin flow and its concentration in blood and milk and how is this influenced by the F:C ratio fed?*

Niacin flows or concentrations in the body were influenced by NA supplementation as well as by the F:C ratio fed. Niacin flow at the duodenum was enhanced by feeding supplemental NA. But only a relatively small proportion of the NA amount supplemented reached the duodenum, which resulted in a negative value for apparent niacin synthesis in the rumen. Depending on the ration fed, between 88% and 94% of the supplement did not arrive at the duodenal cannula. It is assumed that ruminal degradation of the supplement as well as duodenal absorption before the cannula are the reasons for these results, but the contribution of either factor is unknown.

Differences in F:C ratio of the diet influenced the flow of total niacin and NA at the duodenum, but did not change the flow of NAM. Apparent synthesis was always positive in cows fed unsupplemented rations, hence it can be concluded that net niacin synthesis occurred with all three rations. But it was lowest when a ration with a high forage

proportion (LC) was fed. Hence, ruminal niacin synthesis seems to be less in diets containing high proportions of forage. As apparent synthesis consists of real synthesis and degradation, also degradation may be higher in those diets. But to prove this, the contribution of real synthesis and real degradation to apparent synthesis has to be quantified in further research. In groups supplemented with NA, cows receiving the HC diet had the highest amount of niacin reaching the duodenum and the least negative apparent synthesis. Thus, either degradation of supplemental niacin is less with rations containing higher proportions of concentrate or real synthesis is further enhanced.

Except for one cow, in serum only NAM was found. NAM concentrations in serum increased during niacin supplementation and with higher level of concentrate. It is suggested that serum values follow the processes at the duodenum and reflect an enhanced delivery to the duodenum with an increase in serum concentrations. But since only NAM was found in serum and NA is the main vitamer in duodenal fluid, conversion of NA to NAM must have occurred during or shortly after absorption. It is possible that NA can only be found in blood if very high amounts are given orally or are infused behind the rumen. Investigations of these mechanisms are desirable.

NAM concentrations in milk or amounts daily excreted via milk were not modified by NA supplementation, but showed impacts of level of concentrate. It is surprising that even though NA supplementation enhanced serum concentrations, this did not lead to changes in milk NAM concentrations. The reasons for this are unknown and further research is needed, especially on the mechanism of NAM secretion into milk.

For all measurements of niacin flow or concentrations no interactions occurred between NA supplementation and F:C ratio in the present trial. Hence, responses to supplemental NA are not altered by different F:C ratios fed and NA supplementation does not lead to changes in responses to feeding of diets differing in F:C ratio.

## Summary

Niacin is of great importance in the metabolism. However, former experiments with a niacin supplementation to diets of ruminants have led to inconsistent results. But niacin flows or concentrations in various body fluids are often not determined. This impedes conclusions on metabolic effects of altered niacin concentrations in the body after niacin supplementation.

It was suggested that ruminal niacin synthesis is influenced by the ration fed. But amounts of niacin reaching the duodenum with different diets were studied only twice, with inconsistent results. Furthermore, nothing is known about the impact of the diet composition on the metabolism of supplemental niacin.

Hence, in the present thesis, the effect of a niacin supplementation on various parameters is investigated. Special emphasis is placed on the niacin flow to the duodenum and its concentration in blood and milk. Furthermore, it is assessed if feeding of different diets would alter the responses to supplemental niacin. For these purposes, three dry and seven midlactation (102 ± 18 days in milk at the beginning) fistulated Holstein-Friesian cows were used. At the beginning of the experiment, animals had an average weight of 599 ± 79 kg and lactation numbers ranged from second to fifth lactation. They were equipped with cannulas in the dorsal sac of the rumen and in the proximal duodenum. The diets applied differed in forage-to-concentrate ratio (**F:C ratio**), they consisted of either 1/3 concentrate and 2/3 forage (**LC**), 1/2 concentrate and 1/2 forage (**MC**) or 2/3 concentrate and 1/3 forage (**HC**) on dry matter basis. Each diet was fed in one period without and in the following with a supplementation of 6 g nicotinic acid (**NA**) per cow and day. Ruminal fluid was obtained before and six times after the first morning feeding. Duodenal chyme was collected every two hours for five days. $Cr_2O_3$ was used as flow marker for duodenal flow of nutrients. Blood samples were withdrawn from the *vena jugularis externa* just before and three and six hours after first morning feeding. Milk was sampled on two days from morning and evening milking.

NA supplementation caused several changes in ruminal metabolism. In supplemented animals, rumen ammonia concentration increased significantly, while the short chain fatty acid concentration was decreased. Molar proportion of some minor fatty acids was influenced as well. Significant interactions between NA supplementation and the F:C ratio fed occurred for molar proportion of iso-valeric and iso-butyric acid, but due to the

difficulties in analysing the small amounts of these fatty acids, this should not be over-interpreted.

The amount of organic matter reaching the duodenum was enhanced if niacin was added to the ration. NA supplementation also led to higher flows of microbial crude protein as well as undegraded feed crude protein at the duodenum. Furthermore, efficiency of microbial protein synthesis was enhanced in supplemented animals. But for this variable, a trend for an interaction with F:C ratio occurred. This happened due to the fact that the effect of NA on microbial protein synthesis and efficiency of microbial protein synthesis was highest in HC ration and negligible in MC diet.

The amounts of niacin and NA reaching the duodenum were less in LC diet than with higher concentrate level and rose with NA supplementation. Contrary, the flow of nicotinamide (**NAM**) was not influenced by the F:C ratio, but was enhanced during NA feeding. Concentrate proportion in the diet affected only apparent synthesis of NA and thus total niacin, but not NAM. For these vitamin flows, no interactions occurred between F:C ratio and NA supplementation.

Also in intermediary metabolism several changes were found. NA supplementation enhanced the concentrations of glucose and urea in blood. In contrast to previous expectations, concentrations of non-esterified fatty acids and ß-hydroxybutyrate were unaffected by niacin supplementation. Except for one cow, only NAM was found in serum and concentrations were enhanced with higher proportions of concentrate and addition of NA to the ration. Also in milk, only NAM was detected. NAM concentrations or amounts excreted via milk were not significantly influenced by NA feeding, in spite of higher serum concentrations in supplemented animals. But an effect of F:C ratio was seen. Performance parameters were not significantly altered by niacin supplementation, but a trend towards higher milk protein and milk fat concentrations was observed. Milk fat composition showed a significant effect of additional NA, because the proportion of oleic acid in milk fat augmented in milk of niacin supplemented cows. For all serum and milk variables analysed, no interaction between F:C ratio and NA supplementation was detected, hence the different diets applied did not alter the observed responses to supplemental niacin.

From the present investigation it can thus be seen that feeding 6 g NA per cow and day had several effects on the metabolism of dairy cows. Only few trends or significant interactions with the F:C ratio fed were observed. It may thus be assumed, that diets differing in F:C ratio are in general not altering responses of the cows to supplemental nia-

cin. But this may not be valid for microbial protein synthesis, since trends for an interaction were observed for some parameters related with microbial protein synthesis. Effects of niacin seem to be highest for these variables when high proportions of concentrate are fed, but almost absent if the ration contained an intermediate concentrate proportion. Niacin flows or concentrations in various body fractions are affected by concentrate proportion and NA supplementation. No interactions occurred as well for these parameters, hence it may be concluded that diets with different concentrate proportion do not change effects of supplemental NA on vitamin flows or concentrations.

## Zusammenfassung

Niacin besitzt im Stoffwechsel eine große Bedeutung. Allerdings führten bisherige Versuche mit Niacinzulagen bei Wiederkäuern zu inkonsistenten Ergebnissen. Zudem erfolgte bei diesen Versuchen in der Regel keine simultane Bestimmung der Niacinkonzentrationen in verschiedenen Körperkompartimenten. Eventuelle Konsequenzen aus durch eine Niacinzulage veränderten Niacinflüssen im Tier sind daher schwer abzuschätzen.

In einigen Arbeiten wurde angenommen, dass die ruminale Niacinsynthese von der verfütterten Ration beeinflusst wird. Allerdings gibt es bisher nur zwei Studien, in denen der duodenale Fluss an Niacin bei verschiedenen Rationen verglichen wird, mit unterschiedlichen Ergebnissen. Über die Wirkung von verschiedenen Rationen auf die Metabolisierung einer Niacinzulage ist bisher fast nichts bekannt.

Daher wird in der vorliegenden Arbeit der Effekt einer Niacinzulage auf verschiedene Parameter untersucht. Besonderes Augenmerk liegt dabei auf dem Niacinfluss am Duodenum sowie den Niacinkonzentrationen in Blut und Milch. Außerdem wird analysiert, ob ein Einfluss der Rationsgestaltung auf die Niacinwirkung besteht. Zu diesem Zweck wurden drei trockenstehende und sieben laktierende Holstein-Friesian Kühe im mittleren Laktationsabschnitt (102 ± 18 Laktationstage zu Beginn des Experiments) mit einem Gewicht von 599 ± 79 kg, verwendet. Die Kühe waren doppelt fistuliert, mit einer Fistel im dorsalen Pansensack und einer weiteren im proximalen Duodenum. Die verwendeten Rationen unterschieden sich im Grundfutter-Kraftfutter-Verhältnis (**GF:KF Verhältnis**). Sie bestanden entweder aus 1/3 Kraftfutter und 2/3 Grundfutter (**LC**), 1/2 Kraftfutter und 1/2 Grundfutter (**MC**) oder 2/3 Kraftfutter und 1/3 Grundfutter (**HC**) auf Trockensubstanzbasis. Jede Ration wurde in einer Periode ohne und in der folgenden mit Zulage von 6 g Nicotinsäure (**NA**) pro Tier und Tag verfüttert. Die Entnahme des Pansensafts erfolgte einmal kurz vor der ersten Morgenfütterung und an sechs Zeitpunkten danach, während der Duodenalchymus über fünf Tage in zweistündigen Intervallen gesammelt wurde. Als Flussmarker zur Bestimmung des duodenalen Nährstoffflusses diente $Cr_2O_3$. Blutproben wurden kurz vor, sowie drei und sechs Stunden nach der ersten Morgenfütterung aus der *vena jugularis externa* entnommen. Weiterhin wurden an zwei Tagen Milchproben aus dem Morgen- und Abendgemelk gezogen.

Die NA-Supplementierung bewirkte einige Veränderungen im Pansen. Die ruminale Ammoniak-Konzentration stieg bei den supplementierten Tieren, während die Gesamt-

Konzentration an kurzkettigen Fettsäuren vermindert war. Der molare Anteil an Valeriansäure war geringer bei den Tieren mit NA-Zulage. Es gab signifikante Interaktionen zwischen der NA Supplementierung und dem GF:KF Verhältnis für die molaren Anteile an Iso-Valeriansäure und Iso-Buttersäure. Allerdings sind diese Fettsäuren nur in sehr geringen Anteilen vorhanden, was den Nachweis erschwert und daher nicht überbewertet werden sollte.

Die Niacinzulage erhöhte den Fluss an organischer Masse am Duodenum und verminderte damit die scheinbare ruminale Verdaulichkeit der organischen Masse. Weiterhin führte sie zu höheren Flüssen sowohl an mikrobiellem als auch an unabgebautem Futterrohprotein. Außerdem steigerte sich die Effizienz der mikrobiellen Proteinsynthese bei den mit NA supplementierten Tieren. Für diese Variablen trat allerdings eine tendenzielle Interaktion mit dem verfütterten GF:KF Verhältnis auf, da der Effekt einer NA-Zulage sowohl auf die mikrobielle Proteinsynthese als auch auf deren Effizienz am höchsten bei Fütterung der HC Ration war, während er bei der mittleren Ration sehr gering ausgeprägt war.

Die Mengen sowohl an Gesamt-Niacin als auch an NA am Duodenum waren am geringsten bei der Ration mit niedrigem Kraftfutteranteil und stiegen bei einer NA-Supplementierung an. Der Fluss an Nicotinamid (**NAM**) wurde dagegen nicht durch das GF:KF Verhältnis beeinflusst, er war aber bei Niacinzulage erhöht. Der Kraftfutteranteil der Ration beeinflusste nur die scheinbare Synthese an NA und daher Gesamt-Niacin, aber nicht die von NAM. Für alle beschriebenen Vitaminflüsse wurden keine signifikanten Interaktionen zwischen der Vitaminzulage und dem GF:KF Verhältnis der Ration beobachtet.

Weiterhin zeigten sich auch Effekte der NA-Fütterung im Stoffwechsel. So wurden erhöhte Glucose- und Harnstoffkonzentrationen im Serum der supplementierten Tiere gemessen. Im Gegensatz zu vorherigen Erwartungen waren allerdings weder die Konzentrationen an nicht-veresterten freien Fettsäuren noch die von ß-Hydroxybutyrat durch die Niacinzulage beeinflusst. Mit Ausnahme von einer Kuh konnte im Serum nur NAM nachgewiesen werden. Die NAM Konzentration im Serum steigerte sich mit höheren Kraftfutteranteilen und mit Niacinzulage. Auch in der Milch war nur NAM messbar. Allerdings konnten trotz höherer Serum-NAM-Konzentrationen in den Zulage-Gruppen keine signifikanten Einflüsse der NA-Zulage auf den NAM-Gehalt der Milch beobachtet werden. Dieser wurde aber durch das verwendete GF:KF Verhältnis beeinflusst. Die Niacinzulage zeigte keinen signifikanten Effekt auf verschiedene Kennzah-

len der Milchleistung. Es gab aber sowohl einen Trend zu höheren Milchfett- als auch Milchproteingehalten. Die Milchfettzusammensetzung zeigte einen signifikanten Effekt der Niacinzulage, da der Ölsäureanteil im Milchfett bei den mit NA gefütterten Tieren anstieg. Für alle betrachteten Serum- und Milchparameter konnten keine signifikanten Interaktionen zwischen dem GF:KF Verhältnis und der Niacinzulage ermittelt werden, somit wurden die Effekte einer Niacinsupplementierung nicht durch den Kraftfutteranteil der Ration beeinflusst.

Aus der vorliegenden Untersuchung kann daher gefolgert werden, dass die Verfütterung von 6 g NA pro Kuh und Tag Effekte auf den Stoffwechsel der Kühe hat. Es gab nur wenig tendenzielle oder signifikante Interaktionen mit dem verwendeten GF:KF Verhältnis. Daher kann angenommen werden, dass Rationen verschiedener GF:KF Verhältnisse die Effekte einer Niacinzulage im Allgemeinen nicht beeinflussen. Dies gilt allerdings vermutlich nicht für die mikrobielle Proteinsynthese, da tendenzielle Interaktionen für einige Variablen mit Bezug zur mikrobiellen Proteinsynthese vorlagen. Die positiven Effekte einer Niacinzulage auf diese Parameter sind scheinbar am höchsten, wenn hohe Kraftfutteranteile in der Ration vorhanden sind, während sie bei eher ausgewogenen Rationen nur in sehr geringem Maß auftreten. Niacinflüsse oder Konzentrationen am Duodenum, sowie in Blut und Milch werden durch das GF:KF Verhältnis und die Niacinzulage beeinflusst, aber es waren keine Interaktionen sichtbar. Daher scheinen die Effekte einer Niacinzulage auf die Vitaminflüsse oder Konzentrationen nicht durch das GF:KF Verhältnis beeinflusst zu werden.

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