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on the efficiency of nitrogen utilisation

in the rumen of dairy cows

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Effect of niacin on the efficiency of nitrogen utilisation in the rumen of dairy cows

The aim of the present study was to investigate the effect of an oral niacin supplementation of 6 g per day to a diet deficient in ruminally degradable protein (RDP) on rumen metabolism, microbial protein synthesis, nitrogen (N) balance and N utilisation in lactating cows. It should be assessed to what extend a niacin supplementation can compensate for restricting effects of a negative rumen N balance (RNB) on rumen fermentation.

A total of 9 ruminally and duodenally fistulated lactating multiparous German Holstein cows was used and the diets varied as follows: RNB0 with energy, utilisable crude protein (uCP), and RNB (0.08 g N/MJ ME) according to the average requirement of the animals; RNB- with energy and uCP at the duodenum according to the average requirement of the animals, but with a negative RNB (-0.41 g N/MJ ME); and diet NA, which was the same ration as RNB-, but supplemented with 6 g niacin/d.

Reducing the amount of RDP in the diet caused several effects in the N metabolism of the animals. The negative RNB reduced N excretion with urine, the total N excreted with urine and faeces, and the N balance. Plasma and milk urea content were lower as well as ammonia content in rumen fluid. Also the digestibility of the diet, in particular NDF, was reduced. Number of protozoa in rumen fluid was enhanced while the amount of microbial crude protein (MP) and the amount of uCP reaching the duodenum declined during N deficiency, but the use efficiency of N for MP synthesis and for milk production was higher.

Supplemental niacin decreased the daily N excretion with faeces and elevated the N balance. No effects on milk yield and composition were observed, but the ammonia content in rumen fluid was higher. Addition of niacin could compensate for the decline in NDF digestibility. The number of protozoa in rumen fluid was higher in NA treatment as compared to RNB-. The amount of MP reaching the duodenum per day was unaffected by niacin administration, but the efficiency of MP synthesis from RDP was elevated compared to RNB-.

In conclusion, supplemental niacin to diets with a negative RNB induced a more efficient use of rumen degradable N. A shift in the rumen microbial community which was mainly due to an increased number of protozoa may have led to modifications of rumen fermentation and changes in the composition of MP reaching the duodenum.

Effekte von Niacin auf die Effizienz der Stickstoffausnutzung im Pansen von Milchkühen

Das Ziel der vorliegenden Arbeit war es, die Effekte einer oralen Niacingabe von 6 g zu einer Ration mit negativer ruminaler Stickstoff (N)-Bilanz (RNB) auf den Pansenmetabolismus, die mikrobielle Proteinsynthese, die N-Bilanz und die N-Verwertung bei laktierenden Milchkühen zu untersuchen und zu bewerten, in wieweit die Niacinsupplemetation die restriktiven Effekte einer negativen RNB auf die Pansenfermentation kompensieren kann.

Die Studie wurde mit insgesamt neun pansen- und dünndarmfistulierten laktierenden Deutschen Holstein Kühen durchgeführt. Die gefütterten Rationen waren wie folgt konzipiert: RNB0: Energiegehalt und Menge an nutzbarem Rohprotein (nXP) entsprechend dem Bedarf der Tiere und eine ausgeglichene RNB (0,08 g N/MJ ME); RNB-: Energie- und nXP-Gehalt entsprechend dem Bedarf der Tiere, jedoch mit negativer RNB (-0,41 g N/MJ ME); NA: Ration identisch mit RNB-, jedoch mit zusätzlicher Gabe von 6 g Niacin je Tier und Tag.

Die Reduktion der Menge pansenabbaubaren Proteins (RDP) in der Ration RNB- hatte diverse Effekte auf dem Metabolismus der Tiere. Die negative RNB reduzierte die N-Ausscheidung mit dem Harn, sowie die Gesamt-N-Ausscheidung und die N-Bilanz. Plasma- und Milchharnstoff waren verringert, gleiches galt für den Ammoniakgehalt im Pansensaft. Auch die ruminale und die Gesamttrakt-Verdaulichkeit der Ration, insbesondere der NDF-Fraktion, waren herabgesetzt. Während die Protozoenkonzentration im Pansensaft erhöht war, wurde die Menge mikrobiellen Rohproteins am Dünndarm (MP), sowie die Menge nXP durch den N-Mangel reduziert. Allerdings war die Nutzungseffizienz des N für die mikrobielle Synthese und die Milchproduktion erhöht.

Der Zusatz von Niacin verringerte die tägliche N-Ausscheidung mit dem Kot und erhöhte die N-Bilanz. Der Ammoniakgehalt im Pansensaft war höher aber es gab keine Effekte auf Milchmenge und -zusammensetzung. Die Niacingabe konnte die reduzierenden Effekte der negativen RNB auf die Verdaulichkeit der NDF kompensieren. Die Anzahl Protozoen im Pansensaft wurde durch Niacin erhöht, die tägliche Menge MP blieb unbeeinflusst. Jedoch stieg die Effizienz der MP-Synthese aus RDP im Vergleich zur Behandlung RNB-.

Aus den vorliegenden Ergebnissen kann geschlussfolgert werden, dass der Niacin-Zusatz von 6 g je Tier und Tag zu einer Ration mit negativer RNB eine effizientere Nutzung des RDP zur Folge hat. Verschiebungen in der mikrobiellen Population des Pansens auf Grund einer höheren Protozoenkonzentration könnten zu Modifizierungen des Pansenmetabolismus und zu einer veränderten Zusammensetzung des MP geführt haben.

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Abbreviations

ADF	Acid detergent fibre, expressed without residual ash
ARD	Apparent ruminal digestibility
ash	Crude ash
BHB	β-Hydroxybutyrate
C0	Cobalt concentration at time of dosing
CF	Crude fibre
CP	Crude protein
Ct	Cobalt concentration at time t
d	Day
DCF	Digestible crude fibre
DEE	Digestible ether extract
deg	Theoretical degradability *in sacco* (French protein evaluation system)
DM	Dry matter
DMF	Dry matter flow
DMTP	Digestible microbial true protein (UK protein evaluation system)
DOM	Digestible organic matter
DUP	Digestible undegradable protein (UK protein evaluation system)
DVE	Digestible true protein (Dutch protein evaluation system)
EE	Ether extract
EP	Endogenous protein
FCM	Fat corrected milk
FOM	Fermented organic matter
k	Elimination constant
LSMeans	Least square means
ME	Metabolisable energy
MNE	Milk nitrogen efficiency
MP	Microbial crude protein
N	Nitrogen
NA	Treatment “niacin”

NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fibre
nXP	Nutzbares Rohprotein
OEB	Degraded protein balance (Dutch protein evaluation system)
OM	Organic matter
PBV_N	Protein balance in the rumen (NorFor protein evaluation system)
PDIA	Truly digestible rumen undegradable protein (French protein evaluation system)
PDIE	Protein value, calculated from energy (French protein evaluation system)
PDIME	Microbial true protein calculated from energy (French protein evaluation system)
PDIMN	Microbial true protein calculated from rumen degradable protein (French protein evaluation system)
PDIN	Protein value, calculated from rumen degradable protein (French protein evaluation system)
RDP	Rumen degradable protein
rd_cp	Degradation of crude protein (NorFor protein evaluation system)
REML	Restricted maximum likelihood
r_mcp	Microbial crude protein flow out of the rumen (NorFor protein evaluation system)
RNB	Rumen nitrogen balance
RNB-	Treatment RNB-
RNB0	Treatment RNB0
RUP	Rumen undegradable protein
SCFA	Short chain fatty acids
SD	Standard deviation
SEM	Standard error of means
t	Time
uCP	Utilisable crude protein

Chapter 1: General introduction and review of literature

Environmental impact of nitrogen originating from animal production, especially from ruminants

The demand of consumers for more animal products of higher quality is rising constantly and this trend will continue during the next years. While in the affluent societies of the industrial countries animal foodstuffs produced with the highest standards of animal welfare, quality, and hygiene throughout the production chain are desired, the ever-increasing human population in emerging and developing countries will lead to an increasing demand for foodstuffs from animal origin in general (Flachowsky, 2011). These processes will be accompanied by further intensifying of livestock production systems like increasing stocking rates. Therefore, sustainability of animal production, especially the protection against detrimental effects on the environment, is focused by research and policy.

Besides the emission of methane and phosphorus, nitrogen (N) emissions in form of nitrate (NO_3), nitrous oxide (N_2O), and ammonia are directly linked to meat and milk production. The main sources of N input into the environment by farms are manure and fertilizers. Urea, which is the main form of N in manure, is quickly converted into ammonia after excretion because of the presence of urease in the environment (Tamminga, 1992). Ammonia leads to eutrophication of nutrient poor ecosystems and surface water and causes acidification of soil. Under aerobic conditions, ammonia is converted into nitrate, which pollutes groundwater. In the absence of oxygen, as occurring in the deeper layers of the ground, NO_3 is converted into nitrite (NO_2) and subsequently into gaseous N_2 by bacterial denitrification. During this process, N_2O can be formed, which is harmful to the ozone layer (Hristov et al., 2011).

Ruminants are inefficient in converting dietary N into milk and meat protein. On average, in cattle the N use efficiency, which characterizes the amount of N converted into meat- or milk-N per unit of N intake, is approximately 30% (Tamminga, 1992). Kalscheur et al. (2006) reported a milk N efficiency (MNE) in dairy cows between 28 and 35%. Huhtanen and Hristov (2009) estimated the MNE to be on average up to only 28% with a large variation between 16% and 40%. Hence, in intensive feeding systems about 72% of the N ingested by dairy cows is excreted with urine and faeces. As shown by several authors

(Swensson, 2003; Colmenero and Broderick, 2006; Burgos et al., 2007), urinary N losses are positively correlated to the crude protein (CP) content of the respective diet while the N losses with faeces are rather constant (NRC, 2001) and therefore less sensitive to varying N contents of the diet (Aarts et al., 1992; Broderick, 2003). Jonker et al. (2002) investigated the effect of a CP supply of 107% compared to 100% of NRC recommendations and observed a 16% increase in urinary N excretion, whereas N excretion with faeces was only enhanced by 3%. The reduction of N intake provides a possibility to lower the N excretion with manure (Tamminga, 1992) and therefore the volatile N losses from livestock facilities (Frank et al., 2002). Additionally, in a meta-analysis including a wide range of milk performance levels and a huge variety of diets with different CP contents, Huhtanen and Hristov (2009) showed that in general reduced N intakes lead to higher MNE.

Not only the reduction of CP in the diet, but more precise the reduction of ruminally degradable CP (RDP) can provide a possibility to decrease N losses from dairy cows. According to NRC (2001), N excretion with urine can be expressed with a linear relationship to RDP intake as follows: Urinary N excretion [g/d] = (RDP supply [g/d] · 0.0628) + 55.6.

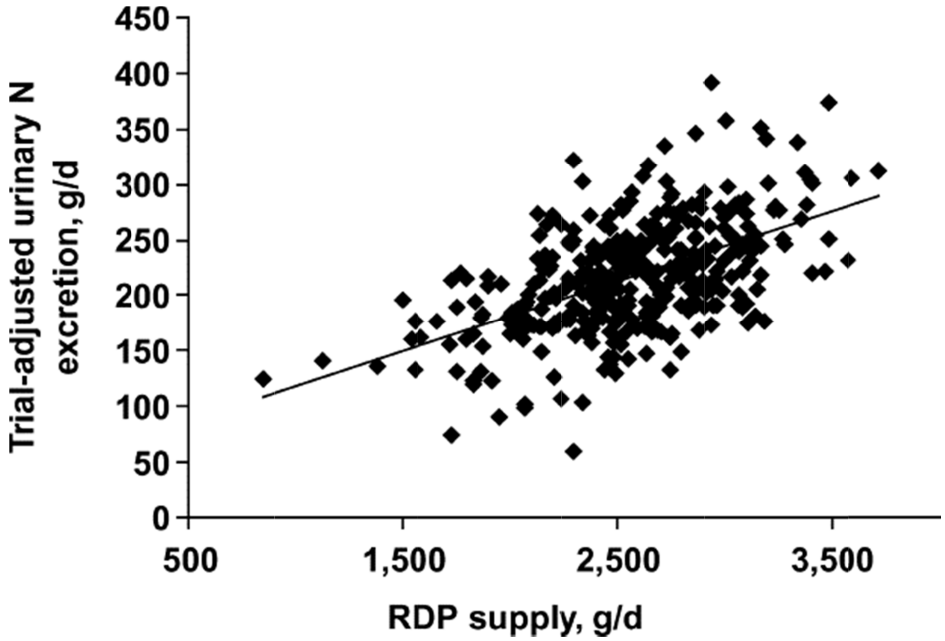

Figure 1: Relationship between the supply with rumen degradable protein (RDP, g/d) and urinary nitrogen (N) excretion [g/d] for lactating cows (n = 372; Nennich et al., 2006). The solid line is equal to urinary N excretion as calculated by NRC (2001): Urinary N excretion [g/d] = (RDP supply [g/d] · 0.0628) + 55.6.

In a meta-analysis of 16 N balance trials with lactating dairy cows, Nennich et al. (2006) confirmed this positive correlation (Figure 1) and van Duinkerken et al. (2005) observed a linear relationship between the RDP supply to lactating cows and emissions of ammonia from dairy cow houses.

Effects of feeding reduced amounts of rumen degradable protein as an approach for reducing N excretions by ruminants

Generally, diets for ruminants have to cover the N requirement of the microbial population in the rumen and meet the amino acids requirement of the host animal (Schwab et al., 2005). The effects of a reduction of RDP in diets for cattle on rumen metabolism and the production performance were investigated intensively during the last few years.

The effects of reduced amounts of RDP on milk performance reported in the literature are inconsistent. In the study of Canfield et al. (1990), milk yield decreased when the diet was calculated to have 9.2% RDP and Cyriac (2009) found a trend for a decreased milk yield in an experiment with ruminally and duodenally fistulated cows feeding a diet containing 7.6% RDP. In contrast, Gressley and Armentano (2007) and Agle et al. (2010) found no effect after feeding a diet calculated to have 7.4% or 7.1% RDP, respectively, according to the NRC (2001) model. Milk composition, except urea-N in milk, was unaffected by RDP in most of the trials investigating different levels of RDP (Armentano et al., 1993; Kluth et al., 2003; Geerts et al., 2004), but Reynal and Broderick (2005) observed reduced milk protein values after feeding a diet with a measured RDP content of 11.7% compared to a diet with 13.2% RDP.

The major fibre digesting microbes in the rumen are the bacterial genera *Ruminococcus*, *Fibrobacter* and *Butyrivibrio* as well as some species of *Eubacterium* and *Clostridium* (Dehority, 2003). These bacterial groups are eminently sensitive to an ammonia-N deficiency in the rumen (McAllan and Smith, 1974). Thus, feeding RDP below requirements can compromise ruminal digestion of carbohydrates, in particular NDF, as shown *in vitro* (Nagadi et al., 2000; Griswold et al., 2003) and *in situ* (Erdman et al., 1986; Caton et al., 1988). In *in vivo* studies, a calculated content of 7.4% RDP in DM was found to be sufficient for ruminal degradation of NDF, but the digestion of starch was reduced (Gressley and Armentano, 2007). In another experiment, Lebzien et al. (2006) showed that the amount of NDF fermented in the rumen was significantly decreased when the rumen

nitrogen balance (RNB) in the fed diet became negative (-0.3 or -0.6 g N/MJ ME). Consequently, passage rate and DM intake can also be influenced by low levels of RDP in the diet. A shortage of RDP decreased feed intake in beef cattle (Wheeler et al., 2002) and sheep (Mehrez and Ørskov, 1978). Reynal and Broderick (2005) observed a lower feed intake when the measured RDP supply was reduced from 11.7% to 10.6% of DM in diets for dairy cows. Cyriac (2009) also found a linear decline in DM intake when RDP content in the diet decreased from 11.3% to 7.6%, while the content of ruminally undegraded CP (RUP) was unchanged. The author suggested that the decreased dietary RDP content may not have met the N requirement of rumen microbes and therefore was not adequate for maintaining DM intake (Cyriac, 2009).

When N for the microbial synthesis was limited due to reduced intake of RDP, the microbial CP flow at the duodenum was decreased in beef cattle (Martín-Orúe et al., 2000), sheep (Chandrasekharaiah et al., 2011), and dairy cows (Aldrich et al., 1993). Nevertheless, at a feed intake of about 15 kg DM/d of lactating dairy cows, Riemeier (2004) found only a low correlation between rumen nitrogen balance and microbial CP synthesis ($r^2 = 0.1$). *In vitro* studies showed that an ammonia-N concentration of 50 mg/L in ruminal fluid is needed for optimal microbial growth of mixed rumen fluid associated bacteria (Satter and Slyter, 1974), but nothing is gained by further supplementation of RDP, whereas particle associated bacteria may need higher ammonia concentrations in rumen fluid (McAllan and Smith, 1983).

Estimation of the N requirements of rumen microbes and the host animal

In light of these inconsistent results from literature, the importance of further understanding the requirements of microbes and host animals for RDP becomes clear. The different national protein evaluation systems for dairy cows in Europe predict different requirements for RDP based on different assumptions to calculate the variables of N supply to the rumen microbial population.

In the German system (GfE, 2001), the protein supply for the host animal is calculated using estimated equations and expressed as utilisable crude protein at the duodenum (uCP, in German nutzbares Rohprotein am Duodenum, nXP), which is the sum of RUP and microbial crude protein. Contrary to other systems, in the estimated equations for nXP, microbial crude protein and RUP are not calculated separately. To characterize the N

supply to the rumen microbes, the German system uses the RNB which is defined as: RNB [g/d] = (CP intake [g/d] - uCP [g/d])/6.25. The GfE (2001) recommended a balanced RNB in diets for lactating dairy cows, but a positive RNB up to 50 g/d was considered as tolerable. Although, the German system is relatively simple compared to others, in a study by Schwab et al. (2005) it predicted milk protein yield better than most of the other systems using feed-specific degradability and digestibility values of UDP.

For characterizing the protein supply to the animal, the French PDI system (INRA, 1989) calculates two individual protein values for each diet or feedstuff, using either RDP (PDIN) or energy (PDIE) as limiting factor for the microbial synthesis. The PDIN is calculated as: PDIN = PDIA + PDIMN and the PDIE = PDIA + PDIME, where PDIA is the truly digestible RUP, PDIMN is the amount of microbial true protein calculated from RDP, and PDIME is the amount of microbial true protein calculated from energy. The lower of the two protein values is used for calculations when the feedstuff is fed alone and the higher value reflects the potential amount of microbial CP that can be obtained if it is fed together with appropriate other feedstuffs. The calculated amount of microbial CP based on the supply with RDP is calculated as: $CP \cdot [1 - 1.11 \cdot (1 - deg)] \cdot 0.9$, whereby deg is the theoretical degradability *in sacco* and it is assumed that the rumen microbes can use 90% of RDP for protein synthesis.

The Dutch DVE system is based on the French PDI system (Tamminga et al., 2007). Each feed has a DVE value, expressing the protein supply to the host animal, composed of the digestible true protein contributed by RUP, microbial CP synthesized in the rumen and a correction for endogenous CP losses in the digestive tract. Each feed also has a degraded protein balance (OEB) reflecting the (im)balance between microbial protein synthesis potentially possible from RDP and that potentially possible from the energy obtained by anaerobic fermentation in the rumen. The recommended optimum for the OEB value in a diet is therefore zero or slightly above.

In the UK system “Feed into milk” (Thomas, 2004), the amount of metabolisable protein for the host animal consists of digestible undegradable protein (DUP) and digestible microbial true protein (DMTP). The DUP is defined as RUP corrected for acid detergent N. Furthermore, it is assumed that 10% of RUP are theoretically rumen degradable. To determine the supply with microbial CP, for each feed in the diet the potential amount of microbial CP arising from ATP and the potential amount of microbial CP arising from effectively degraded N is determined and summed up to get the respective value for the

whole diet. The lower of these two values is used to calculate DMTP. For the calculation of DMTP, it is assumed that the true protein content of microbial CP is 75% and that the digestibility of true protein is 85%.

In the Nordic countries, the NorFor-system (Volden, 2011) is used to predict N supply to the microbes and the host animal. In NorFor, the protein balance in the rumen (PBV_N) is used to evaluate the adequacy of protein supply for microbial growth and is calculated as: PBV_N = rd_CP + (CP intake · 0.046) – r_mcp, whereby rd_CP is the degradation of CP, 0.046 is the proportion of dietary protein (N · 6.25) recycling back to the rumen, and r_mcp is the microbial CP flow out of the rumen. The supply with metabolisable protein for the animal is calculated as the amount of amino acids absorbed from the small intestine. This value is composed of dietary, microbial, and endogenous amino acids digested in the small intestine. To estimate the microbial amino acid supply from the rumen, the efficiency of microbial protein synthesis, the degradation of starch, NDF, carbohydrates, crude fat and CP corrected for urea and ammonia as well as the liberation of feed fermentation products is taken into account.

An essential difference between the above described systems is the consideration of urea recycling in the rumen as N source for the microbes. While in the German system it is assumed that up to 20% of the N requirements for the microbial protein synthesis can be covered by recycled urea N (GfE, 2001), the PDI system in France calculates with 0% to 9% of recycled urea N for protein synthesis in the rumen (INRA, 1989) and in NorFor it is assumed that 4.6% of the dietary N is recycling back to the rumen. In the Dutch DVE system (Tamminga et al., 2007) it is assumed, that between 175 and 280 g of RDP are provided by urea recycling per day. In the Feed into milk system (Thomas, 2004) N reaching the rumen via the rumino-hepatic cycle is not considered. The disregard of urea recycling may lead to significant overestimation of RDP requirements, as shown by Huhtanen and Hristov (2009).

Approaches to manipulate rumen microbial fermentation to increase the use efficiency of nitrogen in ruminants

Several attempts have been undertaken to reduce the N excretion with manure by increasing the efficiency of microbial protein synthesis or modifying the N metabolism in the rumen. Hereafter, some of the most frequently investigated and promising approaches will be addressed.

Synchronizing the availability of N and energy in the rumen was considered as a possibility to improve efficiency of ruminal fermentation (Cabrita et al., 2006; Chumpawadee et al., 2006). In former studies, it was already stated that this strategy may support the establishment of a more consistent fermentation with less diurnal variation (Satter and Baumgardt, 1962) and therefore promote an improved N utilization (Baldwin and Denham, 1979). Sinclair et al. (1993) established an equation for a synchronicity index on an hourly basis and recommended an amount of 25 g N/kg truly rumen digested OM as optimum relation between the supply with N and energy. To achieve this synchronization, feeding management strategies like variation of feeding frequency (Chen et al., 1987; Cecava et al., 1990; Shabi et al., 1998; Thivierge et al., 2002) as well as the combination of carbohydrate (Johnson, 1976; Gozho and Mutsvangwa, 2008; Oba, 2011) and protein sources with different degradabilities (Belasco, 1954; Ahn and Moon, 1990; Froidmont et al., 2009; Kozloski et al., 2009; Budag and Bolat, 2010) have been investigated during the last decades in ruminant nutrition.

Besides dietary composition and feeding management, numerous chemical substances have been tested as supplements to diets for dairy and beef cattle during the last decades to manipulate protein metabolism in the rumen and to improve N utilisation. Antibiotic ionophores like monensin presented a very successful opportunity to reduce protein and energy losses in the rumen, but the use of antibiotics in animal diets is forbidden in the EU since the beginning of 2006 (European Commission, 2003) and furthermore the acceptance for antibiotics in animal nutrition is continuously shrinking among the public.

During the last years, the use of phytochemical substances like essential oils, tannins, or saponins have been focused by research to modify the ruminal fermentation and to improve the use efficiency of nutrients (Calsamiglia et al., 2007).

Essential oils are steam-volatile or organic solvent extracts of plants. The main effects of essential oils in the rumen include the reduction of degradation of protein and starch from the diet and an inhibition of degradation of amino acids, based on selective action on certain rumen bacteria (Hart et al., 2008). One mode of action assumed for essential oils is therefore an effect on the bacterial colonisation of feedstuffs as they enter the rumen, in particular starch rich components (Patra and Saxena, 2009a). Also, an inhibition of hyper ammonia producing bacteria involved in amino acid deamination was observed after the supplementation of diets with essential oils (Wallace et al., 2002). A large variety of essential oils was studied including garlic oil, cinnamaldehyde (active component of

cinnamon oil), and anethol (active component of anise oil) as well as the phenolic substances thymol (active component of thyme oil), eugenol (active component of the clove bud), and capsaicin (active component of hot peppers). Results indicated that garlic oil, cinnamaldehyde, eugenol, capsaicin, and anethol modified proteolysis, peptidolysis, or deamination in the rumen as summarized by Calsamiglia et al. (2007). Problematically, there was considerable variation in the content of active compounds in the extracts used in different studies because of variety in the cultivated plants, growing conditions, and processing methods and therefore, the comparability of results from literature concerning the effects on rumen metabolism is limited (Calsamiglia et al., 2007). Furthermore, after studying a blend of essential oils containing among others thymol and eugenol, McIntosh et al. (2003) suggested that the effective concentration of biologically active compounds in *in vitro* experiments to affect the protein metabolism should be higher than 35 g/L of rumen fluid. That level of supplementation would be difficult to achieve under *in vivo* conditions. Besides this lack of clarity concerning the dosage, in several studies the observed effects of essential oils within 24 to 48 h after supplementation were not always confirmed in long-term tests (Szumacher-Strabel and Cieslak, 2010). It was suspected that the rumen microbial population may adapt to the essential oils already after 6-7 days of supplementation (Busquet et al., 2005; Benchaar et al., 2008a).

Altering the rumen microbial ecosystem by removal of protozoa from the rumen (defaunation) inhibits the predation of rumen bacteria by protozoa and therefore enhances the efficiency of bacterial protein synthesis (Jouany and Ushida, 1998). At present it is difficult to achieve total defaunation because no practical method has been developed to date to eliminate protozoa efficiently and safely (Teferedegne, 2000). Saponins are known to be the most effective naturally occurring substance in plants with the potential to limit the number of protozoa in the rumen and hence to increase the efficiency of microbial protein synthesis and the protein flow to the duodenum (Szumacher-Strabel and Cieslak, 2010). Yet, the effects of saponins on rumen fermentation have not been found to be consistent (Shete et al., 2011). Lu and Jorgensen (1987) observed a decrease in bacterial nitrogen flow to the duodenum after intra ruminal administration of alfalfa saponins (2 or 4% of DM intake) in sheep, whereas Klita et al. (1996) reported increased microbial flow after the same dose and source of saponins. These discrepancies seem to be based on the chemical structure of saponins, the diet composition as well as the microbial population and its adaptation to saponins in the diet (Patra and Saxena, 2009b).

Tannins are able to form stable complexes with proteins. These complexes are resistant to microbial degradation and therefore tannins may provide a possibility to interfere the rumen protein metabolism (Patra and Saxena, 2009a). However, their effectiveness in altering rumen metabolism was not consistent in experimental studies. Microbial protein synthesis observed *in vitro* was increased after supplementation of quebracho tannins (50 or 100 g/kg DM), but tannic acid in the same concentration had no effect (Getachew et al., 2008). Contrary, another *in vitro* study showed that tannic acid even at lower concentrations (0.1%) increased bacterial growth (Hristov et al., 2003). Results of *in vivo* studies are considerable scarcer and the effect of tannins remained unclear. A tannin concentration of 3% of DM (McSweeney et al., 1998) as well as a pure Leucaena hybrid diet containing 11.6% tannins (McNeill et al., 2000) had no effect on microbial protein flow in sheep. Similar to the saponins, the lack of effects of tannin supplementation *in vivo* may be due to the adaptation of microbial population to tannins in the diet during the experimental period or to bacterial degradation of tannins (Benchaar et al., 2008b).

Another possibility to influence the composition of the microbial population and thus the ruminal fermentation, is the addition of fungi or live yeast as probiotics to diets for ruminants. *Saccharomyces cerevisiae* has been used extensively as feed additive for ruminants, especially for dairy cattle (McAllister et al., 2011). From a meta-analysis Desnoyers et al. (2009) concluded that supplemental *Saccharomyces cerevisiae* increased dry matter intake, rumen pH, concentration of volatile fatty acids in rumen fluid and organic matter digestibility. Yeast contains micronutrients that may stimulate the growth of the microbial population (Robinson and Erasmus, 2009) and by utilising small amounts of oxygen occurring in the rumen it has the ability to establish an environment that is more beneficial for anaerobic rumen bacteria (Jouany et al. 1999). Hence, *Saccharomyces cerevisiae* may lead to an incremented bacterial population and cause shifts in rumen bacterial populations towards fibrolytic rumen bacteria, which would result in improved ruminal fiber digestion and higher use efficiency of nutrients (McAllister et al., 2011).

Lately, additional long-term *in vivo* experiments with defined doses of active compounds have to be carried out and the potential adaptation of the rumen microbial population as well as their ability for degradation of these compounds has to be considered.

Niacin in ruminant nutrition

The B-vitamin niacin is part of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) and therefore it is involved in several energy providing processes in the metabolism (Bender, 1992). Ruminants can obtain niacin to cover their requirements from feedstuffs, from the enzymatic conversion of tryptophan and quinolinic acid into niacin (Bender, 1992), and from the microbial synthesis in the rumen (Abdouli and Schaefer, 1986). The niacin content in feedstuffs for ruminants can vary widely. According to the Subcommittee on Feed Composition (1982), niacin content of cereal grains varied between 64 mg/kg DM in wheat and 83 mg/kg DM in barley. Variation in commonly used protein feedstuffs in concentrates for cattle was much higher as solvent-extracted soybean meal contained 31 mg/kg DM and postextraction rapeseed meal 161 mg/kg DM. Niacin content in maize silage was assumed to be 47 mg/kg DM (Subcommittee on Feed Composition, 1982).

The formation of niacin from tryptophan is ineffective since 50-60 mg of tryptophan is needed for 1 mg of niacin (Dreosti, 1984). Therefore, it can be assumed that tryptophan is only used for niacin formation beyond the need for protein synthesis.

Since the 1940s, it is known that the bacterial population in the rumen is able to synthesize niacin (Menke, 1973). The rate of synthesis was estimated to be 1804 mg per day (NRC, 2001). Further, the NRC (2001) estimated the niacin requirement of a lactating cow with 650 kg body weight and 35 kg milk yield to be 289 mg per day. From these calculations it seems to be obvious that microbial synthesis of niacin would exceed the requirements many times over. Hence, it was assumed that the sum of niacin naturally occurring in feedstuffs and niacin synthesized by the rumen microbes would meet the requirements of the animal (GfE, 2001; NRC, 2001). However, the requirements have not been determined experimentally, but derived from the data available for lactating sows (NRC, 2001). Furthermore, ruminal niacin synthesis varied when different diets were applied (Niehoff, 2009).

Although, it was supposed that rumen bacteria are able to synthesis niacin, studies showed that bacteria from the genera *Streptococcus* required niacin or that additional niacin had stimulatory effects on them (Dehority, 2003). The protozoa in the rumen are generally unable to synthesize water-soluble vitamins (Brent and Bartley, 1984) and thus they have been shown to benefit from the supplementation of niacin *in vitro* (Erickson et al., 1990) as well as *in vivo* (Doreau and Ottou, 1996). In more recent *in vivo* experiments with

buffaloes (Kumar and Dass, 2005) and dairy cows (Niehoff, 2009) it was demonstrated that the supplementation of diets with niacin changed the ruminal fermentation pattern and increased the flow of microbial CP to the duodenum. Thus, the supplementation of RDP-deficient diets with niacin may modify the rumen metabolism and enhance the use efficiency of RDP and therefore provide a possibility to lower the N excretion with manure while simultaneously maintaining the uCP supply to the host animal.

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Chapter 2: Scope of the thesis

In consideration of the depicted diverse effects of reduced supply of ruminants with ruminally degraded crude protein (RDP) and having regard to the expected effects of addition of niacin to diets for cattle, the aim of this thesis was to examine the effects of niacin supplemented to a diet deficient in RDP for dairy cows.

Therefore, an animal experiment with fistulated lactating German Holstein cows was conducted and the results are presented in chapters three and four. These chapters comprise manuscripts which have been submitted for publication in scientific journals.

Chapter three focuses the environmental aspect of feeding reduced amounts of RDP and niacin. The effects of a reduced intake of RDP and a niacin supplementation of 6 g/(cow · day) on the nitrogen balance and overall nitrogen use efficiency, expressed as milk nitrogen relative to nitrogen intake, were investigated. Therefore, the nitrogen excretion with urine, faeces and milk were determined. Milk composition, in particular milk fat, milk protein, and milk urea content were evaluated. Several blood variables as well as the total tract nutrient digestibility were measured. The reduced RDP supply was expected to reduce nitrogen excretion and to enhance the nitrogen use efficiency, but also an interfering effect on milk performance may have been expected. The potential of supplemental niacin to compensate for these impairing effects of a reduced nitrogen supply to the rumen was studied in this chapter.

In chapter four the effect of a reduced RDP supply and supplemental niacin on rumen metabolism is emphasized. Hence, rumen fermentation variables were measured and ruminal digestibility of fibre fractions as well as rumen liquid turnover and the nutrient flow to the small intestine were estimated. The microbial crude protein synthesis was assessed and the microbial community, in particular the protozoal population, was investigated. As a reduced nitrogen supply for the rumen microbes decreases the microbial protein synthesis, the supplementation of niacin to the diet was evaluated as a possibility to compensate for this decline and to improve the use efficiency of nitrogen in the rumen.

In chapter five general conclusions are drawn to work out the capabilities for the use of supplemental niacin to compensate for the effects of a reduced amount of RDP in diets for lactating dairy cows.

Chapter 3

Effect of niacin supplementation on digestibility, nitrogen utilisation and milk and blood variables in lactating dairy cows fed a diet with a negative rumen nitrogen balance

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Abstract

The aim of the present experiment was to determine if a niacin supplementation of 6 g/d to lactating dairy cow diets can compensate negative effects of a rumen nitrogen balance (RNB) deficit. A total of 9 ruminally and duodenally fistulated lactating multiparous German Holstein cows were successively assigned to one of three diets consisting of 10 kg maize silage (dry matter, DM, basis) and 7 kg DM concentrate: RNB- (n = 6) with energy and utilisable crude protein at the duodenum (uCP) according to the average requirement of the animals, but with a negative RNB (-0.41 g N/MJ metabolisable energy (ME)); RNB0 (n = 7) with energy, uCP at the duodenum, and a RNB (0.08 g N/MJ ME) according to the average requirement of the animals and, finally, diet NA (nicotinic acid; n = 5), which was the same diet as RNB-, but supplemented with 6 g niacin/d. Samples of milk were taken on two consecutive days, blood samples were taken on one day pre- and post-feeding and faeces and urine were collected completely over five consecutive days. The negative RNB reduced milk and blood urea content and apparent total tract digestibility of DM, organic matter, and neutral detergent fibre (NDF). Also N excretion with urine, the total N excreted with urine and faeces, and the N balance were reduced when the RNB was negative. Supplementation of niacin elevated plasma glucose concentration after feeding and the N balance increased. Supplementing the diet with a negative RNB with niacin led to a more efficient use of dietary N thereby avoiding the negative effects of the negative RNB on the digestibility of DM, organic matter and NDF.

Keywords: niacin, rumen nitrogen balance, nitrogen excretion, nitrogen utilisation, digestibility

Introduction

Nitrogen (N) emissions from animal husbandry lead to rising pollution of groundwater and air. In particular, ruminants cause a major proportion of these N emissions. On average, the efficiency of use of dietary N in cattle amounts only to 23% (Kohn et al., 2005). Hence, improving the N use efficiency in ruminants is a point of focus in research.

Rumen metabolism has been identified as the most important factor contributing to the inefficient use of N in ruminants (Tamminga, 1992). Because N excretion in manure is strongly correlated with N intake, one way to lower N excretion and therefore to enhance the N efficiency is the reduction of the amount of rumen degradable N in diets of dairy cows (Burgos et al., 2007), but a shortage in N supply for the microbes results in a decreased ruminal fermentation (Lebzien et al., 2006). It was stated that maximum efficiency of N utilisation would only occur at the expense of some losses in production performance (Calsamiglia et al., 2010).

Niacin is of great importance in the metabolism of humans and animals. Apart from feedstuffs as a niacin source and niacin synthesis from tryptophan, ruminants can use the niacin synthesised by several species of rumen microbes (Niehoff et al., 2009a). Nevertheless, studies showed that an additional oral niacin supplementation improved the energy balance of high yielding lactating dairy cows (Niehoff et al., 2009a). Furthermore, some microbial species in the rumen, especially the protozoa, are not able to synthesise the essential B-vitamins like niacin and therefore it is assumed that they benefit from supplemental niacin (Doreau and Ottou, 1996). Recent research has shown that microbial protein flow at the duodenum was enhanced after niacin supplementation to diets of buffaloes (Kumar and Dass, 2005) and dairy cows (Niehoff, 2009). Also, greater synthesis of microbial protein relative to fermented organic matter (OM) and feed intake was suspected (Shields et al., 1983). Therefore, the aim of the present study was to investigate whether a niacin supplementation for lactating dairy cows can compensate the negative effects of a deficiency in ruminally degradable N on nutrient digestibility, N utilisation and milk and blood variables. In this way niacin may allow the lowering of N content in diets for dairy cows and therefore provide a possibility for the reduction of N excretion with manure without compromising performance.

Materials and methods

Experimental design and animals

The experiment was conducted at the experimental station of the Friedrich-Loeffler-Institute in Braunschweig according to the European Community regulations concerning the protection of experimental animals and approved by the Regional Council of Braunschweig, Niedersachsen, Germany (file number 33.9.42502-04/057/07). A total of 9 German Holstein cows were used. The cows were equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter: 10 cm) and t-shaped cannulas at the proximal duodenum close to the pylorus (inner diameter 2 cm). The animals were housed in a tethered stall with neck straps and individual troughs with free access to water. Cows were milked at 05:30 and 15:30 h daily.

At the beginning of the experiment, animals had an average body weight of 599 kg (SD ±38 kg). All cows were lactating (79 ± 41.4 days in milk at the beginning) during the whole experimental period. Lactation numbers ranged from second to fourth lactation. The experimental diets consisted of 10 kg dry matter (DM) maize silage and 7 kg DM concentrate. To ensure the intended maize/concentrate ratio, the DM of maize silage was determined twice a week. Maize silage and concentrates were given in two equal portions at 5:30 h and 15:00 h. The pelleted concentrates were hand mixed with the silage in the trough.

In three periods the cows were assigned to the following experimental diets: RNB-, with energy and utilisable crude protein at the duodenum (uCP) according to the average requirement of the animals and a negative rumen N balance (RNB = -0.41 g N/MJ ME); RNB0, with energy, uCP, and RNB according to the average requirement of the animals (RNB = 0.08 g N/MJ metabolisable energy (ME)) by adding urea to the diet RNB-; and diet NA, with the same composition as diet RNB-, but plus 6 g/(cow · d) niacin as nicotinic acid. Due to different calving dates, not every cow could be used in all periods. In period one each of the three treatments was fed to two cows. In the second period two cows were respectively assigned to diets RNB0 and RNB- and one animal to diet NA. In the third period three cows received diet RNB0 and two cows were respectively fed diet RNB- or NA. No cow received the same treatment twice. The allocation of animals to diets and periods is shown in Table 1.

Table 1. Allocation of animals to diets and periods

	Experimental period		
Treatment	A	B	C
RNB0[†]	1[*]	3	5
	2	4	8
			9
RNB-[‡]	3	1	4
	6	5	7
NA[#]	4	7	1
	5		3

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]1-9 are animal ID numbers

The composition of the concentrates is given in Table 2. Niacin was mixed in an additional 100 g of mineral and vitamin mix (without niacin) and one half of this mixture was top-dressed on the concentrate during the morning feeding, the other half in the afternoon. The cows without niacin supplementation only received the additional amount of 100 g of mineral and vitamin mix in the same way.

Table 2. Composition of the concentrates

Components [%]	RNB0[†]	RNB-[‡] / NA[#]
Soybean meal, solvent-extracted	20	20
Barley grain	21.9	22.7
Wheat grain	21.9	22.7
Maize grain	18	18.8
Sugar beet pulp, dried	14.2	14.8
Urea	3	0
Mineral- and vitamin-mix[*]	1	1

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]Composition per kg: 175 g Ca, 100 g Na, 50 g P, 30 g Mg, 1 g Fe, 1.3 g Cu, 6 g Zn, 4 g Mn, 0.05 g I, 0.05 g Se, 0.03 g Co, 1,000,000 IU vitamin A, 100,00 IU vitamin D3 and 4 g vitamin E.

Sample collection

Each experimental period consisted of three weeks of adaptation to the diet followed by three weeks of sample collection. During the first and second sampling week, samples of maize silage and concentrate as well as feed refusals, if any, were collected daily and pooled on a weekly basis. Feed samples and refusals were dried at 60°C before analysis.

During the first sampling week, faeces and urine were collected completely. For that purpose the cows were equipped with urine devices, which were fitted around the vulva and allowed a separate collection of urine and faeces. Urine was piped from the urine device through a tube into a canister with 500 ml of sulphuric acid (10%, v/v). The amount was recorded every day and a subsample was taken and stored at -20°C. Faeces samples were taken every day, homogenized, and weighed. An aliquot of 2% was taken daily, pooled on a weekly basis, and freeze-dried. Milk yields were recorded daily. Milk samples were collected over two days of consecutive morning and evening milking in the first sampling week. A sample of 50 ml from each milking was conserved with bronopol and kept at 8°C until analysis for milk components. For the determination of milk urea, aliquots of the two daily milk samples were mixed and frozen at -20°C. During the second sampling week, duodenal chyme was collected for subsequent investigations. During the third sampling week, blood was collected in heparinised tubes on one day just before feeding at 5:30 h and at 8:00 h from a *Vena jugularis externa*. Sampling procedure was the same for both sampling times and for all animals. Stress level and animal treatment during sampling was exactly the same for all cows. Blood samples were kept at 15°C for 30 min and were centrifuged at 3000 · g for 30 min at 15°C. Afterwards plasma was frozen at -20°C until analysis.

Analysis

Feedstuffs, refusals, and faeces were analysed for dry matter (DM), crude protein (CP), crude ash (ash), ether extract (EE), and crude fibre according to methods of the VDLUFA (2007). The NDF was determined as described by Van Soest et al. (1991) and ADF analysis was done according to method number 6.5.2 of the VDLUFA (2007). Both were expressed without residual ash. Milk samples were analysed for fat, protein, and lactose using an infrared milk analyser (Milkoscan FT 6000 combined with a Fossomatic 5000; Foss Electric, Hillerød, Denmark). Milk urea-N concentration was determined enzymatically (Harnstoff/Ammoniak-Test, R Biopharm, Darmstadt, Germany). The N

concentration in freshly thawed urine was measured according to the method of Kjeldahl. Plasma glucose concentration, plasma urea-N, and β-hydroxybutyrate (BHB) in plasma were analysed photometrically (Eurolyser CCA 180 VET; Greiner Diagnostic, Bahlingen, Germany).

Calculations and statistics

The ME [MJ] content was calculated according to GfE (2001) from the digestion trial:

ME [MJ/kg DM] = 0.0312 g DEE + 0.0136 g DCF + 0.0147 g (DOM - DEE - DCF) + 0.00234 g CP

Where DEE = digestible ether extract [g/kg DM]; DCF = digestible crude fibre [g/kg DM] and DOM = digestible OM [g/kg DM].

Fat corrected milk (FCM) was estimated as follows:

Fat corrected milk [kg/d] = ((% milk fat · 0.15) + 0.4) · kg milk yield (Helfferich and Gütte, 1972).

N balance was calculated with the following equation:

Balance [g/d] = N intake [g/d] - faecal N [g/d] - urinary N [g/d] - milk N [g/d]

The SAS software package (Version 9.1.3., procedure mixed, SAS Institute Inc., Cary, NC, USA) was used to analyse the data. The procedure "MIXED" was applied. Feeding group and period were considered as fixed effects in the model. Additionally, to analyse plasma variables, the sampling time was also included. The fact that the cows were 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 and degrees of freedom were calculated according to the Kenward-Roger method. The "PDIFF" option was applied to test differences between least square means (LSMeans), using a Tukey-Kramer test for post-hoc analysis.

Main effects of the three different feeding regimes were considered as significant if F-statistics revealed $p<0.05$, a trend was considered if $p<0.10$ and >0.05. Because data were unbalanced, results are reported as LSMeans with the standard error of means (SEM), except for chemical composition of feedstuffs.

Results

Diet composition

Table 3 shows the means values for the chemical composition of the diets. The calculated energy [MJ ME] content varied slightly between the diets due to varying digestibility.

Table 3. Nutrient composition [g/kg DM] and realised metabolisable energy[*] of the silage and the experimental diets (arithmetic means of six observations ± standard deviation)

	Silage	Experimental diets	
		RNB0[†]	RNB-[‡] / NA[#]
Organic matter	961 ±3.8	959 ±2.9	958 ±2.9
Crude protein	74 ±2.3	156 ±4.4	122 ±2.3
Ether extract	34 ±1.8	31 ±2.6	32 ±2.7
Crude fibre	196 ±7.0	143 ±4.8	143 ±4.7
ADF	224 ±4.8	167 ±1.8	167 ±1.2
NDF	434 ±25.2	342 ±15.9	343 ±17.5
ME [MJ/kg DM]	-	10.97 ±0.2	10.57[¥]/10.76[§] ±0.2

Notes: [*]Based on the measured digestibility; [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [¥]Energy content of diet RNB-; [§]Energy content of diet NA

Milk yield and composition

No significant effects of rumen degradable N level or supplementation of niacin to the diet were observed for milk yield, FCM yield, milk fat and milk protein content as well as for yields of milk fat, protein, and lactose (Table 4). The milk protein content tended to be 0.15 percentage points higher (p = 0.09) in the NA diet compared to diet RNB-. Diet RNB0 had lower milk lactose content by about 0.11 percentage points compared to diet RNB-, but no effect compared to NA was observed. Both, RNB- and NA significantly decreased milk urea-N concentration.

Table 4. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on milk production and composition (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
Milk [kg/d]	29.3 ±0.93	29.8 ±0.99	28.8 ±1.05
FCM [kg/d]	25.5 ±1.18	25.6 ±1.25	25.0 ±1.31
Milk composition [%]			
Fat	3.14 ±0.19	3.04 ±0.20	3.11 ±0.21
Protein	3.12 ±0.04	2.99 ±0.04	3.14 ±0.05
Lactose	4.67 ±0.05[b]	4.79[a] ±0.05	4.74[ab] ±0.05
Yield [g/d]			
Fat	919 ±62.9	909 ±66.6	898 ±70.1
Protein	909 ±33.5	889 ±37.1	891 ±41.4
Lactose	1375 ±56.1	1430 ±58.8	1370 ±71.3
Urea [mg/L]	215.5 ±9.6[a]	71.9 ±9.8[b]	65.1 ±9.9[b]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [a,b,c] Means in the same row with different superscripts differ ($p<0.05$).

Plasma variables

Plasma urea and glucose data as well as BHB concentrations are shown in Table 5. Cows fed the diets RNB- and NA showed lower urea-N concentrations in plasma compared to diet RNB0 before and after feeding. All diets caused a decline in plasma glucose concentration between the two sampling times, but only RNB- and NA reduced plasma glucose concentration significantly by 35.1% and 30.5%, respectively. Post-feeding, plasma glucose concentration showed differences between the three treatments. The concentration was highest for RNB0 and lowest for RNB-, with NA intermediate. The diet had no influence on the BHB concentration in plasma, but the BHB content was enhanced after feeding in all groups compared to pre-feeding samples (Table 5).

Table 5. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on blood metabolites (LSMeans with their standard errors)

	RNB0[†] (n = 7)		RNB-[‡] (n = 6)		NA[#] (n = 5)		*p*		
	5:30 h	8:00 h	5:30 h	8:00 h	5:30 h	8:00 h	diet	time	diet x time
Plasma glucose [mmol/L]	2.94 ±0.14	2.71 ±0.14	3.07 ±0.15	1.96 ±0.15	3.43 ±0.17	2.26 ±0.17	0.06	<0.01	<0.01
Plasma urea nitrogen [mmol/L]	8.63 ±0.57	10.72 ±0.57	4.32 ±0.62	4.16 ±0.62	3.95 ±0.69	4.57 ±0.69	<0.01	0.09	0.16
BHB[*] [mmol/L]	0.41 ±0.20	1.07 ±0.2	0.21 ±0.22	1.45 ±0.22	0.35 ±0,24	1.70 ±0.24	0.45	<0.01	0.20

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]BHB, β-Hydroxybutyrate.

Apparent digestibility

As shown in Table 6, diet had no effect on apparent total tract digestibility of crude fibre and ADF. The apparent digestibility of DM and OM was higher for RNB0 than RNB- and supplementation of niacin to RNB- tended to increase OM digestibility (p = 0.08). The apparent digestibility of NDF was significantly increased for NA versus RNB-, whereas no effect was observed for diet NA compared to diet RNB0 (Table 6).

Table 6. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on total tract apparent digestibility of nutrients [%] (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
Dry matter	70.8 ±0.65[a]	68.0 ±0.71[b]	69.6 ±0.78[ab]
Organic matter	72.1 ±0.62[a]	68.8 ±0.69[b]	71.0 ±0.76[ab]
Crude protein[*]	64.9 ±1.05	65.3 ±1.25	67.7 ±1.3
Crude fibre	45.8 ±2.78	41.6 ±3.06	46.4 ±3.36
ADF	49.0 ±2.05	43.0 ±2.26	45.4 ±2.50
NDF	53.4 ±1.4[a]	46.6 ±1.45[b]	53.4 ±1.68[a]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*] Crude protein intake corrected for urea nitrogen; [a,b,c] Means in the same row with different superscripts differ (p<0.05).

Partitioning of dietary N

Faecal N excretion was lower for NA (107.7 g/d) compared to RNB- (119.4 g/d). Diets RNB- and NA resulted in a lower excretion of urinary N (Table 7). Milk N excretion did not differ among diets. As a proportion of N intake (i.e., milk N efficiency) the milk protein yield was greater for RNB- and NA and furthermore NA tended (p = 0.08) to be higher (by 3.0 percentage units) in milk N efficiency than RNB-. The N balances showed differences between the three treatments. RNB0 resulted in the highest N balance. Furthermore, NA increased the N balance by 19.2 g/d in comparison to RNB- (Table 7). The live weight gain varied between -27 kg and 30 kg and was unaffected by treatment (p > 0.05, Data not shown)

Table 7. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on nitrogen excretion and nitrogen balance (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
Faecal nitrogen [g/d]	115.3 ±2.48[ab]	119.4 ±2.66[a]	107.7 ±2.85[b]
Urinary nitrogen [g/d]	112.6 ±3.82[a]	48.5 ±4.21[b]	44.0 ±4.70[b]
Inefficiently used nitrogen [g/d][*]	228.2 ±5.18[a]	168.4 ±5.72[b]	152.5 ±6.46[b]
Milk nitrogen [g/d]	141.2 ±5.38	140.6 ±5.81	137.5 ±6.25
Milk nitrogen efficiency [%]	33.1 ±1.17[b]	40.6 ±1.24[a]	41.6 ±1.30[a]
Nitrogen balance [g/d][§]	52.6 ±2.63[a]	20.6 ±2.91[c]	39.8 ±3.12[b]
Efficiently used nitrogen [g/d][¥]	192.8 ±5.21[a]	157.4 ±5.77[b]	170.9 ±6.20[b]
Inefficiently used nitrogen / efficiently used nitrogen	1.18 ±0.05[a]	1.07 ±0.06[ab]	0.89 ±0.06[b]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB- ,ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*] Inefficiently used nitrogen, sum of faecal and urinary nitrogen; [§]Nitrogen balance = nitrogen intake – faecal nitrogen –urinary nitrogen – milk nitrogen; [¥]Efficiently used nitrogen, sum of milk nitrogen and balance nitrogen; [a,b,c] Means in the same row with different superscripts differ ($p<0.05$).

The sum of milk N and N balance, hereafter designated efficiently used N, was greater in treatment RNB0. Although the difference was not significant, diet NA increased the efficiently used N by 13.5 g N/d in comparison to RNB-. Hence, the sum of faecal and urinary N losses, described as inefficiently used N, was highest with RNB0 and lower for NA and RNB- (Table 7).

The ratio between inefficiently and efficiently used N, which indicates the units of N that are excreted in urine and faeces relative to N use for milk and retention, was reduced by NA (0.89) compared to RNB0 (1.18) with RNB- being intermediate (1.07).

Discussion

Milk

Consistent with our results, Zimmerman et al. (1992) found no treatment effects on milk yield, FCM, milk fat content, and milk fat yield in multiparous cows fed an N deficient diet and an N balanced diet with 13.7% and 18.8% CP, respectively after niacin supplementation of 12 g/d. This agrees with other studies where the effect of 6 or 12 g/d of dietary niacin was investigated at a balanced N supply (Christensen et al., 1996; Ghorbani et al., 2008). Other researchers have reported increased yields of FCM when 12 g/d niacin was supplemented (Drackley et al., 1998). Only one study reported an increase of milk fat proportion with 10 g/d of supplemental niacin (Belibasakis and Tsirgogianni, 1996). In the present study, the reduced RNB level of the diet (RNB0 versus RNB-) had no impact on milk yield and composition, which is consistent with the study of Agle et al. (2010). However, it may be assumed that long-term trials with a marked deficit in rumen degradable N will lead to a depression in milk yield. The decreased digestibility of the RNB deficient diet in this study (Table 5) or reduced rumen fermentation (Lebzien et al. 2006) due to a nitrogen deficiency in the rumen may lead to a lack of energy for milk synthesis. Consistent with our results, others have reported trends towards increased milk protein concentrations when niacin was supplemented (Drackley et al., 1998; Niehoff et al., 2009b). Increased microbial protein synthesis in the rumen may lead to higher milk protein proportions or niacin may enhance the amino acid uptake in the mammary gland due to the effect of insulin (Erickson et al., 1992). However, other studies reported that milk protein proportions were neither affected by niacin supplementation (Ottou et al., 1995; Ghorbani et al., 2008) nor by the N level of the diet (Steinwidder et al., 2009; Agle et al., 2010). As expected, a reduced dietary N supply to the rumen decreased milk urea-N content. Significant reductions of milk urea-N concentrations have been previously observed (Roseler et al., 1993; Monteils et al., 2002; Lebzien et al., 2006). Only few authors have studied the effect of a niacin supplementation on milk urea-N. Consistent with our results, Niehoff et al. (2009b) observed no effect of niacin on urea-N concentration in milk

Blood variables

Generally, it must be considered that the blood samples were only taken at two time points and thus they are not representative for the variation during a whole day. The deficit in ruminally degradable N in the RNB- and NA diets decreased plasma urea-N irrespective of sampling time, corroborating previous findings (Lebzien et al., 2006; Agle et al., 2010). Roseler et al. (1993) concluded that plasma urea-N concentration reflects the CP intake in lactating dairy cows and Rodriguez et al. (1997) and Niehoff et al. (2009b) determined a positive correlation between rumen ammonia concentration and concentration of plasma urea-N. Riemeier (2004) found a strong positive correlation (r^2 = 0.83) between the concentration of urea-N in blood and milk of dairy cows, which complies with the present study, where a correlation coefficient of 0.68 was found. In contrast to Belibasakis and Tsirgogianni (1996), who observed a lower urea-N concentration in plasma and Niehoff et al. (2009b), who reported a greater urea concentration in plasma after an oral niacin supplementation, most other researchers found in accordance with the present study no response of plasma urea-N to niacin supplementation neither with an N deficient diet (Zimmerman et al. 1992) nor with N balanced diets (Christensen et al., 1996; Madison-Anderson et al., 1997). Niehoff et al. (2009b) mentioned a potential impact of sampling time in relation to time after feeding on urea-N concentration in plasma. These effects might explain the different findings concerning the effects of niacin supplementation on blood urea nitrogen.

The post-feeding decline in plasma glucose concentration in all feeding groups agreed with other studies (Oba and Allen, 2003; Plaizier et al., 2005). The secretion of insulin, which is responsible for the regulation of plasma glucose concentration, depends on the concentration of short-chain fatty acids, especially propionate, in the blood of dairy cows (Oba and Allen, 2003). Others also observed a reduced plasma glucose concentration when low-N diets were fed (Zimmerman et al., 1992). Perhaps the reduced availability of glucogenic amino acids or a declined yield of propionate due to the impaired microbial fermentation activity in the rumen caused a limitation in the gluconeogenetic activity. However, most researchers reported no significant effects of niacin on plasma glucose concentration (Cervantes et al., 1996; Madison-Anderson et al., 1997). In accordance with this study, Niehoff et al. (2009b) and Ghorbani et al. (2008) observed increased plasma glucose concentrations after niacin administration. Niacin is incorporated into the coenzymes NAD and NADP and therefore it is intimately involved in the Krebs cycle and

gluconeogenesis. Consistent with the present study, neither niacin supplementation (Cervantes et al., 1996) nor a reduced N level of the diet (Rius et al., 2010) had an impact on BHB concentrations. The enhanced after-feeding concentration of plasma BHB in all groups might be related to the production of BHB from butyrate in rumen epithelial cells (Nielsen et al., 2003).

Apparent total tract digestibility

In accordance with the current results, a positive relationship between total tract digestibility of DM and OM and the N supply in dairy cows was reported by others (Röhrmoser et al., 1984; Broderick et al., 2009). A depression in NDF digestibility was observed in the present study when the content of ruminally degradable N in the diet was reduced. Similar results have been reported by Broderick et al. (2009). Cellulolytic bacteria in the rumen need ammonia from dietary protein as N source to build microbial protein, otherwise the fermentation of fibre is restrained (Bryant, 1973).

This restraining effect could be compensated for by niacin supplementation. Similar to this study, Horner et al. (1988) found that niacin supplementation enhanced NDF digestibility, whereas the digestibility of ADF was unaffected by treatment. These authors (Horner et al., 1988) assumed a shift in the rumen microbial population due to higher ruminal availability of niacin, which resulted in an improved digestion of hemicelluloses. As protozoa are niacin consumers (Niehoff et al., 2009a) and known to be strongly involved in the digestion of fibre (Takenaka et al., 2004), particularly hemicelluloses (Bailey and Mac Rae, 1970), a positive effect of supplemental niacin on the efficiency of NDF digestion by the protozoal population could be assumed. However, no effect of niacin on apparent total tract digestibility of ADF and NDF was found by other authors (Erickson et al., 1992; Doreau and Ottou, 1996). Flachowsky (1993) stated that type of diet, level of added niacin and experimental conditions may influence the results of digestibility studies, which may explain the inconsistent findings considered above.

Nitrogen balance

Numerous studies have been performed on the influence of the amount of ruminally degradable N on N losses and N balance of lactating dairy cows. Reduced urinary N excretion has often been observed when the amounts of CP, ruminally degradable N, or rumen degradable protein in the diets were reduced (Röhrmoser et al., 1984; Weiss et al.,

2009). With regard to the environment, it has to be considered that N excreted in urine is more susceptible to N leaching and emission losses than N in faeces (Bussink, 1998). In the dynamic N metabolism model of Kebreab et al. (2001) N output exhibits an exponential relation to increasing levels of N intake with a high coefficient of determination ($r^2 = 0.79$). In accordance with our results, in several other investigations the faecal N output was unaffected by a moderate reduction in the amount of ruminally degradable N (Gressley and Armentano, 2007; Broderick et al., 2009).

Studies concerning the impact of niacin on N balance and N losses of ruminants are scarce. As in the present study, Kung et al. (1980) did not observe changes in the amount of N excreted in urine when niacin was supplemented at 6 g/d to dairy cows. In contrast to Kung et al. (1980), who found no influence of an oral niacin supplementation on the faecal N excretion, a decrease in faecal N excretion by 9.7% was observed in the current study when niacin was added to the RNB- diet. In an analysis of 159 digestion trials with sheep, N excretion in faeces was negatively correlated with OM digestibility of the diets (Wang et al., 2009). Thus, one explanation for the observed decreased faecal N excretion in the niacin supplemented group may be the above mentioned greater NDF digestibility and the trend towards improved OM degradation.

The N excretion with milk did not differ among diets in the present study, corroborating previous findings by Monteils et al. (2002), who investigated the effect of different dietary CP levels (13%, 14.7% and 16.3%) with a deficit in ruminally degradable N on N utilisation in dairy cows. In the study of Kung et al. (1980), milk N excretion was also not affected by a niacin supplementation of 6 g/d. An equal output of milk N with a simultaneous reduction in the sum of urinary and faecal N losses consequently caused an improvement in the efficiency of transformation of dietary N into milk protein by 22% when feeding RNB- (Table 7). Huhtanen and Hristov (2009) performed a meta-analysis including a wide range of diets and animal performance variables and concluded that the level of dietary CP was the most important determinant for milk N efficiency in lactating cows. In accordance with the values for the milk N efficiency, the ratio between inefficiently and efficiently used N, which can be used as an additional measure for the efficiency of N utilisation, decreased numerically when dietary N was deficient and was significantly lowered when diet NA was fed in comparison to RNB0.

The N balance showed differences between the diets. Surprisingly, even the animals with a deficiency of N in the diet showed a positive balance, although the body weight was

unaffected by treatment. Nonetheless, it has to be considered that measured N balances in experiments with adult ruminants are very often greater than expected (Reynolds and Kristensen, 2008). The N balance over a whole lactation is approximately zero, although the calculated N retention can be positive or negative in short-term experiments. Possible reasons for this overestimation of the N balance are numerous. Faecal ammonia N losses, losses in hair and scurf, nitrate formation, and some gaseous losses as well as pregnancy have to be considered (Huhtanen et al., 2008). The total sum of these minor losses may be important (Reynolds and Kristensen, 2008). Spanghero and Kowalski (1997) highlighted another important issue concerning N balance studies. The authors assumed an overestimation of retained N due to losses occurring during collection and analysis (Spanghero and Kowalski, 1997). Nevertheless, in the present trial, sampling procedure and data evaluation were the same for all animals and treatments. Therefore, balance data can depict differences between the treatments within the trial. As expected, the N balance was higher in animals fed the RNB0 diet. Niacin supplementation resulted in increased N balances as compared to RNB-, which indicated a higher amount of N which was not excreted. However, further evaluation of the intra-ruminal processes is needed to clarify the N retention in the body and the effects of N and niacin on the microbial population and the fermentation pattern as well as on the flow of nutrients at the duodenum.

Conclusion

A limitation of the supply with ruminally degradable nitrogen reduced the milk and blood urea content as well as the apparent total tract digestibility of dry matter, organic matter, and neutral detergent fibre. Also, nitrogen excretion with urine and the sum of nitrogen excreted with urine and faeces was lower compared to the diet with a balanced nitrogen supply. Furthermore, the milk nitrogen efficiency was enhanced and the nitrogen balance was reduced.

The supplementation of 6 g niacin per cow and day to a diet with a negative rumen nitrogen balance (RNB-) compensated for the negative effect of RNB- on the digestibility, the N excretion with faeces and tended to increase the milk protein content. Additionally, the nitrogen balance was elevated. Also, the negative effect of a reduction of rumen nitrogen balance on the amount of efficiently used N could be partly compensated by niacin supplementation. With regard to the environment, this seems to be a possible

approach for a reduction of the nitrogen emitting potential in milk production, but long-term experiments with a higher number of observations are needed.

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Chapter 4

Effect of niacin supplementation on rumen fermentation parameters and nutrient flow at the duodenum in lactating dairy cows fed a diet with a negative rumen nitrogen balance

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Abstract

The aim of the present experiment was to determine if a niacin supplementation of 6 g/(cow · d) to lactating dairy cow diets can compensate for the decrease in rumen microbial fermentation due to a negative rumen nitrogen balance (RNB). A total of 9 ruminally and duodenally fistulated lactating multiparous German Holstein cows was used. The diets consisted of 10 kg (dry matter, DM) maize silage and 7 kg DM concentrate and differed as follows: RNB- (n = 6) with energy and utilisable crude protein (CP) at the duodenum (uCP) according to the average requirement of the animals, but with a negative RNB (-0.41 g N/MJ metabolisable energy (ME)); RNB0 (n = 7) with energy, uCP, and RNB (0.08 g N/MJ ME) according to the average requirement of the animals; and diet NA (nicotinic acid; n = 5), which was the same diet as RNB-, but supplemented with 6 g niacin/d. The negative RNB affected the rumen fermentation pattern and reduced ammonia content in rumen fluid and the daily duodenal flows of microbial CP (MP) and uCP. Niacin supplementation increased the apparent ruminal digestibility of neutral detergent fibre. The efficiency of microbial protein synthesis per unit of rumen degradable CP was higher, whereby the amount of MP reaching the duodenum was unaffected by niacin supplementation. The number of protozoa in rumen fluid was higher in NA treatment. The results indicated a more efficient use of rumen degradable N due to changes in the microbial population in the rumen when niacin was supplemented to diets deficient in RNB for lactating dairy cows.

Keywords: niacin, rumen nitrogen balance, microbial protein, nitrogen utilisation, ruminal digestibility

Introduction

Reduction of nitrogen (N) emissions from animal husbandry has been a focus of research in recent times (Kohn et al., 2005). In particular, N losses from ruminants and thus the utilisation of dietary N in the rumen were highlighted as important factors (Tamminga, 1992). The average milk N efficiency (milk N/N intake) in European dairy cattle was estimated to amount to only 28% with a large variation between 16% and 40% (Huhtanen and Hristov, 2009). Furthermore, N intake is positively correlated with N losses (Burgos et al., 2007). Therefore, one approach to reduce N emissions is the limitation of N in the diet for dairy cows. However, the rumen microbial population is very sensitive to deficient N supply. This became obvious as the flow of microbial crude protein at the duodenum (MP) was reduced when the rumen nitrogen balance (RNB) in the diet was -0.6 g N/MJ ME as shown by Lebzien et al. (2006).

Niacin is of great importance for the energy metabolism of the animal because it is an integral part of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) (Bender, 1992). In reduced form, these coenzymes are involved in several essential metabolic pathways like synthesis of fatty acids, supply of amino nitrogen through aspartate, and urea biosynthesis as well as in the pentose phosphate pathway and gluconeogenesis (Bender, 1992). Both NAD and NADP can also be present in an oxidised form as NAD^+ and $NADP^+$. They are important electron acceptors in the Krebs cycle and the glycolytic pathway (Bender, 1992). Apart from dietary niacin, ruminants use endogenous niacin synthesis from tryptophan and microbial niacin synthesis in the rumen as a niacin source (Niehoff et al., 2009). Both GfE (2001) and NRC (2001) assume that these sources are sufficient to meet the niacin requirements of dairy cows. As reviewed by Niehoff et al. (2009), studies concerning the effect of dietary niacin on rumen metabolism provide inconsistent results. Nevertheless, in several studies supplemental niacin improved MP synthesis in the rumen of buffaloes and dairy cows (Riddell et al., 1980; Kumar and Dass, 2005; Niehoff, 2009) and the efficiency of use of dietary N and the N balance (Aschemann et al., 2012). These effects might be attributed to protozoa which are not able to synthesize niacin and thus benefit in particular from additional niacin in the diet (Doreau and Ottou, 1996). Therefore, it was the aim of the present study to determine if a supplementation of 6 g niacin per day could compensate for

the decreased microbial fermentation due to a negative RNB in a diet for lactating dairy cows.

Materials and methods

Experimental design and animals

The experiment was conducted at the experimental station of the Friedrich-Loeffler-Institute in Braunschweig according to the European Community regulations concerning the protection of experimental animals and approved by the Regional Council of Braunschweig, Niedersachsen, Germany (file number 33.9.42502-04/057/07). A total of 9 German Holstein cows were used. The cows were equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter: 10 cm) and t-shaped cannulas at the proximal duodenum close to the pylorus (inner diameter: 2 cm). The animals were housed in a tethered stall with neck straps and individual troughs with free access to water. Cows were milked at 05:30 h and 15:30 h daily.

At the beginning of the experiment, animals had an average body weight of 599 kg (SD 38 kg). All cows were lactating (79 days in milk at the beginning; SD 41.44) during the whole experimental period. Lactation numbers ranged from second to fourth lactation. The experimental diets consisted of 10 kg dry matter (DM) maize silage and 7 kg DM concentrate. To ensure the intended maize/concentrate ratio, the DM of maize silage was determined twice a week. Maize silage and concentrates were given in two equal portions at 5:30 h and 15:00 h. The pelleted concentrates were hand-mixed with the silage in the troughs.

In three periods the cows were assigned to the following experimental diets: RNB-, with energy and utilisable crude protein at the duodenum (uCP) according to the average requirement of the animals and a negative rumen N balance (RNB = -0.41 g N/MJ ME); RNB0, with energy, uCP, and RNB according to the average requirement of the animals (RNB = 0.08 g N/MJ metabolisable energy (ME)) by adding urea to the diet RNB-; and diet NA, with the same composition as diet RNB-, but plus 6 g/(cow · d) niacin as nicotinic acid. Due to different calving dates not every cow could be used in all periods. In period one each of the three treatments was fed to two cows. In the second period, two cows were respectively assigned to diets RNB0 and RNB- and one animal to diet NA. In the third period three cows received diet RNB0 and two cows were respectively fed diet RNB- or

NA. No cow received the same treatment twice. The composition of the concentrates is given in Table 1.

Table 1. Composition of the concentrates

Components [%]	RNB0[†]	RNB-[‡] / NA[#]
Soybean meal, solvent-extracted	20	20
Barley grain	21.9	22.7
Wheat grain	21.9	22.7
Maize grain	18	18.8
Sugar beet pulp, dried	14.2	14.8
Urea	3	0
Mineral- and vitamin-mix[*]	1	1

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]Composition per kg: 175 g Ca, 100 g Na, 50 g P, 30 g Mg, 1 g Fe, 1.3 g Cu, 6 g Zn, 4 g Mn, 0.05 g I, 0.05 g Se, 0.03 g Co, 1,000,000 IU vitamin A, 100,00 IU vitamin D3 and 4 g vitamin E.

Niacin was mixed in an extra 100 g of mineral and vitamin mix (without niacin) and one half of this mixture was top-dressed on the concentrate during the morning feeding, the other half in the afternoon. The cows without niacin supplementation only received the mineral and vitamin mix in the same way.

Sample collection

Each experimental period consisted of three weeks of adaptation to the diets followed by three weeks of sample collection. During the first and second sampling week, samples of maize silage and concentrate as well as any feed refusals were collected daily and pooled on a weekly basis. Feed samples and refusals were dried at 60°C.

During the first sampling week, samples of ruminal fluid (approximately 100 ml) were withdrawn from the ventral sac through the rumen cannula using a hand vacuum pump. Fluid was taken before first feeding at 5:30 h in the morning and 30, 60, 90, 180, 300, and 420 minutes afterwards. Also in the first sampling week, urine and faeces were sampled completely. For that purpose, the cows were equipped with urine devices which were adhered around the vulva and allowed to separate urine from faeces. The amount of urine

and faeces was recorded every day and a subsample was taken and stored at -20°C for further analysis.

During the second sampling week, duodenal chyme was collected over five consecutive days in two-hour intervals. At each sampling, four 100 ml samples were taken through the duodenal cannula from each cow. Immediately after withdrawal, the pH was measured using a glass electrode (pH525, WTW, Weilheim, Germany) and the sample with the lowest pH was added to the daily pooled sample from each cow and stored at -18°C (Rohr et al., 1984). To estimate the digesta flow, chromium oxide (Cr_2O_3) marker (19.8% Cr_2O_3, 79.2% wheat flour and 0.67% Al_2SO_4) was used. The marker was given in two portions of 50 g at 5:15 h and 17:15 h into the rumen beginning 10 days before the start of duodenal chyme collection. One day before and then during the sampling period, 25 g were administered every 6 h at 5:45 h, 11:45 h, 17:45 h, and 23:45 h.

In the third sampling week, rumen liquid turnover and the protozoal population were examined. For estimation of rumen liquid volume and turnover a single dose of 25 g cobalt-EDTA was used as marker. Cobalt-EDTA complex was prepared as described by Udén et al. (1980) and dispersed in the liquid phase of the rumen of each cow via the cannula at 5:30 h, just after the morning feeding. Approximately 100 ml of rumen fluid was taken every hour over 12 h beginning 2 h after marker application using a hand vacuum pump. The samples were centrifuged at 2000 · g for 5 min at 4°C immediately after withdrawal and the supernatant was frozen at -20°C.

For the investigation of the protozoal population, on one day 15 ml of rumen fluid of each cow were taken 3 h after morning feeding, mixed with 15 ml of methylgreen-formalin solution (Ogimoto and Imai, 1981), and stored at room temperature in the dark until protozoa counting.

Analyses

Feedstuffs, faeces, and refusals were analysed according to methods of the VDLUFA (2007) and method numbers are given. Crude ash (ash) was determined using method 8.1. Crude fibre (CF) and ether extract (EE) were analysed according to methods 6.1.1 and 5.1.1, respectively. The NDF was determined as described by Van Soest et al. (1991) and ADF analysis was done according to method number 6.5.2 of the VDLUFA (2007). Both were expressed without residual ash. Crude protein (CP) in feedstuffs and refusals was analysed using Dumas combustion (Method number 4.1.2). The CP content in faeces and

freshly thawed duodenal chyme was measured according to the method of Kjeldahl (Method number 4.1.1). Immediately after collection of rumen fluid, pH of each sample was measured with a glass electrode (pH525, WTW, Weilheim, Germany). Short chain fatty acids (SCFA) were analysed according to Geissler et al. (1976) using a gas chromatograph (Hewlett Packard 5580, Avondale, PA, USA) equipped with a flame ionization detector. Ammonia-N (NH_3-N) in rumen fluid and freshly thawed duodenal chyme was analysed according to DIN 38406-E5-2 (Anonymous, 1998). The following analyses of duodenal chyme were carried out with freeze-dried and ground material. The DM and ash contents were analysed in the daily pooled samples by the same methods described above for feedstuffs. The proportion of microbial-N of the non-ammonia-N (NAN) in duodenal chyme was estimated using near infrared spectroscopy according to Lebzien and Paul (1997). Cr_2O_3 in duodenal chyme was measured using an inductively coupled plasma optical emission spectrometry (ICP-OES; Quantima, GBC Sientific Equipment Pty Ltd, Victoria, Australia) after sample preparation according to Williams et al. (1962). The chromium content was used to calculate the daily duodenal DM flow. According to the daily flows, one aliquot pooled sample per cow per week was generated. In the pooled samples, NDF, ADF, and CF were quantified applying the same methods as for feedstuffs. Protozoa were counted under an optical microscope using a Fuchs-Rosenthal chamber and differentiated into Holotricha and Entodimiomorpha. Concentration of cobalt in rumen fluid samples for the estimation of the rumen fluid volume, outflow rate and turnover rate was also analysed by ICP-OES, using the same equipment as described above.

Calculations and statistics

The ME [MJ] content was calculated according to GfE (2001) from the digestion trial:

ME [MJ/kg DM] = 0.0312 g DEE + 0.0136 g DCF + 0.0147 g (DOM - DEE - DCF) + 0.00234 g CP

Where DEE = digestible ether extract [g/kg DM]; DCF = digestible crude fibre [g/kg DM] and DOM = digestible OM [g/kg DM].

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

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

The daily duodenal flows of organic matter (OM) and nutrients were estimated by multiplication of their respective concentrations in duodenal digesta with DMF.

The uCP at the duodenum was estimated following Lebzien and Voigt (1999):

uCP [g/d] = CP flow at the duodenum [g/d] - NH_3-N · 6.25 [g/d] - endogenous CP (EP) [g/d].

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

EP [g/d] = (3.6 · kg DMF) · 6.25

The RNB and ruminally degraded CP (RDP), ruminally undegraded feed CP (RUP) and ruminally fermented OM (FOM) were calculated with the following equations:

RNB [g/d] = (CP intake [g/d] - uCP [g/d])/6.25

RUP [g/d] = 6.25 · (NAN at the duodenum [g/d] - microbial N [g/d]) - EP [g/d]

RDP [g/d] = CP intake [g/d] - RUP [g/d]

FOM [kg/d] = OM intake [kg/d] - (duodenal OM flow [kg/d] - microbial OM [kg/d])

The microbial OM was estimated as described by Schafft (1983):

Microbial OM [kg/d] = 11.8 · microbial N [kg/d]

Rumen turnover time and pool size were calculated from the concentration of cobalt in sequential samples of rumen fluid. It was assumed that the decline in marker concentration followed first order kinetics (Barboza et al., 2006):

$$C_t = C_0 \cdot e^{-kt}$$

where C_t is the marker concentration at time t (h after dosing), C_0 is the marker concentration at the time of dosing, and k the elimination constant. C_0 was calculated by least square regression method. The size of the ruminal fluid pool was calculated by dividing the dose of cobalt [mg] with the concentration of marker [mg/L] predicted at t_0 from the regression.

The SAS software package (Version 9.1.3., procedure MIXED, SAS Institute Inc., Cary, NC, USA) was used to analyse the data. Feeding group and period were considered as fixed effects. Additionally, to analyse rumen variables, the sampling time was also included. The fact that a cow was 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 squares means, using a Tukey-Kramer test for post-hoc analysis.

Main effects of the three different feeding regimes were considered as significant, if F-statistics revealed $p<0.05$ and differences between the treatments were taken as significant, if Tukey-Kramer test revealed $p<0.05$. Except for chemical composition of feedstuffs, results are reported as least squares means (LSMeans) with standard error of means because data were unbalanced.

Results

There was no effect of the experimental period on any of the variables.

Diet composition

Table 2 shows the mean values of the chemical composition of the silage and the diets. The calculated energy content varied slightly between the diets due to differences in measured

digestibilities. The analysed CP content was 15.6% for the balanced diet and 12.2% for diets RNB- and NA.

Table 2. Nutrient composition [g/kg DM] and realised metabolisable energy[*] of the silage and the experimental diets (arithmetic means of six observations ± standard deviation)

	Silage	Experimental diets	
		RNB0[†]	RNB-[‡] / NA[#]
Organic matter	961 ±3.8	959 ±2.9	958 ±2.9
Crude protein	74 ±2.3	156 ±4.4	122 ±2.3
Ether extract	34 ±1.8	31 ±2.6	32 ±2.7
Crude fibre	196 ±7.0	143 ±4.8	143 ±4.7
ADF	224 ±4.8	167 ±1.8	167 ±1.2
NDF	434 ±25.2	342 ±15.9	343 ±17.5
ME [MJ/kg DM]	-	10.97 ±0.2	10.57[¥]/10.76[§] ±0.2

Notes: [*]Based on the measured digestibility; [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [¥]Energy content of diet RNB-; [§]Energy content of diet NA.

Rumen measurements

The analysed rumen variables showed effects of time after feeding. Because these relationships are well known and have been discussed several times (Nikkaha, 2011; Nikkaha et al., 2011), they will not be presented here in detail.

As shown in Table 3, the pH value was unaffected by the diet. The content of ammonia in rumen fluid was higher over the whole sampling time with diet RNB0 compared to diet RNB- with NA being intermediate.

No effects for ruminal SCFA were observed at the particular measurement times between the three treatments. Therefore, LSMeans over the whole sampling time with their standard error are presented in Table 3. A reduction in RDP (RNB-) reduced the molar proportion of propionic acid, valeric acid, and iso valeric acid, whereas the molar percentage of butyric acid and the ratio between acetic acid and propionic acid was enhanced for RNB-. The supplementation of niacin increased the proportion of valeric acid, iso valeric acid, iso

butyric acid as well as propionic acid, whereas the percentage of acetic acid was reduced. Consequently, NA decreased the ratio between acetic acid and propionic acid. The total concentration of SCFA in rumen fluid was unaffected by treatment (Table 3).

Table 3. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on rumen fermentation parameters (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
pH	6.4 ±0.07	6.4 ±0.07	6.4 ±0.08
NH3 [mmol/L]	14.2 ±0.56[a]	2.3 ±0.64[c]	4.0 ±0.67[b]
SCFA total [mmol/L][*]	135.3 ±4.9	122.3 ±5.8	125.9 ±6.1
Acetic acid [mol%]	58.4 ±1.57[ab]	59.7 ±1.64[a]	57.0 ±1.68[b]
Propionic acid [mol%]	23.2 ±1.17[a]	19.7 ±1.22[c]	21.1 ±1.24[b]
Butyric acid [mol%]	13.7 ±0.99[b]	16.8 ±1.02[a]	17.2 ±1.05[a]
Valeric acid [mol%]	3.9 ±0.42[a]	2.3 ±0.43[b]	2.7 ±0.43[a]
Isobutyric acid [mol%]	0.5 ±0.04[b]	0.5 ±0.04[b]	0.6 ±0.04[a]
Isovaleric acid [mol%]	1.6 ±0.18[b]	1.0 ±0.19[c]	1.4 ±0.19[a]
Acetic acid : propionic acid	2.6 ±0.19[b]	3.2 ±0.20[a]	2.9 ±0.21[b]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]SCFA, short chain fatty acids; [a,b,c] Means in the same row with different superscripts differ ($p<0.05$).

Diet type had no effect on rumen liquid volume (p = 0.72) or the turnover rate of rumen fluid (p = 0.74; Table 4). Average values for rumen fluid volume ranged from 58.0 L (± 4.25 L) in animals fed diet RNB0 to 53.2 L (± 5.11 L) for the animals in group NA. The outflow varied slightly from 9.1 ± 0.68 L/h (RNB-) to 8.5 ± 0.62 L/h (RNB0).

As presented in Table 4, number of Holotricha was unaffected by RNB in the diet, whereas treatment RNB- increased the counts of Entodiniomorpha, the total concentration of protozoa, and the ratio between Entodiniomorpha and Holotricha compared to treatment RNB0. Niacin supplementation increased the concentration of protozoa in rumen fluid. This increase concerned Holotricha as well as Entodiniomorpha and the total number. The ratio between Entodiniomorpha and Holotricha was unaffected by niacin.

Table 4. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on rumen liquid volume, turnover and concentration of protozoa ($\cdot 10^3$/ml) in rumen fluid (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
Rumen liquid volume [L]	58.0 ±4.25	55.1 ±4.65	53.2 ±5.11
Rumen liquid outflow			
Volume [L/h]	8.5 ±0.62	9.1 ±0.68	9.1 ±0.75
Rate [%/h]	14.7 ±0.78	16.5 ±0.85	17.2 ±0.93
Protozoal population			
Entodiniomorpha	292 ±65[c]	465 ±66[b]	702 ±67[a]
Holotricha	9 ±2[ab]	8 ±2[b]	11 ±2[a]
Entodiniomorpha : Holotricha	38.4 ±11.2[b]	70.2 ±12.1[a]	70.9 ±19.4[a]
Total protozoa	301 ±65.1[c]	473 ±66.6[b]	714 ±67.5[a]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [a,b,c] Means in the same row with different superscripts differ ($p<0.05$).

Nutrient flow at the duodenum

Nutrient flows at the duodenum are presented in Tables 5 and 6. Diet had no effect on the proportion of FOM of OM intake. Supplementation of niacin increased apparent ruminal digestibility of NDF while values for digestibility of OM and ADF were unaffected by the respective diet (Table 5).

An inadequate supply of microorganisms with rumen degradable N resulted in decreased daily flows of N, NAN, MP, RUP, and uCP at the duodenum (Table 6). Also, the amount of RUP as percentage of CP intake decreased when diet RNB- was compared to RNB0. Diet RNB- also reduced the efficiency of MP synthesis per MJ ME (-13.7%), but enhanced the amount of MP per g of RDP (+10.4%) compared to animals fed the balanced diet. Diets had no effect on the amount of MP per kg FOM. Supplementation of 6 g niacin per day numerically increased most of the parameters of N and MP flow at the duodenum, but differences were not significant with the exception of amount of microbial protein per unit of RDP, which was elevated when diet NA was fed. The effects of the negative RNB on MP/MJ ME and on RUP were no longer significant after NA supplementation. Realized RNB, based on realized CP intake and measured uCP flow, was 0.26 g/MJ ME (±0.03) in diet RNB0 and -0.15 g/MJ ME (±0.04) in diets RNB- and NA (Table 6).

Table 5. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on nutrient flows at the duodenum, apparent rumen digestibilities and amount of fermented organic matter (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
OM [kg/d][*]	10.6 ±0.24	10.2 ±0.28	9.7 ±0.29
ARD [%][§]	37.5 ±1.12	36.9 ±1.31	39.9 ±1.37
NDF [kg/d]	3.7 ±0.05[ab]	3.8 ±0.06[a]	3.5 ±0.06[b]
ARD [%]	36.0 ±0.78[ab]	33.3 ±0.91[b]	39.1 ±0.95[a]
ADF [kg/d]	1.9 ±0.07	1.9 ±0.08	1.8 ±0.09
ARD [%]	32.2 ±1.83	31.2 ±2.09	34.5 ±2.23
FOM[kg/d][¥]	9.6 ±0.27	9.1 ±0.32	9.4 ±0.34
FOM of OM intake [%]	59.4 ±1.23	56.4 ±1.49	58.6 ±1.56

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]OM, organic matter; [§]ARD, apparent ruminal digestibility; [¥]FOM, fermented organic matter; [a,b,c] Means in the same row with different superscripts differ (p<0.05).

Table 6. Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on nitrogen flow at the duodenum and microbial crude protein synthesis (LSMeans with their standard errors)

	Experimental diets		
	RNB0[†] (n = 7)	RNB-[‡] (n = 6)	NA[#] (n = 5)
Nitrogen [g/d]	437 ±9.54[a]	366 ±12.32[b]	383 ±11.53[b]
Non-ammonia nitrogen [g/d]	415 ±9.11[a]	346 ±10.97[b]	348 ±11.72[b]
MP [g/d][*]	1901 ±53.1[a]	1590 ±65.1[b]	1666 ±62.3[b]
per FOM [g/kg][§]	202 ±8.82	170 ±10.81	185 ±10.35
per ME [g/MJ][¥]	10.2 ±0.27[a]	8.8 ±0.33[b]	9.3 ±0.32[ab]
per RDP [g/g][£]	0.77 ±0.02[c]	0.85 ±0.02[b]	0.91 ±0.02[a]
RUP [g/d][$]	428 ±16.5[a]	333 ±20.3[b]	369 ±19.4[ab]
[% of feed crude protein][1)]	22.0 ±1.00[a]	17.0 ±1.16[b]	18.7 ±1.20[ab]
RDP [g/d]	2423 ±33.4[a]	1776 ±39.2[b]	1745 ±40.9[b]
RNB [g/MJ ME]	0.26 ±0.03[a]	-0.15 ±0.04[b]	-0.15 ±0.04[b]
uCP [g/d][¶]	2335 ±50.9[a]	1930 ±65.3[b]	2022 ±62.2[b]

Notes: [†]RNB0, ruminal nitrogen balance in the diet = 0.08 g N/MJ ME; [‡]RNB-, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME; [#]NA, ruminal nitrogen balance in the diet = -0.41 g N/MJ ME plus 6 g niacin per day; [*]MP, microbial crude protein; [§]FOM, fermented organic matter; [¥]ME, metabolisable energy; [£]RDP, rumen degradable protein; [$]RUP, rumen undegradable protein; [¶]utilisable crude protein; [1)]corrected for urea nitrogen; [a,b,c] Means in the same row with different superscripts differ (p<0.05).

Discussion

Rumen measurements

In the present trial, pH in rumen fluid was unaffected by the amount of RDP in the diet. That complied with previous experiments (Song and Kennelly, 1990; Riemeier, 2004; Steinwidder et al., 2009). Only Monteils et al. (2002) found a decline in rumen pH when the CP level of the diet rose from 12% or 14% up to 16%, whereby the diet composition in that study was different for the different CP levels and the proportion of concentrate increased with increasing CP levels from 26% for the diet with 12% CP to 34% in the diet with 16% CP, which may have had more effects on ruminal pH than the CP content itself and may explain the differences to the findings in the current and the above mentioned experiments.

As in the present study, the supplementation of 6 g niacin per cow and day had no effect on rumen pH in most other trials (Kung et al., 1980; Horner et al., 1988; Doreau and Ottou, 1996). Madison-Anderson et al. (1997) administered 12 g nicotinic acid per cow and day without an impact on rumen pH. Only under *in vitro* conditions Riddell et al. (1980) described a decrease of pH in rumen fluid when niacin was supplemented with 1 g/L to the fermentation vessel, which was a considerably higher supplementation than in the present *in vivo* experiment. Not only higher doses of niacin in *in vitro* studies could be an explanation for the different findings. The absence of effects of supplemental niacin in *in vivo* trials could have been due to sufficient microbial synthesis of niacin in the rumen, which is not present under *in vitro* conditions (Ottou and Doreau, 1996).

Treatment did not alter total SCFA concentration in rumen fluid, according to the pH values. Also in other trials neither level of RNB (Lebzien et al., 2006; Agle et al., 2010) nor supplementation of niacin (Christensen et al., 1996; Ottou and Doreau, 1996; Madison-Anderson et al., 1997) had an effect on concentration of total SCFA in rumen fluid.

In an evaluation of published data, Harmeyer and Kollenkirchen (1989) assumed that *in vitro* experiments showed inconsistent effects of additional niacin on fermentation characteristics in rumen fluid and Niehoff et al. (2009a) summarized that the response of SCFA to niacin supplementation varied greatly among the data sets from *in vivo* trials.

In the present experiment, the molar proportion of acetic acid in rumen fluid was reduced in niacin supplemented animals. Christensen et al. (1996) also found a trend for reduced

acetic acid values after administering 12 g nicotinic acid per day to German Holstein cows. The presence of an enhanced number of protozoa may be an explanation for the current observations. In an *in vivo* study with sheep Dönmez et al. (2003) established a negative relationship between the acetic acid content and the number of protozoa in the rumen. The present results confirm these findings, as the number of protozoa in the NA group was higher (Table 4).

A trend for an enhanced content of propionic acid in rumen fluid after niacin supplementation was found in trials with cows (Christensen et al., 1996) and growing bulls (Flachowsky et al., 1993), while Madison-Anderson et al. (1997) found no effect. According to Itabashi and Kandatsu (1975), there is a positive correlation between number of protozoa and propionic acid release in the rumen which would match the present observations. In a former study, Riddell et al. (1980) found an increase of propionic acid content in the rumen after niacin supplementation only 6 h after feeding. That study (Riddell et al., 1980) demonstrated the possible impact of sampling time in relation to feeding time on the composition of ruminal SCFA and this might be a reason for inconsistent results reported in the literature.

Due to a reduced proportion of acetic acid and an enhanced percentage of propionic acid the ratio of these two SCFA was also reduced in the niacin treatment. This matched the findings of Riddell et al. (1980), who measured a decreased ratio at 3 and 6 h after feeding in cows given a diet based on hay and concentrate with a niacin concentration of 200 mg/kg fresh matter. However, the comparability with the present results is limited because the aforementioned authors (Riddell et al., 1980) did not show niacin concentration in DM.

In contrast to our results, butyric acid was the most affected SCFA by niacin supplementation in *in vivo* trials reviewed by Niehoff et al. (2009a), but the effects were inconsistent. It was assumed that an increasing number of protozoa led to effects on butyric acid concentration (Jouany, 1991) after niacin supplementation. This assumption is not in line with the present study and with the study of Samanta et al. (2000), as an increase in number of protozoa did not affect butyric acid concentration. These findings may indicate that, besides the effect of niacin on protozoa, there could be another way in which niacin altered SCFA production in the rumen.

Contrary to our results, Niehoff (2009) found a decrease in the molar proportions of valeric acid. However, *in vitro* studies showed elevated values for valeric acid proportions (Ottou

and Doreau, 1996) and Horner et al. (1988) found an effect on valeric acid proportion when a diet with a niacin concentration of 400 mg/kg DM was administered, but no effect was found when the same amount of nicotinamide was given to the fermenters. This may indicate different effects of niacin on rumen metabolism depending on the chemical structure of the supplemented niacin.

In most studies, different levels of nitrogen in the diet had no effect on the molar proportions of SCFA (Teather et al., 1980; Gabler and Heinrichs, 2003; Lebzien et al., 2006). In contrast to the aforementioned experiments and the present findings, Zimmerman et al. (1992) found an increase in the proportions of valeric acid, iso valeric acid, and iso butyric acid in cows fed a diet deficient in RDP with a moderate fibre content (35.8% NDF). According to Cline et al. (1958), in *in vitro* studies rumen organisms grown in a medium deficient in urea nitrogen appeared to intensively synthesize valeric acid. Hence, a deficiency in RDP may stimulate the synthesis of valeric acid in the rumen *in vivo.*

It is known that the branched chain fatty acids iso butyric acid and iso valeric acid originate from degradation of protein or amino acids in the rumen (Russell and Hespell, 1981) and that these SCFA are growth factors for several microbial species including cellulolytic bacteria (Allison and Bryant, 1958). In the present study, animals fed the N balanced diet showed a higher content of iso valeric acid in rumen fluid, whereas the proportion of iso butyric acid was unaffected by N content of the diet, which may indicate that the above mentioned production of some branched chain fatty acids is also stimulated by non-protein N.

Consistent with the present study, Cunningham et al. (1996) found an increased ratio of acetate to propionate when the CP content of the diet declined, whereby different amounts of soybean meal were used to change the CP content of the diet. Often, changes in content of CP or RDP of experimental diets were accompanied by changes in composition of the diets. These effects of diet composition may cover the effect of reduced N supply on the composition of SCFA and therefore lead to inconsistent results regarding the effect of reduced RDP supply on ruminal fermentation in literature data.

The measured ammonia concentration in rumen fluid was above the critical concentration of 3.6 mmol/L (Satter and Roffler, 1975) at all sampling times in the treatment RNB0. The ammonia concentration in the groups NA and RNB- were below this value at 90 minutes

and at 90 and 150 minutes after feeding, respectively (data not shown). These results were expected as the amount of RDP was higher in treatment RNB0.

In most studies, no effect of supplemental niacin on the ruminal ammonia concentration was found, neither *in vivo* (Kung et al., 1980; Zimmerman et al., 1992) nor *in vitro* (Hannah and Stern, 1985; Ottou and Doreau, 1996). Similar to the present results, Niehoff (2009) found elevated ammonia concentrations in rumen fluid after administering 6 g niacin per cow per day and in an earlier study a niacin supplementation of 200 mg/kg to a diet consisting of hay and concentrate resulted in an increase of ammonia 6 h after feeding (Riddell et al., 1980). The enhanced values might be explainable by the higher number of protozoa in rumen fluid after niacin administration. In several studies, faunated animals showed higher ammonia concentrations than defaunated animals (Eugène et al., 2004; Firkins et al., 2007) because protozoa contribute significantly to protein degradation and deamination, but are not able to use ammonia as an N source (Hristov and Jouany, 2005). Regarding the rumen ammonia concentration, it has also to be considered that there is a large diurnal variation in relation to time after feeding. According to Gustafsson and Palmquist (1993), the ammonia peak occurred 1.5 h to 2 h after feeding. This may partly explain some of the differences in the literature data because it cannot be excluded that some of the observed effects of niacin were rather due to diurnal variation in the rumen than a response to niacin.

The estimated values for the volume of rumen fluid and the turnover are within the range of those shown in other studies (Gasa et al., 1991; Reynolds et al., 2004), and Hartnell and Satter (1979) observed a high variation among individual cows in liquid fill of the rumen as well as in liquid turnover. In line with the present experiment, no influence of supplemental niacin (12 g per cow per day) on volume and outflow was found by Christensen et al. (1996) and Campbell et al. (1994).

As in several other studies (Erickson et al., 1990; Doreau and Ottou, 1996; Kumar and Dass 2005), number of protozoa was enhanced in niacin supplemented animals. As protozoa are unable to synthesize niacin (Brent and Bartley, 1984), they have to obtain niacin from feed or from rumen bacteria which can synthesize niacin (Menke, 1973).

The decreased protozoa count in animals fed diet RNB0 may be due to the presence of additional urea in the diet. Rumen protozoa are deficient in the enzyme urease which is responsible for hydrolysis of urea (Onodera, 1977).

Duodenal flow of nutrients and microbial protein

Numerous studies are available concerning the effect of RDP level on the apparent ruminal digestibility of nutrients. Lebzien et al. (2006) reported no effect of a reduction of the RNB (-0.3 and -0.6 g N/MJ ME) on degradability of OM and ADF, but they found a decreased apparent ruminal digestibility of NDF. Colmenero and Broderick (2006) also did not detect a change in apparent ruminal digestibility of OM, NDF and ADF when increasing the RDP content of the diet from 9.3 to 12.7% of DM calculated according to NRC (2001). It has to be considered that diet composition changed with changing RDP levels in the aforementioned study (Colmenero and Broderick, 2006) and therefore effects of changes in the diet composition may cover effects of N reduction. In *in vitro* studies, an amount of RDP of 8% of DM reduced the degradability of NDF compared to a diet with 11% of RDP (Griswold et al., 2003). The CP content of 12.2% of DM or rather the RNB of -0.41 g N/MJ ME in diet RNB- in the present study may not have been low enough to decrease the activity of the cellulolytic bacteria in the rumen. Alternatively, the microbial population may have adapted to the reduced N supply during the adaptation period before the sampling weeks because apparent ruminal digestibility of NDF was not affected (Table 5).

In the present study, effects of supplemental niacin on the daily flow of OM and on the amount of FOM as proportion of OM intake were not significant, which complies an *in vivo* study of Doreau and Ottou (1996), who also supplemented 6 g niacin per cow and day without an effect on apparent and true ruminal digestibility. Former *in vitro* studies also found no impact of niacin on OM degradation (Shields et al., 1983; Hannah and Stern, 1985). Niehoff (2009) observed a decreased apparent ruminal digestibility of OM independent of the forage-to-concentrate ratio in the diet when 6 g niacin were administered, but the amount of FOM was unaffected by treatment, which can be explained by the higher flow of MP to the duodenum after NA supplementation in that study. In the recent experiment, the unaffected liquid outflow rates and SCFA production in the rumen (Tables 3 and 4) matched the unchanged OM degradation rates. Studies concerning the effect of niacin on ruminal fibre digestion are scarce. Christensen et al. (1996) found no effect on ruminal degradation of ADF and NDF when 12 g niacin per day were given to dairy cows fed an N balanced diet and in an *in vitro* study an addition of 100 mg niacin per kg DM with sufficient N supply to the microbes resulted in slightly higher, but not significantly different values for the degradation of fibre fractions (Hannah and Stern, 1985). These results, as compared to the present trial where a diet with a negative

RNB was fed, may indicate that niacin had less beneficial effects on ruminal fibre degradation when the N supply for the microbial population is optimal. Since between 25% and 30% of the fibre breakdown in the rumen is performed by protozoa (Lee et al., 2000), this may explain the observed increase in ruminal NDF digestibility in the NA treatment as protozoal counts increased. Removal of protozoa decreased the rate of degradation of plant cell wall components in the rumen (Jouany, 1991) and was found to be associated with an increased retention time of plant particles (Jouany, 1996). Horner et al. (1988) also suspected a shift in rumen microbial population due to higher availability of niacin in the rumen, which resulted in an improved digestion of hemicelluloses.

The measured values for the RNB at the duodenum were slightly higher than calculated at the beginning of the experiment because treatments themselves had an effect on nutrient digestibilities and the ruminal N turnover. The N deficit in the rumen in treatment RNB- was sufficient to reduce microbial protein synthesis as compared to RNB0. In accordance with Lebzien et al. (2006), who worked with an RNB of -0.6 g/MJ ME, the microbes attempted to compensate for the deficit in RDP by increasing the feed CP degradation as can be seen from the decreased ratio of g RUP/g CP intake in the treatments RNB- and NA. As a result of the simultaneous reduction of the daily flows of MP and RUP at the duodenum, the amount of uCP was also reduced when diet RNB- was fed. The efficiency of use of RDP for the MP synthesis was elevated from 0.77 to 0.85 when N supply to the rumen became deficient.

Results from studies observing the effect of niacin on microbial protein flow at the duodenum are inconsistent. As in the present study, no effects have been found after administering 6 or 12 g of niacin/d to dairy cows in some studies (Christensen et al., 1996; Doreau and Ottou, 1996), whereas other researchers reported enhanced daily amounts of MP flow after niacin supplementation *in vitro* (Riddell et al., 1980) and in beef cattle (Kumar and Dass, 2005). Niehoff (2009) observed an increase in the flow of MP by 250 g/d when 6 g of niacin were supplemented, whereby these authors, contrary to the present trial, fed a diet with an RNB according to the requirement of the microbes. The unchanged amounts of MP at the duodenum after niacin supplementation in the current study seem to be contradictory because of higher numbers of protozoa. Faunated animals normally show decreased efficiencies of microbial CP synthesis due to bacterial predation by protozoa (Firkins et al., 2007). Due to the fact that, depending on the diet, between 20 and 40% of the total microbial N at the duodenum could originate from protozoa (Sylvester et al.,

2005), it seems to be possible that the composition of the microbial protein reaching the duodenum changed in NA treatment. The proportion of protozoal protein might have increased and overcompensated for the expected decrease in the amount of bacterial protein. This assumption of an increasing flow of protozoal protein to the duodenum was in line with other results from the same trial (Aschemann et al., 2012) as the faecal N excretion was reduced in the niacin supplemented animals. It is known that the digestibility of protozoal protein ranged between 87 and 91%, while bacterial protein had lower digestibilities between 74 and 79% (Owens and Zinn, 1988). Thus, microbial protein containing a higher proportion of protozoal protein may have been digested more comprehensive in the small intestine and therefore, the N excretion with faeces was reduced in niacin treatment.

As assumed by Firkins et al. (2007) and Niehoff (2009), the predation of bacteria by protozoa may be reduced due to a higher ruminal liquid passage rate after niacin supplementation. This assumption could not be supported by the present trial, as the rumen volume and the outflow rates were not different between the three treatments.

The amount of RUP remained unchanged in NA treatment. Hence, there were no differences in the amount of dietary protein digested in the rumen, which might seem to be inconsequent regarding the higher amounts of ammonia in rumen fluid, but the possible reasons for this higher amount of ammonia, founded in the changes of the microbial community in the rumen, are discussed above. The results from other *in vivo* studies concerning the effect of niacin on the degradation of feed CP are inconsistent and the detailed mechanism of impact of niacin on the proteolytic activity in the rumen is not clarified yet. Doreau and Ottou (1996) found a trend for increased RUP flows when niacin was supplemented, whereas no effect of niacin has been observed *in vitro* (Hannah and Stern, 1985) and the opposite trend has been shown in another *in vivo* trial (Horner et al., 1988).

The amount of MP expressed per g of RDP increased from 0.85 to 0.91, which implies an elevated efficiency of MP synthesis from ruminally degraded protein. These results correspond with additional findings from the same trial (Aschemann et al., 2012), as an elevated N balance and a more efficient use of dietary N were observed when niacin was added to the diet deficient in RNB.

Conclusion

A negative rumen nitrogen balance (RNB) decreased the ammonia content in rumen fluid and the flow of microbial crude protein at the duodenum (MP), whereas the use efficiency of rumen degradable protein (RDP) for microbial synthesis was enhanced. Furthermore, the negative RNB induced changes in the microbial population and therefore the ruminal fermentation pattern was affected.

Addition of 6 g niacin per cow and day to the RNB deficient diet enhanced ruminal degradation of NDF. The amount of MP reaching the duodenum per day was unaffected, but the efficiency of MP synthesis from RDP was elevated. The decreasing effects of the negative RNB on rumen undegradable protein and MP/MJ ME were no longer significant when niacin was administered. The increase in number of protozoa in niacin supplemented animals seems to be a major reason for the observed effects of niacin on rumen metabolism and further research should clarify the detailed impact of supplemental niacin on the rumen microbial community and the composition of MP reaching the duodenum. The use of supplemental niacin may be an attempt to compensate to some extent for the negative effects of a reduced N intake of dairy cows on the supply with utilisable crude protein.

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Chapter 5: General Conclusion

The aim of the present thesis was to evaluate the use of supplemental niacin to improve the efficiency of microbial protein synthesis in the rumen during feeding a diet deficient in rumen degradable protein as a possibility to reduce nitrogen emissions from dairy cows without diminishing the supply with utilisable crude protein.

As expected, the deficit in rumen degradable protein in the diet entailed several effects on rumen metabolism. Since these principal interrelationships are well known, hereafter, general conclusions drawn from the supplementation of niacin to compensate for these effects are focused.

The supplementation of 6 g niacin/(cow · day) to a rumen degradable protein deficient diet changed fermentation patterns in the rumen resulting in a more propionate-pronounced fermentation. In addition, the average ammonia concentration in rumen fluid increased. Niacin supplementation furthermore resulted in greater fibre degradation in the rumen as could be seen from the increased apparent ruminal and total tract digestibility of neutral detergent fibre in niacin treatment.

These effects on rumen metabolism may possibly be traced back to the increased number of protozoa in rumen fluid in supplemented animals, causing a change in activity and/or composition of the rumen microbial community, resulting in higher ammonia production, and a more comprehensive fibre digestion.

Although, normally an increasing number of protozoa reduces the efficiency of the synthesis of microbial protein due to predation of bacteria, in the present study the flow of microbial crude protein to the duodenum was not impaired by a higher concentration of protozoa in rumen fluid. Therefore, an overcompensation of the expected reduced flow of bacterial protein by increasing amounts of protozoal protein appeared likely.

The use efficiency of rumen degraded protein for microbial synthesis was elevated when niacin was supplemented, as could be seen from higher amounts of microbial crude protein per g of rumen degraded crude protein. Furthermore, the decreasing effects of deficient supply with rumen degradable protein on the efficiency of microbial crude protein synthesis (g of microbial crude protein per MJ metabolisable energy) and on rumen undegraded protein were no longer significant.

Faecal nitrogen excretion was the lowest in animals receiving the niacin supplemented diet, although, the amount of utilisable crude protein reaching the duodenum was unaffected. Consequently, the digestibility of utilisable crude protein was higher when niacin was administered, which may be the result of a better digestibility of protozoal protein compared to bacterial protein in the small intestine. Another explanation for the reduced nitrogen content in faeces may be that, due to a more comprehensive degradation in the rumen, less amounts of carbohydrates, in particular neutral detergent fibre reached the colon and resulted in restrained bacterial growth.

Contrary to the nitrogen excretion with faeces, urinary and milk nitrogen excretions were unchanged by niacin administration. Thus, the nitrogen balance was enhanced which indicated that more nitrogen was retained in the body of the animals.

Further research should concentrate on the fate of this balance nitrogen in the animal and on possible metabolic losses, as well as on methodical aspects of estimating the nitrogen balance. Additional studies should clarify the detailed impact of supplemental niacin on the rumen microbial community, especially the effect on proteolytic and cellulolytic activity. Also the shift in the composition of the microbial population and the composition of microbial crude protein reaching the duodenum should be investigated.

The use of supplemental niacin may be an attempt to compensate to some extent for the restraining effects of a reduced supply with rumen degradable protein on the ruminal fermentation and the amount of utilisable crude protein.

With regard to the environment and from an animal nutrition point of view, this seems to be a possible approach for a reduction of the nitrogen emitting potential in milk production.

Summary

Nitrogen (N) emissions from agricultural animal husbandry have attracted increasing attention during the last few years. In particular, N losses from ruminants were coming into focus because the average use efficiency of dietary N in dairy cattle amounts only to 28% with a wide range of variation between 16 and 40%. Because N losses with manure are rather high and positively correlated with the N intake of the animals, one attempt to reduce the N emissions of dairy cows is to reduce the N intake. However, this approach would result in decreased ruminal fermentation as the microbial population in the rumen responds very susceptible to limited N supply. Consequently, the flow of microbial protein to the duodenum would be reduced and the degradation of feed components would be restrained when the rumen nitrogen balance (RNB) in the diet is negative.

The B-vitamin niacin is part of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) and therefore it is involved in several energy providing processes in the metabolism. Besides niacin from feed and tryptophan catabolism, ruminants can use niacin synthesized by rumen microbes. However, several studies showed that additional oral administration of niacin not only improved the energy balance of lactating dairy cows, but also enhanced the flow of microbial crude protein at the duodenum (MP).

Therefore, in this thesis, the effect of an oral niacin supplementation to a diet deficient in ruminally degradable protein (RDP) on rumen metabolism and microbial protein synthesis as well as on N balance and N utilisation in lactating cows was investigated and it should be assessed to what extend a niacin supplementation can compensate for the above mentioned restricting effects of a negative RNB on rumen fermentation.

A total of 9 ruminally and duodenally fistulated lactating multiparous German Holstein cows was used. The fed diets varied as follows: RNB0 with energy, utilisable crude protein (uCP), and RNB (0.08 g N/MJ ME) according to the average requirement of the animals; RNB- with energy and uCP at the duodenum according to the average requirement of the animals, but with a negative RNB (-0.41 g N/MJ ME); and diet NA which was the same ration as RNB-, but supplemented with 6 g niacin/d.

For determining the N balance, the N excretion, and the total tract digestibility, faeces and urine were collected completely over five consecutive days and samples of milk were taken on two consecutive days. Furthermore, blood samples were taken from a *Vena jugularis externa* on one day prefeeding and 2.5 h postfeeding. Rumen fluid was taken on one day just before feeding and 6 times after feeding in the morning. The rumen liquid volume and liquid turnover rate were determined using cobalt-EDTA as a marker and the protozoal population was investigated. Duodenal chyme was collected every two hours over five consecutive days, using chromium oxide as a marker to calculate ruminal digestibilities of nutrients and MP synthesis.

Reducing the amount of RDP in the diet caused several effects in the N metabolism of the animals. The negative RNB in diet RNB- reduced N excretion with urine, the total N excreted with urine and faeces, and the N balance. The supplementation of 6 g niacin per cow and day to the diet with a negative RNB had no effect on N excretion with urine, but the daily amount of N excreted with faeces was lower, whereas the N balance was elevated.

Plasma urea content was decreased with deficient N supply, but remained unchanged by addition of niacin, whereas the postfeeding plasma glucose concentration was higher after niacin feeding.

Milk yield and contents of fat and protein were not altered by treatment, but a negative RNB enhanced milk N efficiency and reduced milk urea content.

Total tract digestibility of dry matter and organic matter were decreased with diet RNB- as compared to treatment RNB0. The degradability of neutral detergent fibre (NDF) also decreased with reduced N supply, but niacin supplementation could compensate for this decline which could be seen from an enhanced NDF digestibility in treatment NA, which was due to a more comprehensive fermentation of NDF in the rumen.

Ammonia content in rumen fluid was lower with diet RNB-, but enhanced after niacin administration. The negative RNB in treatment RNB- affected the composition of the short chain fatty acids in rumen fluid and increased the number of protozoa. Furthermore, the daily duodenal flows of MP, rumen undegradable protein (RUP) and uCP as well as the amount of MP per MJ ME were reduced as compared to treatment RNB0. However, the use efficiency of ruminally degradable N for microbial synthesis was enhanced.

The amount of MP reaching the duodenum per day was unaffected by niacin administration, but the efficiency of MP synthesis from RDP was elevated compared to RNB-. After niacin supplementation, the effects of the negative RNB on the amount of MP per MJ ME and on RUP were no longer significant. The number of protozoa was higher in NA treatment as compared to RNB-. This observed change in the microbial population in niacin supplemented animals may be the reason for reduced effects of the negative RNB on rumen fermentation parameters

In conclusion, supplemental niacin to diets with a negative RNB induced a more efficient use of rumen degraded N. This effect may mainly be attributed to a shift in the rumen microbial community or a change in the fermentation activity due to an increased number of protozoa, which may have led to modifications of rumen metabolism and changes in the composition of MP reaching the duodenum. Regarding the environmental impact of milk production, these results may provide a potential approach for the reduction of nitrogen emissions originating from dairy cows without compromising production performance.

Zusammenfassung

Die Emissionen von Stickstoff (N) aus der landwirtschaftlichen Tierhaltung haben in den letzten Jahren immer mehr an Beachtung gewonnen. Insbesondere die Stickstoffausscheidungen von Wiederkäuern gerieten zunehmend ins Blickfeld, denn die Nutzungseffizienz von Futter-N bei Milchkühen beträgt im Durchschnitt nur etwa 28 % mit großen Schwankungen zwischen 16 und 40 %. Da die N-Verluste mit Kot und Harn sehr hoch sind und stark positiv mit der N-Aufnahme korrelieren, ist die Reduzierung der N-Aufnahme ein möglicher Ansatzpunkt zur Reduzierung der Emissionen. Aus einer reduzierten N-Aufnahme würden jedoch negative Effekte auf die Pansenfermentation resultieren, da die mikrobielle Population sehr empfindlich auf eine restriktive N-Versorgung reagiert. So wurde beispielsweise der Fluss von mikrobiellem Protein am Dünndarm reduziert und die ruminale Verdaulichkeit der Ration wurde herabgesetzt, wenn die ruminale N-Bilanz (RNB) der Ration negative war.

Das B-Vitamin Niacin übernimmt als Bestandteil der Co-Enzyme Nicotinamid-Adenine-Dinucleotide (NAD) und Nicotinamid-Adenin-Dinucleotid-Phosphat (NADP) eine wichtige Rolle in zahlreichen Vorgängen des Energiemetabolismus. Neben Niacin aus dem Futter und dem Tryptophan-Katabolismus können Wiederkäuer als zusätzliche Quelle auch das von den Mikroben im Pansen synthetisierte Niacin nutzen. Dennoch zeigten Studien, dass eine zusätzliche Supplementation von Niacin nicht nur die Energiebilanz laktierender Milchkühe verbesserte, sondern auch den Fluss von mikrobiellem Rohprotein am Dünndarm (MP) erhöhte.

Daher war es Ziel der vorliegenden Arbeit, den Effekt einer Niacin-Supplementation zu einer Ration mit negativer RNB auf den Pansenmetabolismus und die mikrobielle Proteinsynthese, sowie auf die N-Bilanz und die N-Verwertung von laktierenden Kühen zu untersuchen. Des Weiteren sollte abgeschätzt werden, in welchem Ausmaß eine Supplementation von Niacin zu einer Ration mit negativer RNB die oben genannten restriktiven Wirkungen eines Mangels an pansenverfügbarem N auf die Pansenfermentation kompensieren kann.

Die Studie wurde mit insgesamt neun pansen- und dünndarmfistulierten laktierenden Deutschen Holstein Kühen durchgeführt. Die gefütterten Rationen waren wie folgt konzipiert: RNB0: Energiegehalt und Menge an nutzbarem Rohprotein (nXP)

entsprechend dem Bedarf der Tiere mit einer ausgeglichene RNB (0,08 g N/MJ ME); RNB-: Energie- und nXP-Gehalt entsprechend dem Bedarf der Tiere, jedoch mit negativer RNB (-0,41 g N/MJ ME); NA: Ration identisch mit RNB-, jedoch mit zusätzlicher Gabe von 6 g Niacin je Tier und Tag.

Zur Kalkulation der N-Bilanz, der N-Ausscheidung und der Verdaulichkeit der Rohnährstoffe wurde eine Totalsammlung von Kot und Harn über einen Zeitraum von fünf Tagen durchgeführt und es wurden an zwei Tagen Milchproben gewonnen. Außerdem wurden an einem Tag, jeweils direkt vor und zweieinhalb Stunden nach der Morgenfütterung, Blutproben aus einer *Vena jugularis externa* entnommen. Pansenflüssigkeit wurde an einem Tag direkt vor und zu 6 Zeitpunkten nach der Fütterung entnommen, außerdem erfolgte eine Probenahme zur Untersuchung der Protozoen-Population. Zur Abschätzung des Pansenflüssigkeitsvolumens und zur Berechnung des Flüssigkeits-Turnovers im Pansen wurde Cobalt-EDTA als Marker verwendet. Darmchymus wurde im zweistündigen Intervall an fünf aufeinanderfolgenden Tagen entnommen, wobei Chromoxid als Flussmarker verwendet wurde um die ruminale Verdaulichkeit sowie die synthetisierte Menge MP abschätzen zu können.

Die Reduzierung des Gehaltes an pansenverfügbarem Stickstoff in der Ration führte zu verschiedenen Effekten auf den Stickstoffmetabolismus der Tiere. Die negative RNB in der Behandlung RNB- reduzierte die Stickstoffausscheidung mit dem Harn, sowie die Gesamtausscheidung über Kot und Harn und die Stickstoffbilanz. Die zusätzliche Gabe von 6 g Niacin pro Tag zu dieser Ration hatte keine Effekte auf die tägliche Stickstoffausscheidung mit dem Harn, während die Ausscheidung mit dem Kot verringert und die Stickstoffbilanz erhöht war.

Der Harnstoffgehalt im Plasma wurde durch die negative RNB reduziert, wurde aber durch Niacin nicht beeinflusst, während die Glukosekonzentration im Plasma in der Behandlung NA nach dem Füttern erhöht war.

Die Behandlung zeigte keinen Effekt auf die Milchleistung, sowie die Gehalte an Fett und Eiweiß in der Milch. Jedoch erhöhte die negative RNB die Milch-N-Effizienz und verringerte den Milchharnstoffgehalt.

Die Verdaulichkeit der Trockensubstanz und der organischen Masse waren bei reduzierter Versorgung mit pansenverfügbarem N in der Gruppe RNB- herabgesetzt. Auch die totale Verdaulichkeit der Neutralen-Detergenzien-Faser (NDF) war in dieser Behandlung

verringert. Jedoch konnte die Supplementation von Niacin zur Ration mit negativer RNB (Behandlung NA) diesen Rückgang durch eine bessere Verdaulichkeit der NDF im Pansen kompensieren, was anhand der höheren ruminalen Verdaulichkeit der NDF nach Fütterung der supplementierten Ration deutlich wurde.

Der Ammoniakgehalt der Pansenflüssigkeit war bei reduzierter N-Versorgung verringert, die Niacin-Supplementation erhöhte jedoch den Ammoniakgehalt. Die reduzierte N Versorgung beeinflusste das Fettsäuremuster der Pansenflüssigkeit und erhöhte die Protozoenkonzentration. Außerdem waren der MP-Fluss, sowie die Mengen an nXP und unabgebautem Futterprotein (RUP) geringer als bei ausgeglichener RNB und die Menge MP pro MJ ME war reduziert. Jedoch zeigte sich eine höhere Nutzungseffizienz des im Pansen abgebauten Proteins für die mikrobielle Synthese.

Die synthetisierte Menge MP war durch die Niacingabe nicht erhöht aber die Effizienz der mikrobiellen Synthese aus pansenverfügbarem Stickstoff war gesteigert und Effekte der negativen RNB auf die Menge an RUP und die Menge an MP pro MJ ME waren nicht mehr signifikant. Die Protozoen-Konzentration in der Pansenflüssigkeit der supplementierten Tiere war im Vergleich zur Behandlung RNB- höher. Die beobachteten Veränderungen in der Zusammensetzung der mikrobiellen Population durch den Zusatz von Niacin könnte der Grund für die Reduktion der oben genannten Effekte des N-Mangels auf die Pansenfermentation sein.

Aus der vorliegenden Arbeit kann geschlussfolgert werden, dass eine Niacin-Supplementation bei Rationen mit negativer RNB zu einer effizienteren Nutzung des pansenverfügbaren N führt. Dieser Effekt ist vermutlich auf eine Veränderung der Mikrobenpopualtion im Pansen zurückzuführen, die hauptsächlich in einer steigenden Anzahl von Protozoen begründet ist und zu einer Modifikation des Pansenmetabolismus, sowie zu einer veränderten Zusammensetzung des am Dünndarm anflutenden MP führt. Im Hinblick auf die umweltrelevanten Emissionen aus der Milchviehhaltung liefern diese Ergebnisse einen möglichen Ansatz zur Reduzierung der N-Ausscheidungen ohne die Leistung von Milchkühen nachhaltig zu beeinträchtigen.

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