The effect of a Niacin supplementation to different diets on ruminal fermentation and flow of nutrients to the duodenum 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 ruminal metabolism of dairy cows. The rations consisted of either 2/3 forage and 1/3 concentrate, 1/2 forage and 1/2 concentrate or 1/3 forage and 2/3 concentrate on dry matter basis. Each diet was fed in one period without and in the following period with a supplementation of 6 g niacin (nicotinic acid, NA) per cow and day. Three dry and seven mid-lactating 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 consecutive 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. Furthermore, the efficiency of microbial protein synthesis was enhanced in supplemented animals.

The amounts of total niacin (the sum of NA and NAM) 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.

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

Zusammenfassung

Der Einfluss einer Niacin Zulage zu unterschiedlichen Rationen auf die Pansenfermentation und den Nährstofffluss am Duodenum von Milchkühen

Ziel der Untersuchung war, den Einfluss einer Niacinzulage zu drei Rationen mit unterschiedlichem Grundfutter-Kraftfutter Verhältnis (F:C Verhältnis) auf den Pansenmetabolismus von Milchkühen zu untersuchen. Die Rationen bestanden aus 2/3 Grund- und 1/3 Kraftfutter, 1/2 Grund- und 1/2 Kraftfutter bzw. 1/3 Grund- und 2/3 Kraftfutter auf T-Basis. Jede Ration wurde in einer Periode ohne und in der folgenden mit einer Zulage von 6 g Niacin (Nicotinsäure, NA) pro Tier und Tag verfüttert. Eingesetzt wurden drei trockenstehende und sieben laktierende Holstein–Friesian Kühe im mittleren Laktationsstadium mit Fisteln im dorsalen Pansensack und proximalen Duodenum. Pansensaft wurde vor der Morgenfütterung und zu sechs Zeitpunkten danach, Duodenalchymus über fünf Tage alle zwei Stunden entnommen. Cr_2O_3 wurde als Flussmarker eingesetzt.

Der NA-Zusatz führte zu einem Anstieg der ruminalen Ammoniak-Konzentration, die Gesamtmenge an kurzkettigen Fettsäuren war dagegen vermindert. Die Menge an organischer Masse sowie die Flüsse an mikrobiellem Protein und unabgebautem Futterprotein am Duodenum waren durch den Niacinzusatz erhöht. Außerdem steigerte sich die Effizienz der mikrobiellen Proteinsynthese.

Die Mengen an Gesamt-Niacin (Summe aus NA und NAM) am Duodenum stiegen mit zunehmendem Kraftfutteranteil und bei einer NA-Zulage an, während der Fluss an Nicotinamid lediglich durch eine NA-Zulage und nicht durch das F:C-Verhältnis beeinflusst wurde.

Schlüsselwörter: *Niacin, Grundfutter-Kraftfutter-Verhältnis, Milchkühe, Nicotinsäure*

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1 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).

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 rumen fermentation parameters and the amount of nutrients, especially of niacin, arriving at the duodenum of dairy cows.

2 Materials and Methods

2.1 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 ± 79 kg. Seven cows were in lactation (102 ± 18 days in milk at the beginning), and three were non-lactating. 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. 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. Non-lactating cows were fed restrictedly according to their energy requirement for maintenance.

In six periods 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. Composition of the diets is given in Table 1. 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 period 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 during the evening feeding. In periods without supplemental NA, 100 g of extra mineral and vitamin premix only were given in the same way. The seventh and last period was used to fill gaps in animal number per group.

Table 1
Planned composition of the diets in % (DM basis)

Components	LC[2]	MC[3]	HC[4]
Grass silage	26.7	20.0	13.3
Corn silage	40.0	30.0	20.0
Wheat	8.3	12.5	16.7
Corn	8.3	12.5	16.7
Soybean meal	5.7	8.5	11.3
Peas	5.0	7.5	10.0
Dried sugar beet pulp	5.0	7.5	10.0
Mineral and vitamin premix [1]	0.7	1.0	1.3
Calcium carbonate	0.23	0.35	0.47
Urea	0.10	0.15	0.20

[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 D3 , 1,500 mg alpha tocopherol acetate
[2] LC = low concentrate, 1/3 concentrate, 2/3 forage on DM basis
[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

2.2 Sample Collection

During the first three weeks cows were adapted to the respective concentrate level. Afterwards each period consisted of four weeks: 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. Rumen 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 in the last sampling week, four 100 mL samples were taken through the duodenal cannula at two hour intervals for five consecutive days, beginning at 6:00 h Monday morning until 4:00 h on Saturday morning. Immediately after withdrawal, pH was measured using a glass electrode (pH 525, WTW, Weilheim, Germany). The sample with the lowest pH (Rohr et al., 1984) was added

to the daily pooled sample from each cow and stored at -18 °C. For the calculation of the daily digesta flow, Cr_2O_3 as a flow marker was mixed with wheat flour (ratio 1:4). 50 g were distributed into the rumen every 12 h, beginning 10 d before the duodenal digesta sampling period, whereas one day before and during the sampling period, 25 g were administered every 6 h. In a comparison of measurements of duodenal flow in dairy cows (Rohr et al., 1984), the spot-sampling procedure has shown only small differences in flow as compared to the total collection. During the duodenal digesta sampling week, samples of concentrate and forage as well as occurring feed refusals were collected daily and pooled on a weekly basis. 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 to pass through a 1-mm screen.

2.3 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 by the LUFA Speyer. For the calculation of energy (ME) content, digestibility of the forage was determined in a digestibility trial 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 rumen fluid was determined according to DIN 38406-E5-2 (Anonymous, 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, ash and EE 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-nitrogen (NAN) in duodenal chyme was determined via near infrared spectroscopy according to the method of 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. The daily duodenal DM flow was then used as a measure for the preparation of one pooled sample per cow per week, in which concentrations of CF, NDF, ADF and starch were analysed applying the same methods as 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., 5µm) 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 2M phosphoric acid) and 6 % acetonitrile at a flow rate of 0.5 mL/min. The detection wavelength was 260 nm.

2.4 Calculations and Statistics

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

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

Where DEE is digestible ether extract; DCF digestible crude fibre and DOM digestible OM.

Digestibility values for forage were obtained from the wether digestibility 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:

$$\text{uCP (g/d)} = \text{crude protein flow at the duodenum} - \text{endogenous protein (EP)}$$

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

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

Rumen-degradable protein (RDP), rumen-undegradable 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.

3 Results

3.1 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 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 2, feed analyses were pooled over the course of the study for this calculation.

Table 2
Nutrient composition and energy content of the different rations fed (n = 7)

Nutrients	LC[1]		MC[2]		HC[3]	
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 native niacin concentration of the rations seemed to be nearly the same (Table 2). 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). The arithmetic mean of organic matter intake (OMI) per day was almost equal for all rations and is shown in Table 3 together with the niacin intakes in the respective diets.

Table 3
Daily niacin and organic matter intake of the six experimental groups; arithmetic means, miminum- and maximum-values

	Niacin intake g/d		Organic matter intake, kg/d	
Experimental group	Mean	MIN - MAX	Mean	MIN - MAX
LC [1]	553	222 - 1133	12.1	7.0 - 15.5
LC + NA [2]	6449	6233 - 6839	12.4	7.0 - 15.5
MC [3]	325	177 - 412	12.3	6.7 - 16.3
MC + NA	6337	6178 - 6426	12.6	6.7 - 16.3
HC [4]	476	233 - 838	12.2	6.4 - 16.3
HC + NA	6370	6210 - 6509	12.4	6.4 - 15.6

[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

3.2 Rumen Fermentation Measurements

Because of the scope of this study, the effects of OM intake and time after feeding will not be presented in the following.

The results of ruminal measurements over the whole sampling time are shown in Table 4. 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 rumen fluid ($P < 0.001$). Ammonia concentration increased, whereas valeric acid and SCFA decreased with NA feeding. Interactions with F:C ratio were not significant for these variables.

Iso-valeric acid decreased with the LC, but 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.

Table 4
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 = 9)	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
Propionicacid, 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-butyricacid, 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

Table 5
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 [5] (n = 9)	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

3.3 Nutrient Flow at the Duodenum

Nutrient flows at the duodenum are presented in Tables 5 and 6. OMI influenced most duodenal values, but this will as well not be presented.

The ruminal digestibility of fibre fractions and most measurements of nitrogen metabolism were affected by the F:C ratio. As shown in Table 5, 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 5.

Several effects of niacin supplementation were observed on nitrogen metabolism (Table 6). Even though the proportion of microbial-N in NAN was not influenced by NA, the amount of N at the duodenum and NAN 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 (1,166 g MP without and 1,412 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, and it was 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).

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 only 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

Table 6
N flow at the duodenum and efficiency of microbial protein synthesis; LS MEANS and (standarderror)

	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 [5] (n = 9)	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 [10], 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:FOM ratio [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:ME ratio, 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:RDP ratio, 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 protein
[8] FOM = fermented organic matter
[9] uCP = utilizable crude protein
[10] NAN = non-ammonia-nitrogen

HC ration (0.67 g/g without NA; 0.92 g/g with NA supplementation), whereas values for MC diet differed only marginally.

3.4 Niacin Flow at the Duodenum

The measured niacin flow and calculated apparent synthesis are presented in Table 7. 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, the increasing NA flow led to a significant rise in total niacin ($P < 0.01$). The apparent synthesis was also influenced by the proportion of concentrate and 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 such as the total niacin flow ($P < 0.001$). 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, as disappearance of supplemental niacin was least with HC ration. Calculated from the LS MEANS given in Table 7, 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.

4 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.

Table 7
Duodenal flow and apparent synthesis of niacin; 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 [5] (n = 9)	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

4.1 Rumen

Descriptions of the effects of a niacin supplementation on ruminal ammonia concentration in the literature are miscellaneous. As in this trial (Table 4), 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 the own 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.

Supplementation of 6 g NA did not lead to significant changes in molar proportions of major SCFA (acetic, propionic and butyric acid; Table 4), 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 4). 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.

The decrease in total SCFA concentration in niacin supplemented groups was not expected (Table 4). 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 the own 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). 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).

4.2 Nutrient Digestibility

Apparent ruminal digestibility of nutrients was not significantly influenced by niacin supplementation, except for OM (Table 5). 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 to 60 % concentrate). This is in contrast to the own results, 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 supplementation. 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.

4.3 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 6). 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 the own 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 the own 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 degradation of bacteria by protozoa and thus greater N-recycling inside the rumen (Eugene 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 example 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 6). Furthermore, as we hypothesized an increase in digesta passage after niacin supplementation in the present trial, this may also reduce the protozoal degradation of bacteria. But it was also suggested that an increase in the passage rate directly influences microbial protein synthesis instead of a mediated effect through less degradation 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 6). 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 6) 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).

4.4 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 1,602 mg/d with LC – NA until 2,630 mg/d with HC + NA (Table 7). 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 cows, as in the own experiment, reported higher values (Campbell et al., 1994; Santschi et al., 2005a; Schwab et al., 2006). In these studies, total niacin flow lay between 1,908 mg/d (Schwab et al., 2006) and 2,946 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 1,716 mg/d and 4,902 mg/d in that trial. Hence the own values are low to middle range, compared with those previously found in dairy cows.

In the present experiment, total niacin and NA flow to the duodenum increased with increasing proportion of concentrate, but differences between MC and HC ration were small in unsupplemented groups (Table 7). For total niacin, this increase is consistent with results of Schwab et al. (2006). If the ration contained 35 % instead of 60 % forage, these authors 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. The kind of niacin compound, which is influenced by the ration seems to vary. In the present experiment, NA flow increased with higher concentrate proportions, whereas NAM was not influenced (Table 7). But 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.

Amounts of NA, NAM and thus total niacin at the duodenum rose with vitamin supplementation in all three levels of concentrate (Table 7). Numeric increase after vitamin supplementation was 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 NA and NAM. Campbell et al. (1994) did not detect NAM in duodenal fluid, which disagrees with the own results and those of Kollenkirchen et al. (1992). Santschi et al. (2005a) also stated, that NAM could be detected, if the whole duodenal content is taken.

In the present 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.

The apparent synthesis was calculated by subtracting the amount reaching the duodenum from the intake. No other surveys with a niacin supplementation to diets with different F:C ratios and calculation of apparent synthesis are known. But several studies exist where niacin was added to one ration. As 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 -5,922 mg/d. Furthermore, if these calculations were also done for results of Zinn et al. (1987), where either 0, 200 or 2,000 mg niacin per d were supplemented for feedlot calves, apparent synthesis was 210 mg/d, -60 mg/d or -1,666 mg/d. Thus, substantial amounts of a supplementation disappeared before the duodenal cannula. There seems to be a specific rate of vitamin degradation (plus absorption) and a specific rate of microbial synthesis. There out a specific point and concentration exists where both processes compensate each other resulting in 0 net synthesis. 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).

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 7). However, apparent synthesis was higher with the MC than with the HC diet, due to a lower 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.

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 carbohydrate (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 the own 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 the own 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 the 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. 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.

5 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 the present study with a rumen - protected NA with different F:C ratios in future studies.

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