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der
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Methods for estimation and nutritional influencing of thermoregulation and heat
stress in dairy cows and sheep as well as impacts of changing climatic
conditions on the feed value of maize silage

Dissertation

zur Erlangung des akademischen Grades
doctor agriculturarum (Dr. agr.)

vorgelegt von

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Verteidigung am:

21.01.2013

Halle/Saale 2013

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ABBREVIATIONS

Ash	Crude ash
ADL	Acid detergent lignin
ADF	Acid detergent fibre
ADF D	Acid detergent fibre digestibility
A_w	Water activity
B	Biomass
BPM	Breaths per minute
CC	Climatic conditions
CF	Crude fibre
CF D	Crude fibre digestibility
CI	Confidence interval
CP	Crude protein
DEE	Digestible ether extract
DCF	Digestible crude fibre
DIM	Days in milk
DMI	Dry matter intake
DOM	Digestible organic matter
DON	Deoxynivalenol
DM	Dry matter
DS	Drought stress
EE	Ether extract
ELISA	Enzyme-linked Immunosorbent Assay
Ery	Erythrocytes
FACE	Free air carbon dioxide enrichment
FCM	Fat corrected milk
FLI	Friedrich-Loeffler-Institute
GfE	Society of Nutrition Physiology
GPR109A	G protein-coupled receptor 109A
HPLC	High performance liquid chromatography
I	Irrigation
IAC	Immunoaffinity columns
IPCC	Intergovernmental Panel on Climate Change

ISFET	Ion-selective field-effect transistor
IU	International units
Y	Yield
KLIFF	Climate impact and adaptation research in Lower Saxony
LAVES	Lower Saxony State Office for Consumer Protection and Food Safety
LC-ESI-MS /MS	Liquid chromatography-electrospray ionization tandem mass spectrometry
LSmeans	Least square means
ME	Metabolizable energy
MH	Mild heat
N.d.	Not determined
NDF	Neutral detergent fibre
NDF D	Neutral detergent fibre digestibility
NEL	Net energy lactation
N.s.	Not significant
OD	Optical density
OM	Organic matter
OM D	Organic matter digestibility
OTA	Ochratoxin A
PEP	Phosphoenol pyruvate
PMR	Partial mixed ration
ppb	Parts per billion
ppm	Parts per million
REML	Restricted maximum likelihood method
Rectal temp/T	Rectal temperature
RH	Relative humidity
RPMI	Roswell Park Memorial Institute
RR	Respiration rate
RUBISCO	Ribulose-1,5-biphosphate-carboxylase/-oxygenase
RUBP	Ribulose 1,5-biphosphate
SARA	Sub-acute ruminal acidosis
SCFA	Short chain fatty acid
SD	Standard deviation
SE	Standard error

SH	Severe heat
Skin temp	Skin temperature
Starch D	Starch digestibility
Subcutaneous T/ Sub T	Subcutaneous temperature
T	Temperate
TD	Dry bulb temperature
THI	Temperature-humidity index
TMR	Total mixed ration
UV	Ultraviolet
VTI	Johann Heinrich von Thünen-Institute
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten
WW	Well watered
ZON	Zearalenone

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Introduction

“A huge increase in the demand of animal production is expected in the next decades. Food and water security will be one of the other priorities for humankind in the 21st century. Over the same period the world will experience a change in the global climate that will cause shifts in local climate that will impact on local and global agriculture“.

(Nardone et al., 2010)

The climatic changes in the last 150 years and the projected alterations within the next decades are drastic and will probably be largely irreversible for more than 1000 years (Solomon et al., 2009). As a consequence, considerable changes in biological and physical systems are already occurring throughout the world and the majority of these shifts are in the direction expected with increasing temperature (Rosenzweig et al., 2008). Though the implications of climatic alterations will obviously not be limited to effects on agriculture, potential impacts on feed and food production are numerous, may in parts interact in a complex manner and will challenge future agriculture in adapting to substantial changes of the known environmental conditions.

Some of the most important modifications are changes in atmospheric CO₂ concentration, global surface temperature and precipitation. The atmospheric CO₂ concentration has increased from a pre-industrial level of approximately 280 parts per million (ppm) to 385 ppm in 2008 and is predicted to approach 1000 ppm by the end of the 21st century (Meehl et al., 2007). Mean global surface temperatures have increased by about 0.6 °C since the 1970s. Moreover, the projected elevations in dependence of the prospective scenario of greenhouse gas emissions are in the range of 1.8 to 4.0 °C until 2100 but the warming over land will be approximately twice than the mean total warming (Meehl et al., 2007). Shifts in annual precipitation patterns are likely to occur and summer rainfall and soil moisture are prognosticated to be reduced in central and northern Europe probably leading to a growing incidence of drought in these regions (Raisanen et al., 2004; Boe et al., 2009).

Elevated carbon dioxide concentrations were found to increase the rates of photosynthesis but to decrease the water loss of C₃ plants, a process often referred to as “CO₂-fertilization effect” (Long et al., 2004). However, the yield-stimulating impacts of CO₂ were accompanied by substantial reductions of the feed quality of C₃ crops due to decreased contents of protein and amino acids (Högy and Fangmeier 2008; Erbs et al., 2010; Manderscheid et al., 2010). In C₄ plants such as maize (*Zea mays L.*), CO₂ is concentrated 3 to 10 times higher than in the current atmosphere and due to this local concentration C₄ photosynthesis should be saturated

at present atmospheric CO₂ (Ghannoum et al., 2000). However, uncertainty exists about the actual responses of the feed value of ensiled maize, which is used prevalently as a major component of ruminant diets, to increased carbon dioxide. For example, rising concentrations of CO₂ were observed to decrease the stomatal conductance of maize plants (Leakey et al., 2006) and may thus reduce the adverse effects of increasing future drought on maize growth and composition (Zinselmeier et al., 1999; Monneveux et al., 2006). Furthermore, both CO₂ concentration and water activity (a_w) can affect the growth of fungi such as *Fusarium* moulds that may produce toxic secondary metabolites, mycotoxins, like deoxynivalenol (DON) and zearalenone (ZON) which diminish the quality of contaminated feedstuff (Kokkonen et al., 2010; Magan et al., 2011). It is unknown, whether modified growth conditions of maize and increased ambient temperatures during feeding of the generated silages will have interactive effects on blood and immunological parameters of ruminants or the metabolization of DON in the rumen.

Heat stress is known to impair health and productivity of dairy cows (Kadzere et al., 2002). Elevated future ambient temperatures will probably increase both incidence and severity of heat and therefore effective countermeasures are necessary to alleviate its adverse effects. Increasing the proportion of concentrate at the expense of roughage can reduce the dietary heat increment and thus contribute to heat stress relief in ruminants (West, 1999). In dairy cows, the dietary supplementation of niacin was tested as niacin is known to have vasodilatative effects and could thus elevate the dissipation of body heat by a rising skin blood flow (DiCostanzo et al., 1997; Zimbelman et al., 2010). However, experimental results were inconsistent and more information is needed to assess the potential of niacin to alleviate the negative impacts of heat.

In ruminants, the applicability of high concentrate diets is generally restricted as they induce decreases in ruminal pH and may lead to sub-acute ruminal acidosis (SARA), a digestive disorder that was shown to affect cow health and performance in a negative fashion (Enemark, 2008). In the last years, several wireless probes were tested which serve to measure ruminal pH and temperature continuously. Such devices may contribute to the diagnosis of SARA and improve the current understanding of heat stress implications. However, the validity of the obtained results may be reduced by low pH sensor accuracy, pH sensor drift or pH gradients within the reticulorumen (Enemark et al., 2003; AlZahal et al., 2008; Kaur et al., 2010).

Background

1 Climate change

1.1 Past observations

The term “climate change” refers to alterations in the mean climate or its variability that last for extended periods and has to be differentiated from internal climatic variation that is typically present at all times (Hegerl et al., 2007).

Current climatic warming is evident basing on observations of elevations in mean air and ocean temperatures, prevalent melting of ice and snow and rising average sea levels and is contrary to the previous trend of global cooling during the last 2000 years (**Figure 1**). Global surface temperatures maintain a strong trend towards continuous warming and they have risen by approximately 0.6 °C since the 1970s. The total temperature increases in 2001-2005 compared to 1850-1899 were approximately 0.76 °C (IPCC, 2007). Moreover, the trend of linear warming during the last 50 years is almost twice than for the last 100 years and the 25 years trend for the period ending 2008 was an ongoing warming of approximately 0.2 °C per decade. This process is reflected in mean annual temperatures as, for example, every year between 2001 and 2008 has been among the 10 warmest years since the beginning of instrumental recording despite a decrease of solar forcing (Allison et al., 2009).

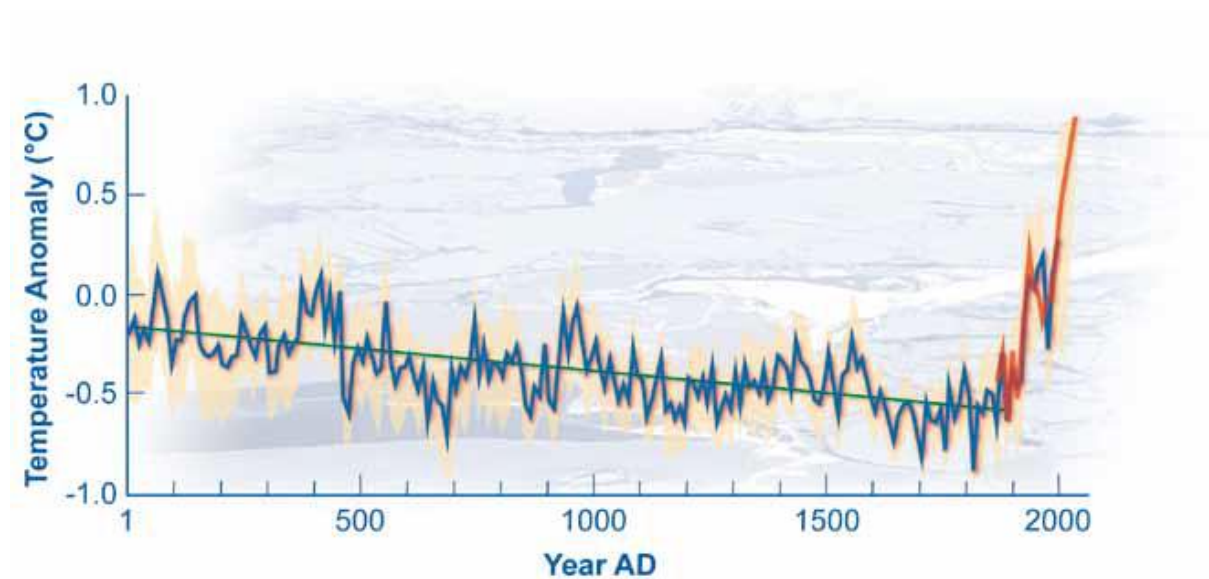


Figure 1: Estimates of Arctic air temperatures over the last 2000 years based on proxy records from lake sediments, ice cores and tree rings. The best-fit line shows the long-term cooling trend for the period ending 1900. Adapted from Allison et al. (2009)

Substantial climatic alterations are not a new phenomenon because the system of the earth was subjected to different large-scale climate changes throughout the history. For example, the Mid-Pliocene (About 3.3 to 3 million years ago) was the most recent period with considerably warmer temperatures than nowadays in the range of 2 to 3 °C (Sloan et al., 1996; Haywood et al., 2000). Current climatic reconstructions for the so-called last glacial maximum, between 26500 and 19000 years ago, are of relatively high quality and simulations demonstrated a colder and dryer climate with a global cooling of about 3.5 to 5.2 °C (Hegerl et al., 2007). Alterations in the concentrations of so-called greenhouse gases, gases that absorb and emit radiation within the thermal infrared range, accounted for approximately 50 % of the simulated cooling in the tropics during this period (Shin et al., 2003). Various climatic changes in the past were initiated by different factors long before human impacts might have been substantial, a fact that does not necessarily imply that the current modifications are natural. Hegerl et al. (2007) suggested three pivotal factors that may have played a role in the modifications of the global climate: (1) Changed amounts of the incoming solar radiation (Alterations associated to the sun itself or changes in the orbit of the earth, so-called Milankovitch cycles). (2) Changes in the reflected solar radiation (Changes in cloud cover, land cover or aerosols, for example). (3) Changes in the long-wave energy that is radiated back to space (By alterations in concentrations of greenhouse gases).

1.2 Driving forces and human impact

Are the current changes and the climatic alterations observed in the past decades of anthropogenic origin or induced by natural processes?

Though not all aspects of the present climate change are unusual, there is a high probability of an enormous anthropogenic contribution. Hegerl et al. (2007) suggested that it is extremely unlikely (<5 %) that the global warming during the last 50 years can be explained without the impact of external forces such as impacts related to human acting. Furthermore, it is very unlikely that natural forces alone may be responsible for the fast changes in a period where natural processes should otherwise have generated a cooling effect. The atmospheric concentrations of different greenhouse gases such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), halocarbons and sulphur hexafluoride (SF₆) have mainly increased dramatically within the last 250 years and it is very likely that they are responsible for the main part of global surface warming during the last decades (Hegerl et al., 2007). The increases in the concentration of atmospheric CO₂ are primarily due to the human use of fossil fuel and, to a smaller extent, to changes in land use. Agriculture is likely to be the main cause

of methane and nitrous oxide emissions (IPCC, 2007). Attribution analysis has revealed that the warming induced by greenhouse gases alone actually would have been greater than the increases observed in the past 50 years if opposing cooling effects of, for example, aerosols would not have reduced the rises in global surface temperature (Stott et al., 2006).

CO₂ is the most important anthropogenic greenhouse gas. Its concentration is now known from Antarctic ice cores for the last 650000 years and the present concentration represents the highest value at any time in that period and possibly the last 3 to 20 million years (Raymo et al., 1996; Luthi et al., 2008; Tripati et al., 2009). It has increased rapidly from a pre-industrial level of about 280 ppm by approximately 100 ppm or 36 % to 379 ppm in 2005 (Forster et al., 2007). Furthermore, atmospheric methane has risen from varying levels during the last 650000 years approximating 400 parts per billion (ppb) during glacial periods to 700 ppb during interglacials (Spahni et al., 2005) to the highest values throughout the complete period approximating 1770 ppb in 2005 (Forster et al., 2007). In the meantime, the annual growth rate of CH₄ has decreased from about 1 % at the late 1970s (Blake and Rowland, 1988) to almost zero at the end of the 1990s (Dlugokencky et al., 1998). Basing on ice core data from Antarctica, the atmospheric N₂O concentration was relatively stable until the year 1800 and was then subjected to fast elevations, though the increases were almost linearly during the last decades (Approximately 0.26 % per year) and the total rises since 1750 (270 ppb) cumulated to a concentration of 319 ppb in 2005 (Forster et al., 2007).

1.3 Future projections

Projections of future climate involve various models which consider a variety of possible emission scenarios of greenhouse gases as well as other atmospheric constituents and relevant factors. The concentration of CO₂ is likely to increase to 730 - 1020 ppm until 2100 (Meehl et al., 2007). An atmospheric CO₂ concentration of more than 1000 ppm by the end of the century would represent a 300 % elevation of the central greenhouse gas since 1750. Carbon dioxide is known to have a very long lifetime in the atmosphere and its concentration will be to a large extent irreversible for 1000 years after emissions stop (Solomon et al., 2009). Therefore, the current greenhouse gas output defines the climate many future generations have to face and CO₂ emissions similar to the known reserves of fossil fuel would take more than 2000 years to be absorbed from the atmosphere (Eby et al., 2009). Within the twenty-first century, the mean global surface temperature is predicted to increase in the range of 1.8 to 4.0 °C and the temperature at which the warming will stop depends on the total amount of greenhouse gas emissions since the beginning of industrialization and the

overall burning of fossil fuel (Meinshausen et al., 2009; Allen et al., 2009). The warming is likely to show explicit spatial differences (**Figure 2**) because the increases in average surface temperatures will be highest over land and at high northern latitudes. The warming over land will be approximately twice than the mean warming of surface temperatures (Meehl et al., 2007), a forecast that drastically illustrates the fundamental future alterations. In the same report it was stated that an increased incidence of extreme events of surface temperatures and precipitation is likely as, for example, heat waves are expected to occur more frequently, to last longer and to be more intensive. Though the total future global precipitation is projected to rise, considerable regional differences are likely to occur. Different areas such as the Mediterranean region that are already frequently affected by drought will face considerable reductions in precipitation of about 20 % (Rowell and Jones, 2006).

In Europe, the observed increases in mean surface temperatures were higher than the global mean and amounted 0.91 °C from 1901 to 2005 (Jones and Moberg, 2003). Future Europe undergoes a trend of warming in the range of 1 to 5.5 °C in all seasons until the end of the century and beyond (Alcamo et al., 2007). The European temperature change is characterized by substantial spatial distinctions. For northern Europe climatic modeling revealed larger warming in winter than in summer but the reverse for central and southern Europe (Christensen and Christensen, 2007). For example, southern Europe including parts of France and Spain will be affected by incisive increases in summer temperatures that partially exceed 6 °C (Kjellstrom, 2004; Good et al., 2006). In general, average annual precipitation is likely to rise in northern but to decrease in southern Europe with a high seasonal and regional variability (Alcamo et al., 2007). Winter precipitation is projected to be elevated in both northern and central Europe (Raisanen et al., 2004). However, summer rainfall and soil moisture will likely be substantially reduced in central and northern Europe which includes major parts of Germany (Raisanen et al., 2004; Boe et al., 2009; Rowell, 2009).

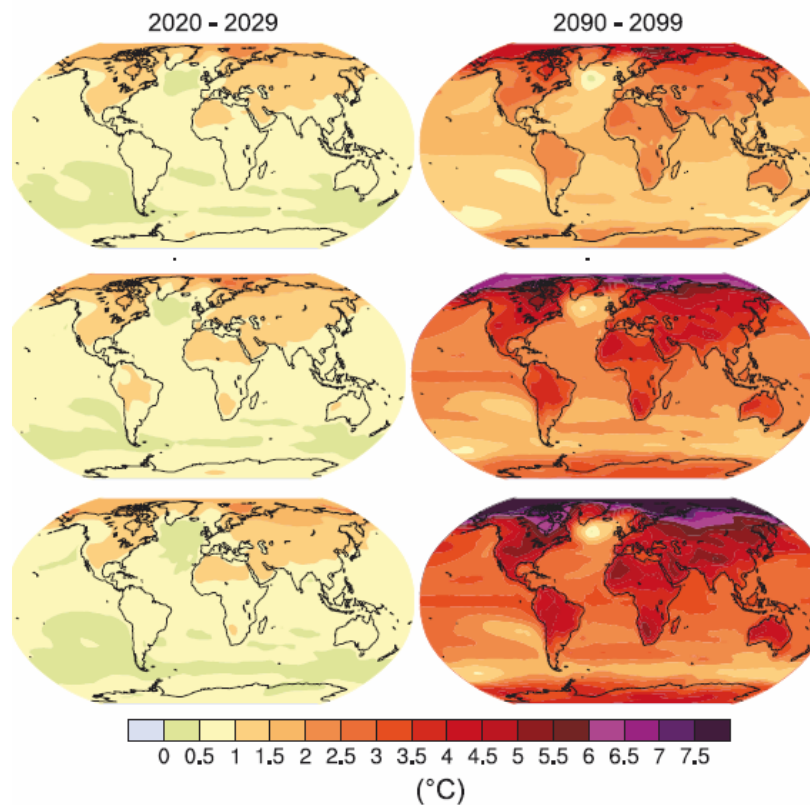


Figure 2: Projections of mean global surface warming relative to the period 1980 to 1999 for three different greenhouse gas emission scenarios (Surface air temperature, °C; Adapted from IPCC, 2007)

2 Climate change: Agricultural implications

2.1 Increasing atmospheric CO₂ concentration: Effects on feed production and quality

2.1.1 “CO₂ fertilization effect” and experimental facilities

Rising atmospheric CO₂ concentrations are not only related to changes in global surface temperatures but were reported to cause substantial but not necessarily beneficial effects on yield and quality of various important plants that are used for feed and food production purposes. C₃ and C₄ plants differ in the primary uptake and fixation of CO₂ and hence the responses of both plant types to elevated carbon dioxide are highly different as shown in **Figure 3**. These unequal reactions are likely to generate very complex future alterations in the production of feed and food of plant origin and it is difficult to prognosticate whether in total these effects will be rather positive or adverse.

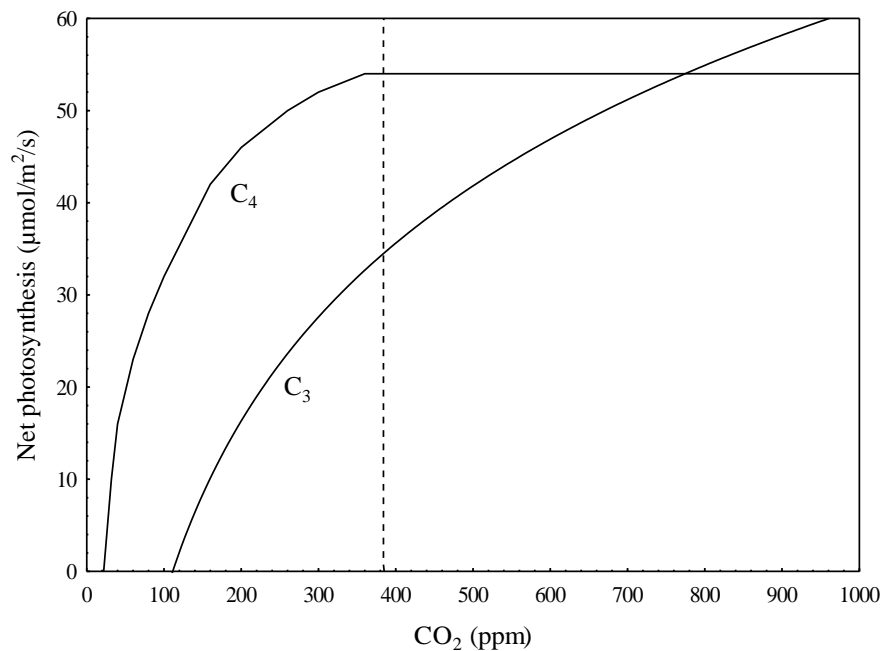


Figure 3: Stylized response of C₃ and C₄ plant photosynthesis to atmospheric CO₂ concentrations (Redrawn from Kimball et al., 1983). Dotted line approximates the atmospheric CO₂ concentration in 2012

Elevated atmospheric CO₂ is known to increase rates of photosynthesis, improve water-use efficiency and decrease stomatal conductance and transpiration at least of C₃ plants if all other conditions remain equal. These observations are often referred to as the “CO₂-fertilization effect” and almost all other effects of rising atmospheric carbon dioxide on plants are derived from the two fundamental responses, increased photosynthesis and reduced stomatal conductance (Long et al., 2004). Already today this photosynthetic stimulation is used in many commercial greenhouses which are often enriched to contain CO₂ concentrations of about twice the current atmospheric content and several greenhouse operators are known to elevate carbon dioxide to 1000 ppm or more (Newman et al., 2011). Early researchers investigating the effects of varying atmospheric carbon dioxide concentrations often used controlled environments in open-top chambers or enclosures but such growth conditions can produce a “chamber-effect” that may lead to an overestimation of the actual influence of high CO₂ atmospheres (Ainsworth and Long, 2005). In contrast, modern free air carbon dioxide enrichment (FACE) technology enables the exposure of plants to enriched CO₂ under natural and full open-air field conditions. In most FACE experiments vertical or horizontal pipes are used to release pure carbon dioxide or air enriched with CO₂ to the periphery of vegetation plots to meet the target concentrations.

2.1.1.1 C₃ plants

C₃-plants comprise approximately 95 % of all known plant species and intensive recent research evaluated the responses of various C₃ species to elevated carbon dioxide. In C₃ plants, the Calvin cycle serves to assimilate CO₂ directly (**Figure 4**). The uptake of CO₂ is catalysed by Ribulose-1,5-biphosphate-carboxylase/-oxygenase (Rubisco). Two possible reactions are catalysed by Rubisco, either the carboxylation of the acceptor molecule ribulose 1,5-biphosphate (RuBP) or a process called photorespiration which includes the oxygenation of RuBP and only the carboxylated molecules can be transformed into carbohydrates (Newman et al., 2011). The CO₂-fertilization effect is induced by an elevation of the carboxylation rate of Rubisco and a competitive inhibition of the oxygenation of Ribulose-1,5-biphosphate (Drake et al., 1997). However, the underlying mechanism which is responsible for the decreases in stomatal conductance due to variations in leaf stomatal aperture is still unclear (Long et al., 2004).

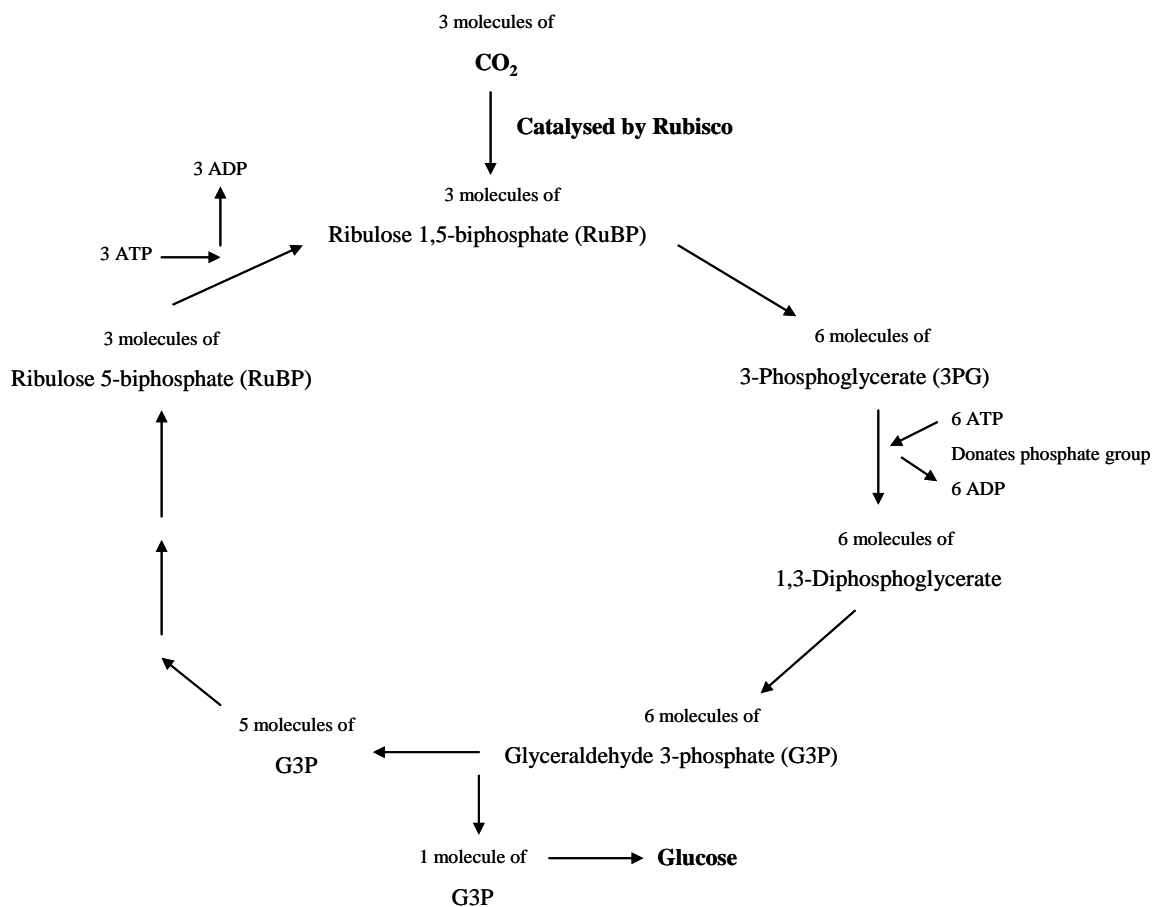


Figure 4: The Calvin cycle (Redrawn from Newman et al., 2011)

All other things being equal, the exposure to atmospheres containing 550 ppm CO₂ considerably elevated the yields of important agricultural C₃ plants such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) or sugar beet (*Beta vulgaris* L.) (Weigel et al., 1994; Wu et al., 2004; Yang et al., 2007; Erbs et al., 2010; Manderscheid et al., 2010). This concentration of carbon dioxide will likely be exceeded in the atmosphere by the middle of the twenty-first century and is predominantly used in current FACE experiments. The induced increases in yields of most C₃ crops were found to be in the range of 10-20 % (Long et al., 2004; Gifford, 2004). However, such yield-stimulating effects were not limited to crops because the above-ground biomass of trees was found to be enhanced by up to 30 %, though the positive responses to carbon dioxide elevation were primarily observed for young trees and the effects on mature natural forests were low or negligible (Nowak et al., 2004; Korner et al., 2005). Moreover, the effects of CO₂ elevation on pasture were in principle consistent with the general vegetative responses and pasture above-ground biomass was stimulated by approximately 10 % (Ainsworth and Long, 2005; Izaurrealde et al., 2011). For example, Milchunas et al. (2005) investigated the effects of increased atmospheric CO₂ on yield and forage quality of a shortgrass steppe where grazing by livestock is the dominant land use. The authors demonstrated that the yields of different plant constituents which are relevant for the nutritive value of ruminant feed such as cellulose and lignin partially increased by carbon dioxide enrichment. However, the effects strongly depended on the respective plant species and both crude protein concentration and digestibility were influenced in a negative fashion. However, experimental results are in parts inconsistent as Hanson et al. (1993) used different model approaches to simulate the responses of rangeland livestock production to climate change and concluded that doubling atmospheric carbon dioxide alone would not significantly elevate pasture plant production. The interpretation of experimental results should imperatively consider the observation that long-term exposure of C₃ plants to elevated CO₂ may be associated with a reduction in the increases of yields, a response often termed down-regulation or acclimation (Ghannoum et al., 2000). Moreover, rising atmospheric carbon dioxide decreases the stomatal conductance, a fact that may mediate indirect positive effects on yields of C₃ plants via enhanced water use efficiency and an amelioration of drought stress (Leakey et al., 2006). This process was confirmed in a variety of experiments (Eamus, 1991; Morison, 1998; Wu et al., 2002) and contributes to stimulations of crop yields in particular during periods of restricted plant water availability.

Increased future yields could be considered as a positive aspect of climate change and may to a certain extent help to meet the probably increasing future food demand either directly or mediated by quantitative elevations in feed production. However, in most experiments the positive effects of increased CO₂ concentrations on yields of several C₃ plant species were accompanied by substantial reductions in the quality of the products generated for food and feed purposes. The effects of CO₂ enrichment on the composition of wheat, which is one of the most important crops worldwide with a total annual harvest of almost 598 million tonnes in 2006 (FAOSTAT, 2007) and an enormous importance for both human nutrition and feeding of various livestock species, are relatively well described. The major part of previous research focused on implications related to food production, though approximately one-sixth of the global annual wheat production is used for livestock feeding. Recently, Porteous et al. (2009) investigated the effects of an atmospheric CO₂ concentration of 550 ppm on composition and feed value of wheat grain and straw and concluded that the feed quality of both products was altered in a negative manner because, for example, grain protein contents were strongly reduced at low levels of N fertilization. Högy and Fangmeier (2008) reviewed the effects of CO₂ elevations on the grain quality of wheat and reported several alarming alterations. The protein concentration of grain grown under natural conditions was reduced by 2.3 to 4.2 %, a fact that will likely reduce both feed and food value. Gluten, substantially influencing the baking properties of wheat flour (Stafford, 2007), was decreased by up to 7.5 %. Furthermore, elevated CO₂ was associated with reductions in the contents of various amino acids (7.7 to 21.8 %) which are known to be essential for humans such as threonine, valine, isoleucine, leucine and phenylalanine. In pig diets, lysine is usually regarded as the first limiting amino acid. However, all mentioned amino acids are essential for pigs as well and large reductions of these amino acids in main components of pig rations such as wheat and barley would cause serious adverse impacts on their future feed value. In ruminants, the amino acid supply is primarily ensured by the microbial protein production in the reticulorumen. Nevertheless, a reduced N intake due to depleted plant protein contents would typically reduce the substrate for microbial amino acid formation in the forestomach and may thus indirectly impact the feed value of the respective C₃ plants. Furthermore, the concentrations of major parts of macro- and micro-elements such as Mg, Ca, Na, S, Fe and Zn tended to decrease as well but the content of starch partially increased, though Högy and Fangmeier (2008) described a high experimental variability.

Homologous adverse effects of enriched carbon dioxide on the quality of barley and rice were reported. In a FACE experiment, Erbs et al. (2010) reported reductions of approximately 12 %

in crude protein concentrations of barley grain due to the exposure to 550 ppm CO₂. In the same trial, the concentrations of starch and several macro- and micro-elements except S were not altered by enriched atmospheric carbon dioxide. Similar carbon dioxide concentrations induced reductions in the protein contents of rice grain, the first staple food especially in Asia that will probably face a strongly increasing future demand (Terao et al., 2005; Yang et al., 2007). The global importance of rice as a feedstuff is limited. However, reduced protein concentrations in a major supplier of food protein for a large part of the human population may contribute to malnutrition especially if the quantitative access to food is restricted since the total yield of rice protein was not necessarily depleted by carbon dioxide enrichment (Yang et al., 2007).

The decreased N and protein contents in C₃ plants are to a large extent the result of an accumulation of carbohydrates and other organic compounds in leaves and other plant organs due to the described CO₂-induced photosynthetic stimulation and could be referred to as a dilution effect (Korner, 2000). These processes are well established since Cotrufo et al. (1998) reviewed 75 previous studies and concluded that 82 % of the evaluated experiments reported reductions in plant nitrogen concentration under conditions of elevated CO₂ with a mean N reduction of 14 %. In addition, the uptake of N from the soil could be decreased indirectly by enhanced carbon dioxide due to lower transpiration rates (Manderscheid et al., 1995). There are indications that reductions in enzymes of the Calvin cycle may contribute to the decreased plant N concentration, possibly an effect of a reduced carboxylation enzyme requirement in high CO₂ atmospheres (Idso and Idso, 2001). Future increases in CO₂ are likely to stimulate yield and photosynthesis of both agricultural and wild C₃ plants. However, the quality of the generated products is often alarmingly reduced and considerable alterations in the chemical composition of food and feed are to be expected and will challenge future diet formulation.

2.1.1.2 C₄ plants

C₄ plants comprise less than 5 % of the known plant species but include some of the most important agricultural plants such as maize, sorghum (*Sorghum bicolor* L.) and sugarcane (*Saccharum officinarum* L.). They contribute about 20 % to the global primary production because C₄ grasslands are highly productive (Ehleringer et al., 1997). In C₄ photosynthesis, the assimilation of carbon dioxide and the Calvin cycle are spatially separated and the primary uptake of CO₂ is not catalysed by Rubisco but by another enzyme called phosphoenol pyruvate (PEP) carboxylase (Newman et al., 2011). This process takes place in outer mesophyll cells of the leaves where carbon dioxide is converted into a 4-carbon acid,

oxaloacetate. Afterwards, oxaloacetate is converted into another acid and this acid is transferred into inner bundle sheath cells of the leaves, where CO_2 is removed from the 4-carbon acid and subsequently enters the normal C_3 pathway. The cell walls of bundle sheath cells are coated with suberin and hence have a low conductance to CO_2 which is concentrated in these cells 3 to 10 times higher than in the current atmosphere (Ghannoum et al., 2000). Based on this local carbon dioxide saturation already at present atmospheric levels it was partially suggested that C_4 species would not be substantially affected by rising CO_2 because a photosynthetic stimulation should not be expected (Bowes, 1993). Interestingly, C_4 plants were proven to be limited in their ability to assimilate carbon at low atmospheric CO_2 concentrations and respond strongly to elevations up to approximately 300 ppm but were found to be CO_2 -saturated at about 400 ppm (Kimball, 1983; Newman et al., 2011).

However, uncertainty about the actual responses of C_4 plants to increasing future CO_2 concentrations persists. Experimental results were inconsistent because in some studies yields and biomass production of several C_4 species were reported to be heightened due to the exposure to enriched carbon dioxide, though most studies did not reveal effects in terms of quantity (**Table 1**). The major part of research that demonstrated increased yields used controlled environmental conditions such as growth chambers (Maroco et al., 1999; De Souza et al., 2008) which can lead to overestimations of the actual carbon dioxide effects. Leakey et al. (2009) suggested that such overestimations may in parts be attributable to a restriction of the typical deep rooting of maize and sorghum resulting in an inadequate root volume to absorb enough water. Growth at elevated CO_2 reduces plant water requirement and may thus result in an apparent photosynthetic stimulation at sufficient water supply in such experiments. Accordingly, various investigations partly using FACE technology did not report significant alterations in yields of agricultural C_4 plants (Leakey et al., 2004; Barbehenn et al., 2004; Leakey et al., 2006; Chun et al., 2011). In contrast, Ghannoum et al. (2000) reviewed the effects of rising CO_2 on the growth response of C_4 plants and concluded that there is growing evidence that increased carbon dioxide can lead to accumulations of more biomass, though the underlying mechanisms are to a large extent unclear. The stimulation of the growth of C_4 weeds was reported to be higher than that of C_4 crops and many wild C_4 species and grasses responded to CO_2 enrichment. It was suggested that their photosynthesis is not necessarily saturated at ambient carbon dioxide (Wand et al., 1999).

Despite of the enormous global relevance of several agricultural C_4 species most research conducted to assess the effects of altered future climatic conditions on food and feed value primarily focused on C_3 plants. However, the controversy about potential impacts of elevated

atmospheric CO₂ on photosynthesis and yield of C₄ plants also highlights both the importance and the considerable gap of knowledge about responses of the respective species in terms of quality, chemical composition and feed value. The limited available literature reflects the inconsistent responses of plant yields and biomasses to increased CO₂. As summarized in **Table 1**, enriched carbon dioxide did not have significant effects on fibre constituents and non-fibrous carbohydrate fractions of most investigated C₄ plants. In contrast, Barbehenn et al. (2004) reported increases of both starch (50 %) and NDF (10 %) concentrations in the C₄ grass *Digitaria sanguinalis* L. (Crabgrass), though the concentrations of the respective fractions were not affected in diverse other C₄ grasses. Furthermore, cellulose content in sugarcane partially increased and non-structural leaf carbohydrates of some C₄ grasses were elevated (LeCain and Morgan, 1998; De Souza et al., 2008). Thus, carbohydrate contents remained either unaffected by CO₂ enrichment or increased, but reductions were not reported in any of the evaluated studies. Inconsistent CO₂ effects were described for plant protein as well but the concentration of protein in C₄ plants such as maize or several grass species was not altered by increased atmospheric CO₂ in different experiments (Akin et al., 1994; Leakey et al., 2006). However, maize leaf protein was reported to decrease by 22-29 % if the plants were grown in controlled environments or subjected to high concentrations of 1100 ppm carbon dioxide (Maroco et al., 1999; Driscoll et al., 2006) and reductions in protein contents of total plants or single organs were described for sudangrass and crabgrass (Akin et al., 1994; Barbehenn et al., 2004).

Background

Table 1. Effects of atmospheric CO₂ enrichment on yield and composition of C₄ plants by different authors

Reference	Enrichment facility ¹	CO ₂ -concentration	Plant species	Yield/Biomass ²	Studied fraction ³
Akin et al. (1994)	FACE	550 ppm	Sudangrass (<i>Sorghum bicolor</i> L.)	n.d.	NDF: n.s. ADF: n.s. ADL: n.s. Leaf protein: n.s. Stem protein: ↓(12-28%)
Barbehenn et al. (2004)	Open-top chambers	740 ppm	C ₄ grasses (<i>Bouteloua curtipendula</i>) (<i>Panicum virgatum</i> L.) (<i>Paspalum notatum</i>) (<i>Buchloe dactyloides</i>) (<i>Digitaria sanguinalis</i> L.)	B.: n.s. B.: n.s. B.: n.s. B.: n.s. B.: n.s.	Protein, Starch, NDF: n.s., n.s., n.s. n.s., n.s., n.s. n.s., n.s., n.s. n.s., n.s., n.s. ↓(17%), ↑(55%), ↑(10%)
Chun et al. (2011)	Growth chambers	800 ppm	Maize (<i>Zea mays</i> L.)	B.: n.s.	n.d.
De Souza et al. (2008)	Open-top chambers	720 ppm	Sugarcane (<i>Saccharum officinarum</i> L.)	B.: ↑(40%)	Sucrose: ↑(29%) Cellulose: ↑(19%)
Driscoll et al. (2006)	Controlled rooms	700 ppm	Maize (<i>Zea mays</i> L.)	n.d.	Leaf protein: ↓(29%)
Leakey et al. (2006)	FACE	550 ppm	Maize (<i>Zea mays</i> L.)	Y.: n.s.	Leaf starch: n.s. Leaf protein: n.s. Leaf amino acids: n.s.
LeCain and Morgan (1998)	Growth chambers	700 ppm	C ₄ grasses (<i>Bouteloua gracilis</i>) (<i>Buchloe dactyloides</i>) (<i>Panicum virgatum</i> L.) (<i>Andropogon gerardii</i> L.) (<i>Schizachyrium scoparium</i>) (<i>Sorghastrum nutans</i> L.)	B.: n.s. B.: n.s. B.: n.s. B.: ↑(50%) B.: n.s. B.: ↑(25%)	Leaf non-structural carbohydrates: n.s. n.s. n.s. ↑(40%) ↑(240%) ↑(60%)
Maroco et al. (1999)	Plexiglass chambers	1100 ppm	Maize (<i>Zea mays</i> L.)	B.: ↑(20%)	Leaf protein: ↓(22%)
Vu et al. (2009)	Greenhouses	720 ppm	Sugarcane (<i>Saccharum officinarum</i> L.)	n.d.	Leaf starch: n.s.

¹ : FACE, free air carbon dioxide enrichment

² : Y, yield; B, biomass; n.s., not significant; n.d., not determined;

³ : NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin

2.1.2 Mycotoxin contamination

Food and feed quality of agricultural plants can be impaired by the contamination with toxic secondary metabolites, mycotoxins. Their production follows plant colonization and infection by fungi from various genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* or *Claviceps*. Most mycotoxins are heat-stable and very difficult to destroy during processing and their ingestion can have serious adverse consequences for human and animal health which include carcinogenic and immune-modulating effects (Magan et al., 2011).

In animal nutrition, several mycotoxins are of considerable importance because they can contaminate a variety of feedstuff and lead to intoxications that may depress animal performance of diverse livestock species. In central Europe, prevalent *Fusarium* species are *F. graminearum* and *F. culmorum*, which are able to produce several toxic metabolites including deoxynivalenol (DON) and zearalenone (ZON) that are often detected in maize, wheat and triticale (Dänicke, 2010).

DON can inhibit the synthesis of proteins and induce vomiting (Rotter et al., 1996; Goyarts 2006). ZON has estrogenic properties and may be related to hyperestrogenism (Döll et al., 2003). The exposure of monogastric animals such as pigs and poultry to high dietary concentrations of DON and ZON was reported to have serious adverse effects on animal health and performance (Goyarts et al., 2005; Dänicke et al., 2007; Dänicke and Döll, 2010). In contrast, the susceptibility of ruminants to both DON and ZON is known to be relatively low, as in the rumen DON is converted almost completely into a less toxic de-epoxidized metabolite (de-epoxy-DON) and ZON is converted into the less absorbable α -zearalenol and the less potent β -zearalenol (Kiessling et al., 1984; Kennedy et al., 1998; Fink-Gremmels, 2008). In spite of the detoxification potential of the reticulorumen, the intake of high doses of DON may affect metabolism and immunological parameters of dairy cows (Korosteleva et al., 2007; Korosteleva et al., 2009). Furthermore, it is unclear whether rising future ambient temperatures will influence the ruminal detoxification of DON and ZON indirectly as hot summer periods are usually associated with the incidence of heat-stress that is well known to affect feed intake, performance and metabolism of ruminants (West, 2003; Marai et al., 2007).

Conceivable climate change effects on plant infection by moulds and subsequent mycotoxin production are complex and the specific impacts will strongly depend on the local climatic development. In general, all main aspects of global climate change, rising surface temperatures, increasing atmospheric CO₂ concentrations and altering precipitation patterns exhibit the potential to modify the growth conditions of various fungi species not only in

agricultural plants. Besides such direct effects of altered climatic conditions indirect implications such as shifted crop rotations may change the occurrence of fungal infection (Chakraborty and Newton, 2011).

Ambient temperatures are well known to influence the growth of various moulds such as *Aspergillus* and *Fusarium* species and often warm temperatures in the range of 20 to 30 °C were found to be optimal conditions (Marin et al., 1995; Giorni et al., 2007). However, alterations in ambient temperatures are complex to simulate in full open-air trials like FACE experiments and the present work will focus primarily on effects related to future changes in carbon dioxide concentration and water availability.

In general, fungi are able to withstand relatively high concentrations of CO₂ and levels in the range of 700 to 1000 ppm as expected by the end of the 21st century will likely not induce inhibitions of fungal growth (Magan et al., 2011). However, very high concentrations may affect *Fusarium* fungi as Magan and Lacey (1984) demonstrated that the latent period prior to growth of *F. culmorum* was increased by a treatment of more than 5000 ppm CO₂. In the same study, CO₂ appeared to interact with the water activity of the media as the growth of *F. culmorum* was significantly inhibited by enriched carbon dioxide at 0.98 and 0.95 a_w but seemed to be unaffected at 0.90 a_w. Moreover, the production of ZON by *F. equiseti* in maize grain was inhibited by high CO₂ levels above 20 % but a lower amount of carbon dioxide inhibited mould development and mycotoxin formation at relatively low oxygen concentrations (Paster et al., 1991). The actual impact of CO₂ may differ among *Fusarium* species because Samapundo et al. (2007) reported that the production of fumonisin B₁ by *F. verticillioides* was completely inhibited in a high carbon dioxide atmosphere of 10 % but the inhibition of the same mycotoxin by *F. proliferatum* required 40 %, 30 % and 10 % CO₂ at a_w 0.984, 0.951 and 0.930.

Water availability is one of the most important factors that influences the life cycles of mycotoxigenic fungi including *Fusarium* species and affects both mould growth and mycotoxin production (Kokkonen et al., 2010) via two possible pathways. First, drought can increase the susceptibility of plants such as maize to fungal infection (Arino and Bullerman, 1994). Secondly, the growth of fungi in different media directly depends on the respective a_w. Accordingly, Mateo et al. (2002) reported that *F. sporotrichioides* produced the largest amount of mycotoxins at the highest a_w (0.99) in wet maize grain.

2.2 Drought: Plant responses and CO₂-interactions

Drought-associated losses of crop yields are likely to exceed the losses induced by all other factors and thus drought may be referred to as the most critical single menace to global food security (Farooq et al., 2009). Moreover, the rising global food demand will probably increase the severity of the consequences of future drought and climatic projections revealed that considerable yield losses may occur in various regions (Somerville and Brisco, 2001). Duration, severity and timing of drought incidents and the response of plants after the ending of drought stress are crucial factors in the assessment of the impacts of water restriction. Consequently, experimental drought-induced losses of maize yield varied considerably but may exceed 50 % and even reach drastic reductions of 80 % or more (Chapman and Edmeades, 1999; Monneveux et al., 2006). The foremost effect of drought is an impaired germination and severe drought stress can inhibit cell elongation and growth of plants (Nonami, 1998). The negative impact of drought on maize is not limited to quantitative depressions of yields because limited water availability may as well affect the nutritive value of both food and feed products and the silage making properties of whole maize plants. Drought stress was reported to deplete the intermediates of starch synthesis and to reduce grain numbers per ear and soluble sugar concentrations (Zinselmeier et al., 1999; Boyer and Westgate, 2004; Yin et al., 2012). Starch is a main constituent of maize silage, a feedstuff that is used prevalently in both northern American and European dairy cow diets to meet the energy requirements in particular of high-yielding animals. The proportion of starch often exceeds 30 % of maize silage dry matter (DM) and an increased future occurrence of drought-associated reductions in starch concentrations exhibits the potential to considerably reduce the feed value of ensiled maize.

The adverse effects of drought on plant growth and composition could be diminished by rising atmospheric CO₂ concentrations which are known to improve plant water-use efficiency and to decrease stomatal conductance and transpiration. Same as for C₃ species, the photosynthesis of C₄ plants that dominate in hot and arid regions is very sensitive to drought stress. However, current literature suggests that elevated carbon dioxide levels indirectly reduce the negative effects of limited water availability on plant productivity by improved soil moisture and plant water status (Wand et al., 1999; Leakey et al., 2004; Leakey et al., 2006). Most research assessing interactions between atmospheric CO₂ and drought focused on C₃ plants (Ghannoum, 2009) and the fewer studies that investigated the responses of C₄ plants primarily evaluated effects on photosynthesis and growth. Thus very few information exists whether congruent interactions are to be expected for chemical composition and nutritive

value of C₄ species such as maize, in particular if elevated CO₂ will help to maintain the future feed value of maize silage in periods of drought.

2.3 Heat stress in dairy cows

2.3.1 Emergence and impacts of heat stress

Heat stress is an enormous financial burden to livestock production in diverse regions of the world (Bernabucci et al., 2010). For example, the dairy industry of the United States of America alone records annual losses due to heat stress which approximate 900 million US-Dollar (Collier et al., 2006). Obviously, globally rising ambient temperatures will increase the occurrence of heat stress especially in regions that already have to face heat in summer months or throughout the year. Considering the projected drastic increases in mean surface temperatures in the range of 1.8 to 4.0 °C until the end of the 21st century and keeping in mind that the warming over land will be about twice than the average warming (Meehl et al., 2007) one must conclude that future heat stress, first, will arise in diverse areas that did not experience respective stressors yet and, second, that the overall economic and physiological impact of climate change induced heat stress on animal production will likely be tremendous and in parts threatening to the supply of food of animal origin.

The enormous genetic progress in milk production of dairy cows and rising animal bodyweights were closely related to elevations in feed intake (Heinrichs and Hargrove, 1987). Increases in feed consumption and the associated metabolism of nutrients result in higher body heat production which requires effective thermoregulatory adaptations in order to dissipate excess body heat if the cows are exposed to warm or hot environmental conditions. The animals lose heat via conduction, convection, radiation and evaporation. Thus increases in the dissipation of body heat are generated by sweating, panting, cooler environmental conditions, elevated skin circulation or vasodilatation, alterations in coat insulation, raised water loss, increased radiating surface and rises in air movement. If the animals fail to discharge sufficient thermal energy, hyperthermia can occur. Kadzere et al. (2002) summarized that heat stress indicates all forces related to high ambient temperatures that induce adjustments from the sub-cellular to the whole animal level and help to maintain the status of physiological functions.

It is complicated to determine the exact and individual threshold where thermoneutrality, a prerequisite for the maintenance of normal physiological processes, is terminated and the animals begin to suffer from heat stress. Lactating dairy cows prefer ambient temperatures in

the range of 5 to 25 °C (Roefeldt, 1998), their so-called thermoneutral zone (**Figure 5**). The thermoneutral zone can be referred to as the zone of minimal heat production at normal core body temperature. It depends on different variables including milk performance leading to the conclusion that calving and peak milk yield that is associated with high heat production should preferably take place in cold seasons. The upper critical temperature is the ambient temperature at which the animals begin to increase heat production in order to dissipate excess body heat. Another useful measure of the thermal impact of an environment can be the calculation of temperature-humidity indices (THI) that consider air relative humidity which can limit evaporative heat dissipation especially in hot and humid climates. A variety of equations were suggested to calculate THI for the use in dairy production (Bohmanova et al., 2007; Dikmen and Hansen, 2009). THI below 70 were usually considered as comfort zone, mild heat stress is likely to be initiated by THI higher than 71 and severe heat stress occurs if THI exceed 75 (Armstrong, 1994).

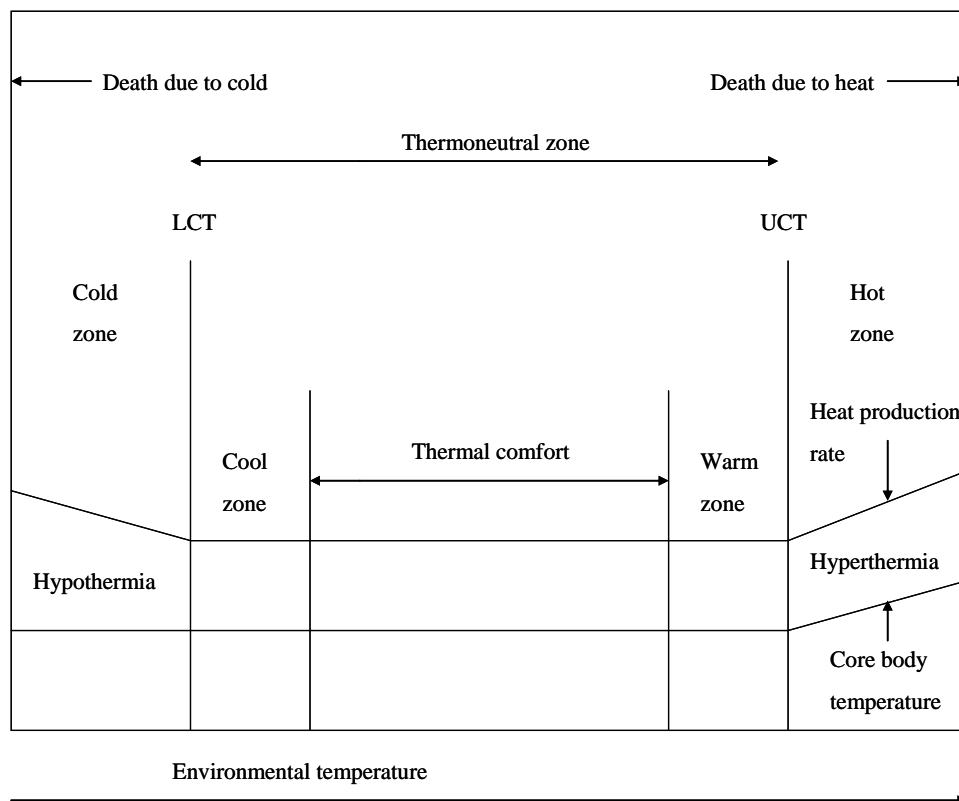


Figure 5: Schematic relationship of the animal's core body temperature, heat production and environmental temperature (Redrawn from Kadzere et al., 2002). LCT, lower critical temperature; UCT, upper critical temperature

The exposure of homoiotherm organisms to warm or hot environments induces diverse homeostatic adaptations including thermoregulatory, humoral, metabolic, nutritive and behavioral changes. In dairy cows and sheep, heat stress can manifest in increases in sweating rates, skin and rectal temperatures, respiration rates, alterations in blood pH, blood gas concentrations, heart rate, hormone secretion, mineral metabolism, water metabolism and changes in ruminal fermentation (Schneider et al., 1988; Blazquez et al., 1994; Kadzere et al., 2002; Marai et al., 2007; Dikmen et al., 2008). Moreover, heat was found to affect the immune cell function in bovines and sheep (Niwano et al., 1990; Lacetera et al., 2005; Booth et al., 2007) and the thermal environment was reported to influence the apparent digestibility of nutrients or DM in cattle and sheep diets in diverse experiments (Christopherson and Kennedy, 1983). For example, the digestibilities of DM, organic matter, NDF and crude protein in steers were increased by 9 to 16 % if the animals were kept at 28 °C compared to 10 °C (Miaron and Christopherson, 1992). Similar results were reported for sheep in a trial of Westra and Christopherson (1976) as the digestibilities of DM and ADF of both hay and pelleted hay diets were higher at 17.7 °C than at 0.8 °C. Impaired animal health and reproduction is mostly accompanied by two major effects of heat stress in relation to milk performance of dairy cows, reduced voluntary feed intake and declining milk yield (**Table 2**). The heat-associated daily reductions in milk production were reported to vary in the range of 0.16 to 0.57 kg/ THI unit above the respective thresholds that indicated the onset of heat stress.

The assessment of future heat stress impacts on dairy cattle should not exclusively focus on direct animal effects but imperatively has to integrate potential alterations in quantity and quality of important ruminant feedstuff. It needs to be elucidated whether changes in the chemical composition of feed may interact with a rising occurrence and duration of heat stress and its physiological effects on the animals. Current information about such complex interactions is very rare but would be valuable in estimating the actual consequences of climate change on dairy production.

Table 2. Effects of heat stress on dry matter intake and milk performance of dairy cows by different authors

Reference	Experimental facility	Heat stress treatment ¹	Effects ²
Bernabucci et al. (2002)	Commercial farm (Italy)	THI > 72	Milk yield: ↓ Milk protein percent: ↓ Milk casein percent: ↓ Dry matter intake: ↓
Bohmanova et al. (2007)	Commercial farms (USA)	THI 68-83	Milk yield: ↓ (0.22-0.57 kg/THI unit)
Bouraoui et al. (2002)	Dairy farm (Tunisia)	THI > 69	Milk yield: ↓ (0.41 kg/THI unit) Dry matter intake: ↓
Igono et al. (1992)	Commercial farms (USA)	THI > 72	Milk yield: ↓ (0.16 kg/THI unit)
Ominski et al. (2002)	Metabolic crates in ventilated rooms	THI > 80	Milk yield: ↓ Dry matter intake: ↓ Milk fat percent: n.s.
Ravagnolo et al. (2000)	Commercial farms (USA)	THI > 72	Milk yield: ↓ (0.2 kg/THI unit) Milk fat yield: ↓ (0.012 kg/THI unit) Milk protein yield: ↓ (0.009 kg/THI unit)
Rhoads et al. (2009)	Environmental chambers	THI 73 - 82	Milk yield: ↓ Milk protein percent: n.s. Milk fat percent: ↑ Milk lactose percent: ↓ Dry matter intake: ↓
Spiehrs et al. (2004)	Tie stalls in climatic laboratory	THI 76 - 79	Milk yield: ↓ Dry matter intake: ↓

¹ : Temperature humidity index, THI

² : Not significant, n.s.

2.3.2 Heat stress alleviation

Various strategies to reduce the adverse impacts of current and increasing future heat on dairy cows were discussed in past literature. Some of the most effective methods to counteract heat stress are shading, cooling and genetic selection (West, 2003). However, several nutritional measures can contribute to heat stress relief and include both diet composition and the use of supplements. Feeding of heat stressed dairy cows generally has to take into account diet reformulation due to decreases in feed intake, greater nutrient requirements and feedstuff specific heat increment. Nutritional countermeasures against excess body heat in warm environments can be divided into two subsections, either direct reductions of dietary body heat increment or the triggering of physiological mechanisms that mediate alleviations of heat

stress via, for example, increases in skin blood flow that lead to elevated convective and conductive dissipation of thermal energy.

Feeding dairy cows with rations that have a high concentrate to roughage ratio should reduce the dietary heat increment because there is a greater heat production associated with the metabolism of acetate compared to propionate (West, 1999). This was demonstrated by Reynolds et al. (1991), who reported that feeding growing heifers with a 75 % concentrate diet resulted in a lower production of heat energy than feeding the animals with a high fibre diet containing 75 % alfalfa. Furthermore, feeding rations with high proportions of concentrate is typically related to an elevated density of metabolizable energy that will be beneficial if heat-stressed animals have to withstand reductions in feed intake (**Table 2**) but increases in heat production rate to dissipate excess energy as shown in **Figure 5**.

Niacin comprises two vitamers, nicotinic acid and nicotinamide, is of great metabolic importance due to its incorporation into the coenzymes NAD and NADP (Niehoff et al., 2009a) and was recently discussed for its potential to reduce the impact of heat stress in cattle. In humans, niacin is used therapeutically for more than 50 years and is well known to induce a side effect called flushing, a strong cutaneous vasodilatation that manifests itself as redness or warmth of the skin (Kamanna et al., 2009). Two pilot studies demonstrated that the supplementation of varying doses of niacin can reduce body temperatures of heat stressed lactating cows (DiCostanzo et al., 1997; Zimbelman et al., 2010). It was hypothesized that the vasodilatative effects of niacin may result in an increased skin blood flow that leads to an improved peripheral dissipation of body heat. However, experimental observations were inconsistent because higher intakes of niacin were not necessarily related to respective reductions of skin temperatures and the highest tested daily supplementation of 36 g niacin per animal did not affect rectal and skin temperatures or respiration rates of Holstein cows (DiCostanzo et al., 1997). Though differing results were reported, the supplementation of niacin can induce a variety of further effects which include increases in milk and fat corrected milk yield, alterations in milk protein and fat content as well as yields, rumen fermentation and microbial populations (Minor et al., 1998; Niehoff et al., 2009a; Niehoff et al., 2009b). However, further research is needed to evaluate the capability of niacin in reducing the adverse effects of heat stress in cattle and to generate an extended basis of knowledge to cope with rising future ambient temperatures. Such investigations should consider different environmental conditions and physiological stages because uncertainty exists about the thermoregulatory responses of growing cattle such as heifers which usually have lower bodyweights and DM intakes than adult cows (Ahn et al., 2005).

2.4 Applicability and restrictions of continuous ruminal pH and temperature measurement

In ruminant feeding, the applicability of high dietary concentrate proportions is limited because the intake of low fibre diets is well known to be associated with increases in the production of short chain fatty acids and depressions in ruminal pH. That may lead to SARA, a digestive disorder with a considerable prevalence of 11 to 26 % even in well-managed European and US-American dairy cow herds (Kleen et al., 2003; Enemark, 2008). SARA was reported to impair animal health and productivity and is related to decreases in feed intake and milk production, premature culling and death loss. Clinical signs of SARA are complicated to detect and include reduced DMI, laminitis and diarrhea. However, prolonged periods of low ruminal pH values may serve as indicators of SARA (Kleen et al., 2003; Morgante et al., 2007). For example, Garrett et al. (1999) considered pH 5.5 to be the critical value and Gozho et al. (2005) suggested that SARA may be identified by ruminal pH below 5.6 for at least 3 hours per day. Common field methods used for SARA detection via determination of ruminal pH are rumenocentesis and the use of oral stomach tubes to measure pH in gained rumen fluid (Duffield et al., 2004) but both fail in depicting the dynamic development of pH over time and thus in monitoring the time spent below specific thresholds. The validity of results obtained by both techniques is generally restricted because their use is time-consuming and expensive and usually limited to infrequent or spot sampling (AlZahal et al., 2008). Moreover, saliva contamination may falsify the results gained via stomach tubes and restrictions in animal numbers may lead to insufficient representation of the situation in complete feeding groups or herds.

Accordingly, Enemark (2008) suggested that a continuous determination of ruminal pH may be a promising tool for the diagnosis of SARA. This can be realized by the application of indwelling rumen probes (boluses) which serve to monitor ruminal pH continuously as influenced by varying diets and feeding schemes. The onset of ruminal pH measurement is represented by an early work of Dado and Allen (1993) who used a prototype device consisting of electrode and transmitter. In the last years, technical progress has enabled the implementation and evaluation of several self-constructed or commercially available probes for wireless *in vivo* measurement of ruminal pH and in parts temperature and pressure in both small ruminants and cattle (Enemark et al., 2003; Penner et al., 2009; Kaur et al., 2010; Phillips et al., 2010). Though such techniques may be promising, the accuracy and validity of pH measurement can be impaired by different confounders. First, method comparison methods revealed that in several experiments pH determination via intraruminal boluses had only low or moderate linear relationships to traditional manual pH measurement (Duffield et

al., 2004; Kaur et al., 2010). Secondly, the accuracy of pH monitoring may be affected by the occurrence of time-dependent pH sensor drift (Penner et al., 2006; Kaur et al., 2010). Thirdly, the exact site of measurement can influence the results obtained via unfixed probes that move freely in the reticulorumen because pH gradients may occur. For example, reticular pH may be higher than ruminal pH due to saliva flooding into the reticulum. Furthermore, the ingesta in the top stratum of the rumen is usually recently consumed and should be subjected to a higher fermentative activity than the material in the ventral rumen sac (Tafaj et al., 2004). Thus evaluation and use of new wireless devices for intraruminal pH measurement imperatively require a comparison to established methods of pH determination in rumen fluid, a verification of potential pH sensor drift and should consider a possible development of pH gradients within the reticulorumen.

The determination of ruminal temperature by means of wireless boluses can contribute to understanding direct ration effects on heat production in the rumen and thus help to optimize dietary adaptations to hot environmental conditions. Varying proportions of concentrate may not only induce alterations in metabolic heat production as mentioned in section 2.3.2 but can also influence ruminal temperatures. For example, AlZahal et al. (2008) reported significant increases in ruminal temperatures of lactating Holstein cows due to feeding high amounts of grain in comparison to a control diet. In the same experiment, the nadirs of both ruminal pH and temperature had a close negative relationship ($R^2=0.77$) and the authors concluded that therefore ruminal temperature could help in the diagnosis of SARA. Furthermore, the temperatures in the reticulorumen were shown to be positively correlated to core body or rectal temperatures of cows (Burns et al., 2002; Bewley et al., 2008a) and could thus theoretically display increased core body temperatures in periods of severe heat stress.

Scope of the thesis

Based on the current literature it can be deduced that future climate change will likely have substantial, multifarious and mainly adverse effects on feed quality and productivity of dairy cows. Considerable gaps of knowledge about the actual and complex impacts of some of the most important climatic alterations such as increased atmospheric CO₂ concentrations, regional rises of drought incidents and global elevations in ambient temperatures on feedstuff, health and performance of dairy cows persist and challenge global agriculture to adapt to and cope with modified future production conditions.

Therefore the first objective of the present thesis was to investigate the effects of increased atmospheric CO₂ concentrations and drought on feed value, mycotoxin contamination and nutrient digestibility of ensiled whole maize plants fed to sheep in consideration of varying climatic conditions during feeding (**Paper I**).

Moreover, the effects of atmospheric CO₂ concentration and drought during maize growth as well as the climatic conditions during feeding on thermoregulation, blood parameters and metabolization of DON in sheep were to be assessed (**Paper II**).

The applicability and accuracy of a new commercially available device for wireless intraruminal pH and temperature measurement were to be evaluated in consideration of the localization of measurement in the rumen and varying proportions of dietary concentrate (**Paper III**).

The impacts of a supplementation of niacin and differing dietary concentrate proportions on the thermoregulation of heat stressed primiparous dairy cows was aimed to be tested and the effects of both treatments on milk performance as well as ruminal pH and temperature were evaluated (**Paper IV**).

Paper I

**Effects of free air carbon dioxide enrichment and drought stress on the
feed value of maize silage fed to sheep at different thermal regimes**

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Archives of Animal Nutrition

2012

Volume 66

335 – 346

Doi:10.1080/1745039X.2012.697352

Abstract

Information about the effects of rising atmospheric CO₂ concentration and drought on the feed value of maize silage and interactions with the thermal environment during feeding is limited. A free air carbon dioxide enrichment facility was operated in a maize field to generate an elevated CO₂ concentration of 550 ppm. Drought was induced by the exclusion of precipitation in one half of all experimental plots. Plants were harvested, chopped and ensiled. In a balance experiment on sheep, the nutrient digestibility was determined for three climatic treatments (temperate, temperature humidity index (THI) 57–63; mild heat, THI 68–71; severe heat, THI 75–80). The CO₂ concentration and drought did not alter the crude nutrient content of silage dry matter (DM) or nutrient and organic matter (OM) digestibility. Drought increased the concentration of deoxynivalenol (DON, $p < 0.001$). The drought-associated increase of DON was reduced by CO₂ enrichment ($p = 0.003$). The lowest digestibility of acid detergent fibre ($p = 0.024$) and neutral detergent fibre ($p = 0.005$) was observed during the coldest climate. OM digestibility increased during mild heat ($p = 0.023$). This study did not indicate considerable alterations of the feed value of maize silage due to increased atmospheric CO₂ and drought. Enriched CO₂ may decrease DON contaminations during drought. The thermal environment during the balance experiment did not interact with feeding maize silage grown under elevated CO₂, but may affect cell wall and OM digestibility.

Keywords: Carbon dioxide enrichment, drought, thermal environment, sheep, maize silage

1. Introduction

Climate change includes the potential to affect both crop cultivation and livestock husbandry. The atmospheric CO₂ concentration has risen to a current value of 390 parts per million (ppm) and may double within this century (IPCC 2007). Furthermore, the global surface temperature is likely to increase in the range of 1.8–4.8 °C until 2090–2099 and altering precipitation patterns are predicted to generate augmented emergence of aridity in various regions. Elevated atmospheric CO₂ concentrations were reported to stimulate photosynthesis and growth of C₃ plants but to reduce forage and grain protein (Weigel and Manderscheid 2005). Information about the effects on C₄ plants such as maize (*Zea mays* L.) are sparse but necessary as besides the immense importance of maize for human food supply maize silage is used prevalently as a major component of ruminant diets. The photosynthesis of C₄ plants is likely to be saturated at ambient CO₂ concentrations (Ghannoum 2009) and therefore the

impact of increasing CO₂ on C₄ crops may be limited. Under controlled environmental conditions yields of C₄ crops were enhanced in a range of 6–10% (Kimball 1983). On the other hand, in a more recent FACE (free air carbon dioxide enrichment) experiment, maize photosynthesis, biomass and yield were not affected by CO₂ elevation. However, a reduced stomatal conductance was observed in this experiment resulting in decreased plant water loss (Leakey et al. 2006). That may alleviate the known negative effects of drought on maize plant growth and grain yield (Azeez et al. 2005; Efeoglu et al. 2009) and contribute to the maintenance of starch formation and feed value of maize silage. Varying atmospheric CO₂ concentration and water activity (aw) were found to influence the growth of fungi from various genera and to impact the production of several toxic secondary metabolites as mycotoxins (Magan and Lacey 1984; Cairns-Fuller et al. 2005; Magan et al. 2011). Since the colonisation of maize kernels by *Fusarium* spp. was reported to be higher during dry conditions (Arino and Bullerman 1994), the incidence of *Fusarium* toxins such as deoxynivalenol (DON) and zearalenone (ZON) may be increased in periods of drought. Furthermore, changes of the thermal environment may be associated with alterations in the activity and the function of the digestive system which are independent of feed intake (Christopherson and Kennedy 1983) and induce effects on the nutrient digestibility of both sheep and cattle (Westra and Christopherson 1976; Miaron and Christopherson 1992). It remains unclear, whether interactions between anticipated future growth conditions of maize such as increased CO₂ concentrations and drought and an altered thermal environment during the feeding of the generated silages are to be expected. Therefore, the objective of the present study was to investigate the potential effects and interactions of elevated CO₂ and drought during cultivation on feed value, concentration of selected mycotoxins and nutrient digestibility of maize silage fed at varying thermal regimes.

2. Materials and methods

2.1. Plant cultivation

The experiment was conducted in 2008 on an experimental field site of the Johann Heinrich von Thuenen-Institute in Braunschweig, Germany (52°18'N, 10°26'E). Agricultural measures of the 10 ha field site were carried out according to local farm practices. Maize (*Zea mays* L., ‘‘Romario’’) was sown on 9 May with a plant density of 10 plants per m² and a row distance of 0.75 m. Mineral nutrients were added according to local fertilising practices based on soil analysis in springtime (Manderscheid et al. 2012). After plant emergence in mid of May, a FACE system as described in detail by Lewin et al. (1992) was installed. Treatments

included three rings with blowers and CO₂ enrichment to meet a target CO₂ concentration of 550 ppm during daylight hours (FACE) and three control rings operated under ambient air (Control), respectively. The mean seasonal CO₂ concentration in the Control treatment was 379 ppm. The rings had a diameter of 20 m and each ring was split into a wellwatered (Well watered) and a dry semicircular subplot (Drought stress). In the well watered subplots water content in the 0.6 m soil profile was kept above 50% of maximum plant available soil water content by drip irrigation. Drought stress was achieved by the operation of rain shelters (Erbs et al. 2011). The dry subplots received 48% less water than the well watered subplots over the whole vegetation period (Manderscheid et al. 2012). Whole maize plants (n = 5) were harvested manually on 6 October from every semicircle within an inner diameter of 16 m and chopped afterwards. Subsequently, ensiling was executed for at least 12 months in plastic barrels (O-Top™ Open Head Drums, Mauser AG, Bruehl, Germany) containing a volume of approximately 0.2 m³ each.

2.2. Balance experiment

A balance experiment, which was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) in Oldenburg, Germany (File number 33.9.42502-04/060/06), was performed in observance of the European Community regulations concerning the protection of experimental animals in temperature but not relative humidity (RH) controlled rooms of the Institute of Animal Nutrition, Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Braunschweig, Germany. The climatic conditions were characterized by room temperature [°C] and relative humidity [%], which were combined by the calculation of temperature humidity indices (THI). Factors were CO₂ concentration (FACE vs. Control) and irrigation (Well watered vs. Drought stress) during plant cultivation as well as the climatic conditions during the balance experiment (Temperate, temperature 14–16°C, RH < 50%, THI 57–63; Mild heat, temperature 24–26°C, RH < 50%, THI 68–71; Severe heat, temperature 34–36°C, RH < 50%, THI 75–80). Room dry bulb temperature (Td) and relative humidity were recorded every 10 min via data loggers (Tinytag Plus 2, TGP-4500, Gemini Data Loggers, Chichester, UK). Mean temperature, relative humidity and THI values met the aimed experimental ranges and were generally characterised by a low variation (Table 1). The balance experiment was conducted following the guidelines for determining the digestibility of crude nutrients in ruminants (GfE 1991). Adult castrated male sheep (German blackheaded mutton sheep) with an initial bodyweight of 100.1 ± 7.4 kg were allocated randomly to experimental groups of four animals each. Both adaptation feeding and

subsequent sampling periods were performed for seven consecutive days. The animals were kept in individual boxes during adaptation and were transferred into metabolism cages for sampling. Individual abdominal fleece lengths were measured prior to each sampling period. All sheep had free access to water and were fed 1 kg dry matter (DM) of experimental maize silage and 20 g urea per day and animal, presented in two equal portions at 6:30 h and 13:30 h. Presented diets were consumed completely. Representative samples of maize silages fed were pooled by daily aliquot retaining. Total faeces were collected daily on an individual basis to generate collective samples. Both silage and faeces samples were oven-dried (60°C for 72 h) and then ground to pass through a one mm sieve for further analyses of nutrients and mycotoxins.

Table 1. Climatic conditions during the balance experiment (Means \pm standard error).

Climatic conditions	Climatic conditions		
	Temperate	Mild heat	Severe heat
Temperature [°C]	15.1 \pm 0.01	25.6 \pm 0.02	35.1 \pm 0.01
Relative humidity [%]	39.6 \pm 0.14	23.4 \pm 0.10	17.8 \pm 0.09
THI*	58.8 \pm 0.02	69.5 \pm 0.02	78.2 \pm 0.03

Notes: *Temperature humidity indices (THI) were calculated according to Hahn (1999): $THI = 0.8 \cdot td + RH \cdot (td - 14.4) + 46.4$ where td is the dry bulb temperature [°C] and RH is the relative humidity.

2.3. Analyses

Dry matter (DM), crude ash (Ash), crude fibre (CF), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch in maize silage and feces samples were analyzed according to the protocols of the Association of German Agricultural Analysis and Research Centres (Naumann and Bassler 1993), whereat ADL, ADF and NDF were expressed without residual ash.

DON and ZON in maize silage samples were analysed using HPLC with UV and fluorescence detection, respectively, after a clean-up of the sample extracts with immunoaffinity columns (IAC) (DON: DONprepTM, R-Biopharm Rhone, Darmstadt, Germany. ZON: ZearalatestTM, Vicam, Milford, USA) as described in detail in previous publications (Dänicke et al. 2001; Valenta et al. 2003). The limits of detection were 30 $\mu\text{g kg}^{-1}$ DM for DON and 1 $\mu\text{g kg}^{-1}$ DM for ZON, respectively.

Ochratoxin A (OTA) in silages was analysed by HPLC with fluorescence detection as described by Valenta et al. (1993) after a clean-up of the sample extracts with IAC (OtaCLEAN, LC Tech, Dorfen, Germany). The limit of detection was 0.1 $\mu\text{g kg}^{-1}$.

2.4. Calculations

Apparent nutrient digestibility was calculated according to GfE (1991).

Calculated nutrient digestibility was utilized to estimate silage metabolizable energy (ME) following the regression equation published by GfE (2001):

$$\text{ME (MJ/kg)} = 0.0312 \cdot \text{DEE [g kg}^{-1}\text{]} + 0.0136 \cdot \text{DCF [g kg}^{-1}\text{]} + 0.0147 \times (\text{DOM} - \text{DEE} - \text{DCF}) \text{ [g kg}^{-1}\text{]} + 0.00234 \cdot \text{CP [g kg}^{-1}\text{]}$$

where DEE = digestible ether extract, DCF = digestible crude fibre, DOM = digestible organic matter, CP = crude protein.

THI were calculated according to Hahn (1999):

$$\text{THI} = 0.8 \cdot \text{td} + \text{RH} \cdot (\text{td} - 14.4) + 46.4$$

where td represents the dry bulb temperature [°C] and RH is the relative humidity expressed as a decimal.

2.5. Statistical Analysis

Statistical analyses were performed using the software package SAS version 9.1 (SAS Institute Inc., 2004). Crude nutrient and mycotoxin concentration of the experimental maize silages were analyzed by the GLM procedure of SAS. The evaluation of nutrient digestibility and silage ME was conducted utilizing the MIXED procedure. CO₂ concentration and irrigation during plant growth and climatic conditions during the balance experiment were considered as fixed effects. Interactions between these variables were investigated. The “random” statement was used for the individual animal effect. Fleece length was included as covariate. The restricted maximum likelihood method (REML) was used to evaluate variances. Degrees of freedom were calculated by the Kenward-Roger method. To investigate differences between means and least square means, the “PDIFF” option was used applying a Tukey-Kramer test for post-hoc analysis. Differences were considered to be significant at $p < 0.05$. Trends were declared at $p < 0.1$.

3. Results

3.1. Chemical composition

Increased atmospheric CO₂ did not affect the concentration of crude protein ($p=0.944$, Table 2) or the contents of the analysed nutrient and cell wall fractions of the investigated maize silages. The concentration of starch was not significantly influenced by carbon dioxide treatments ($p=0.642$). Drought stress during cultivation resulted in an increased subsequent silage DM concentration ($p=0.002$). However, drought stress did not alter the crude nutrient, cell wall or starch contents of silage DM and no significant CO₂ x irrigation interactions were observed for crude nutrients.

The concentration of DON was generally characterized by a considerable variation. Though the CO₂ treatments did not affect DON significantly, a numerical decrease was observed due to CO₂ enrichment ($p=0.058$). Drought stress induced a significant increase of DON in silage DM ($p<0.001$). Furthermore, the interaction of CO₂ concentration and irrigation was found to be significant for DON, which was subjected to a greater increase due to drought stress in the Control compared to FACE treatment ($p=0.003$). Silage ZON was not influenced by CO₂ concentration, irrigation or interactions, though it was increased numerically in the maize silages which were affected by drought stress during growth ($p=0.179$). OTA was found to be lower than the limit of detection in all examined samples.

3.2. Nutrient digestibility

Increased atmospheric CO₂ and drought stress during plant growth did not significantly alter the digestibility of the investigated crude nutrient and cell wall fractions or the estimated silage ME as shown in Table 3. The digestibility of starch was found to be generally almost 100% and was not influenced by treatments. The CO₂ x irrigation interaction was significant for ME content ($p=0.014$), however the nominal differences between both irrigation treatments were below 0.15 MJ kg⁻¹ DM each.

The most pronounced effects were conveyed by the thermal environment since the climatic conditions during the balance experiment had a significant effect on NDF ($p=0.005$), ADF ($p=0.024$) and OM ($p=0.023$) digestibility as well as on silage ME ($p=0.009$). The coldest climate (T) resulted in the lowest digestibility of ADF, NDF and CF. However, the negative effects of moderate cold on the digestibility of the analysed fibre fractions were not reflected in OM digestibility. Though the climatic conditions had a significant impact, the numerical effects were low and the increase of OM digestibility during MH treatment was below 2%. Consequently, the highest ME was calculated during MH as well.

No CO₂ x climatic conditions interactions were observed. A significant interaction of irrigation and climatic conditions was calculated for CF digestibility ($p=0.012$), which was reduced during T treatment if the plants were grown well watered. Furthermore, undirected interactions of CO₂ concentration, irrigation and climatic conditions were calculated for NDF ($p=0.002$) and OM ($p=0.020$) digestibility as well as for silage ME ($p=0.001$).

Table 2. Effects of increased atmospheric CO₂ concentration and drought stress on DM content (g/kg fresh matter) as well as crude nutrient (g/kg DM) and mycotoxin concentration (µg/kg DM) of experimental maize silages (N=3. Means ± standard error).

Parameter	Treatment [#]								p-value [#]
	FACE				Control				
	Well watered	Drought stress	Well watered	Drought stress	Well watered	Drought stress	Well watered	Drought stress	
DM	359.2 ± 7.1	400.1 ± 5.3	377.0 ± 1.3	401.0 ± 11.4	0.234	0.002	0.277		
Crude ash	39.4 ± 1.6	34.3 ± 1.3	37.4 ± 1.3	38.0 ± 2.8	0.655	0.250	0.159		
Crude protein	75.7 ± 4.3	75.0 ± 3.9	71.2 ± 2.2	79.6 ± 2.6	0.986	0.284	0.215		
Ether extract	29.9 ± 1.3	31.5 ± 1.2	28.2 ± 1.5	28.3 ± 3.8	0.299	0.703	0.734		
Crude fibre	200.6 ± 1.3	210.6 ± 10.2	196.1 ± 1.9	198.3 ± 4.1	0.176	0.309	0.511		
Neutral detergent fibre	449.9 ± 15.0	424.7 ± 16.8	430.9 ± 8.6	437.2 ± 24.7	0.856	0.598	0.389		
Acid detergent fibre	236.0 ± 3.7	235.5 ± 14.9	211.3 ± 5.7	229.2 ± 4.9	0.109	0.343	0.307		
Acid detergent lignin	29.8 ± 1.6	30.1 ± 3.6	28.1 ± 0.6	30.3 ± 1.5	0.748	0.580	0.674		
Starch	347.0 ± 9.6	353.0 ± 8.6	354.6 ± 13.1	333.1 ± 17.6	0.642	0.558	0.310		
DON*	590.3 ± 133.3	897.3 ± 60.7	383.7 ± 74.4	1565.7 ± 129.8	0.058	<0.001	0.003		
ZON [‡]	118.0 ± 28.5	129.3 ± 9.2	139.3 ± 7.5	183.3 ± 21.4	0.799	0.170	0.410		
OTA [†]	< 0.1	< 0.1	< 0.1	< 0.1	/	/	/		

Notes: [#]Treatments were CO₂ concentration (CO₂, FACE (Free air carbon dioxide enrichment) vs. Control) and irrigation (I, Well watered vs. Drought stress); *DON, deoxynivalenol; [‡]ZON, zearalenone; [†]OTA, ochratoxin A.

Table 3. Effects of increased atmospheric CO₂ concentration and drought stress during maize cultivation and climatic conditions during balance studies on apparent nutrient digestibility (% DM) and metabolizable energy (MJ kg⁻¹ DM) of maize silages (LSmeans ± standard error).

CO ₂	Treatment [#]		Parameter*							
	I	CC	CFD	NDF D	ADF D	Starch D	OM D	ME		
FACE	WW	T	47.3 ± 3.1	45.9 ± 2.9	41.2 ± 3.6	99.0 ± 0.3	67.8 ± 1.6	10.0 ± 0.2		
		MH	55.6 ± 3.4	55.6 ± 3.3	50.5 ± 4.0	98.8 ± 0.3	71.9 ± 1.8	10.5 ± 0.2		
		SH	54.6 ± 3.9	56.5 ± 3.8	50.2 ± 4.7	99.4 ± 0.4	70.4 ± 2.1	10.4 ± 0.3		
Control	DS	T	58.4 ± 3.7	52.5 ± 3.4	47.9 ± 4.2	99.2 ± 0.3	72.0 ± 1.9	10.7 ± 0.3		
		MH	56.5 ± 2.9	51.7 ± 2.7	51.4 ± 3.3	99.3 ± 0.3	72.7 ± 1.5	10.7 ± 0.2		
		SH	59.8 ± 3.0	55.0 ± 2.8	54.4 ± 3.4	99.0 ± 0.3	70.6 ± 1.5	10.3 ± 0.2		
Control	WW	T	52.9 ± 2.7	55.3 ± 2.5	48.0 ± 3.0	99.7 ± 0.3	73.9 ± 1.4	10.8 ± 0.2		
		MH	59.2 ± 2.9	59.2 ± 2.8	53.7 ± 3.4	99.5 ± 0.3	75.1 ± 1.5	11.1 ± 0.2		
		SH	54.7 ± 3.3	53.5 ± 3.3	46.6 ± 4.0	99.6 ± 0.3	70.6 ± 1.8	10.3 ± 0.2		
Control	DS	T	55.8 ± 3.5	50.5 ± 3.4	51.7 ± 4.1	99.6 ± 0.3	72.6 ± 1.9	10.5 ± 0.3		
		MH	57.3 ± 3.4	58.7 ± 3.2	54.8 ± 3.9	99.6 ± 0.3	72.7 ± 1.8	10.6 ± 0.2		
		SH	60.3 ± 3.4	59.7 ± 3.2	55.5 ± 3.9	99.7 ± 0.3	73.2 ± 1.8	10.7 ± 0.2		
<i>p</i> -value										
CO ₂			0.699	0.311	0.504	0.168	0.234	0.323		
I			0.262	0.921	0.350	0.830	0.726	0.839		
CC			0.055	0.005	0.024	0.478	0.023	0.009		
CO ₂ •I			0.086	0.994	0.803	0.623	0.072	0.014		
CO ₂ •CC			0.702	0.192	0.107	0.797	0.328	0.914		
I•CC			0.012	0.207	0.218	0.249	0.196	0.130		
CO ₂ •I•CC			0.238	0.002	0.458	0.116	0.020	0.001		

Notes: [#]Treatments were CO₂ concentration (CO₂, FACE (Free air carbon dioxide enrichment) vs. Control) and Irrigation (I, Well watered (WW) vs. Drought stress (DS)) during maize cultivation and climatic conditions during balance studies (CC; T, temperate; MH, mild heat; SH, severe heat); *CF D, crude fibre digestibility; NDF D, neutral detergent fibre digestibility; ADF D, acid detergent fibre digestibility; Starch D, starch digestibility; OM D, organic matter digestibility; ME, metabolizable energy.

4. Discussion

4.1. Chemical composition

Intensified recent research was conducted in order to investigate the responses of photosynthesis and growth of C₄ plants to increasing atmospheric CO₂ (Leakey et al. 2006; Prasad et al. 2009). However information about effects on chemical composition and feed value of C₄ plants are rare. For example, the protein concentrations of various plant parts of sudangrass (*Sorghum bicolor L.*) except stem protein were generally not affected by CO₂ enrichment (Akin et al. 1994). That is coherent with the results obtained in the present study since the crude protein content of the analysed whole plant maize silages was not influenced by CO₂ enrichment. Considering the distinctly decreased protein concentrations of C₃ plants due to elevated atmospheric CO₂ (Weigel and Manderscheid 2005), an unaltered protein content may be regarded as a positive aspect for the future feed value of maize silage. Nevertheless, the exposure to atmospheres characterized by high CO₂ concentrations exceeding 1000 ppm as expected by the end of the century (IPCC 2007) might affect the protein contents of whole maize plants because under controlled environmental conditions a significant reduction of maize leaf protein was reported due to a treatment of 1100 ppm CO₂ (Maroco et al. 1999). The lack of effects of elevated CO₂ on maize silage fibre content in the present trial is congruent with previous results since the concentrations of different fibre compounds in sugarcane (*Saccharum officinarum L.*) (De Souza et al. 2008), sudangrass (Akin et al. 1994) and various C₄ grasses (Barbehenn et al. 2004) were generally not influenced by rising CO₂. Furthermore, the content of starch was not affected by elevated atmospheric CO₂ as reported for different C₄ species by Barbehenn et al. (2004).

In the present study, the successful induction of restricted water availability was reflected by a significant reduction of silage water content due to drought stress treatment. Nevertheless, the crude nutrient contents of the analysed DM were not affected and hence no indication for a decreased feed value was observed. In maize, drought was reported to decrease grain number per ear (Boyer and Westgate 2004) and deplete the intermediates of starch synthesis (Zinselmeier et al. 1999). This has also been observed in the present study, in which grain yield was decreased under drought by 35% in the Control CO₂ treatment and by 8% in the FACE treatment (Manderscheid et al. 2012). A reduced plant water loss due to elevated CO₂ (Leakey et al. 2006; Ghannoum 2009) may theoretically mitigate the potentially adverse effects of drought stress on the chemical composition of maize silage. Actually, in the present study under drought stress and Control CO₂ crude protein content increased and starch

content decreased as compared to the other treatments. However, these changes were small and not significant. Thus, the water deficit might have been too low to result in considerable alterations of the crude nutrient contents.

In several experiments, the growth of various fungi species and subsequent mycotoxin production in different media were inhibited by very high CO₂ concentrations (Cairns-Fuller et al. 2005; Giorni et al. 2008). Paster and Bullerman (1988) suggested that rather the biosynthetic pathways for the production of mycotoxins than fungal growth may be blocked by high CO₂ levels. However, 800 to 1000 ppm was discussed to be unproblematic for mycotoxigenic fungi (Magan et al. 2011). Accordingly, increasing the atmospheric CO₂ concentration to 550 ppm had only a numerical effect on silage DON concentration and did not impact silage ZON in the present study. Drought stress was reported to increase *Fusarium spp.* colonization of maize kernels (Arino and Bullerman 1994). Therefore, the more than twofold increase of DON due to restricted water availability may represent an indication for plant drought stress supporting maize infection by *Fusarium* fungi and DON formation. However, maize a_w was sufficient to enable *Fusarium* proliferation and mycotoxin production since severe osmotic and matric water stress were reported to lag the germination of *Fusarium graminearum* (Ramirez et al. 2004) and extreme drought will likely impact mycotoxigenic fungi (Magan et al. 2011). CO₂ enrichment resulted in a reduced DON concentration in ensiled maize samples which were exposed to drought stress during growth. Considering the improbability of direct CO₂ effects on fungal growth and mycotoxin production a decreased plant water loss due to increased CO₂ as observed in previous experiments (Leakey et al. 2006) may have alleviated the impacts of the generated drought stress and thus counteracted plant infection by *Fusarium* fungi and the production of DON indirectly. Accordingly, DON was not reduced by CO₂ enrichment in the absence of drought and the different DON contaminations of the silages grown under well watered conditions may rather reflect natural variations. In general, the susceptibility of ruminants to DON is known to be relatively low and, for example, feeding lambs with a diet containing 15.6 mg DON/kg feed was not related to adverse effects on performance parameters (Harvey et al. 1986). Thus, the considerably lower concentrations of DON in the silages fed in the present experiment were not reflected in effects on the digestibility of the investigated nutrients and may not be problematic for adult sheep during short term exposure.

4.2. Nutrient digestibility

In an experiment of Simsek et al. (2011), drought stress was reported to affect the *in vitro* DM digestibility of maize silage. Nevertheless, the absence of effects of both drought stress and CO₂ enrichment on the digestibility of the investigated nutrients was consistent since both factors did not alter the crude nutrient contents of the investigated silage DM. If the physiological mechanisms of sheep fail to negate the heat load during periods of elevated ambient temperatures, such exposure evokes a series of drastic changes in the biological functions, which include alterations in feed efficiency and utilization (Marai et al. 2007). In several experiments, warmer environmental conditions were observed to increase the digestibility of plant cell walls or DM of diets fed to both sheep (Westra and Christopherson 1976) and cattle (Miaron and Christopherson 1992). Similar positive effects of body heat increment were observed in the present study since the coldest experimental treatment resulted in the lowest digestibility of NDF, ADF and CF, respectively. However these alterations were not reflected in OM digestibility, which was highest during MH treatment indicating a compensation of the adverse effects of the colder climate on fibre digestibility by an increased digestibility of other constituents. Christopherson and Kennedy (1983) suggested that decreases in diet digestibility caused by exposure of ruminants to low environmental temperatures are related to increases in the rate of passage of ingesta through the gastrointestinal tract induced by enhanced motility and, in contrast, opposite changes in these parameters appear to occur in response to heat stress. Accordingly, the retention time of ingesta in Suffolk wethers was reported to decrease from 38.5 h in a warm environment to 32.5 h in a cold treatment (Westra and Christopherson 1976). In the same experiment, the mean number of reticulum contractions was found to increase from 60 at 21.2 °C to 72.5 at 1.3 °C. However, the thermal environment does not necessarily affect the digestibility of ruminant diets. In a recent experiment, Lourenco et al. (2010) investigated the effects of two temperature treatments (25.0±0.26 °C vs. 10.8±0.01 °C) and did not observe significant alterations of DM or NDF digestibility using ewes from different breeds. Christopherson and Kennedy (1983) suggested that the magnitude of temperature changes in several experiments may not be large enough to cause measurable alterations in digestion.

5. Conclusions

An increased atmospheric CO₂ concentration of 550 ppm did not affect the feed value of whole plant maize silage. Furthermore, the effects of drought stress on the chemical composition of silage DM were generally low and did not indicate considerable alterations of

the nutritive value. CO₂ enrichment may reduce the DON content of maize silage which can be increased in periods of drought. In sheep, the thermal environment may influence both OM and fibre digestibility of maize silage. Based on the present experiment, no interactions of altered ambient temperatures during feeding of sheep and changed growth conditions of maize silage are to be expected. Further investigations of the effects of increased atmospheric CO₂ should preferably consider continuous and long-term elevations potentially exceeding 1000 ppm by the end of the century.

Acknowledgements

The study was supported by the Ministry for Science and Culture of Lower Saxony within the network KLIFF – climate impact and adaptation research in Lower Saxony. Furthermore, the assistance of the co-workers of the Institute of Animal Nutrition and the Experimental Station of the Friedrich-Loeffler-Institute in Braunschweig, Germany in performing experiment and analysis and the provision of FACE maize samples by the staff of the Institute of Biodiversity, Johann Heinrich von Thuenen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries in Braunschweig, Germany is gratefully acknowledged.

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Paper II

Effects of the thermal environment on metabolism of deoxynivalenol and thermoregulatory response of sheep fed on corn silage grown at enriched atmospheric carbon dioxide and drought

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Abstract

Future livestock production is likely to be affected by both rising ambient temperatures and indirect effects mediated by modified growth conditions of feed plants such as increased atmospheric CO₂ concentrations and drought. Corn was grown at elevated CO₂ concentrations of 550 ppm and drought stress using free air carbon dioxide enrichment technology. Whole plant silages were generated and fed to sheep kept at three climatic treatments. Differential blood count was performed. Plasma DON and de-epoxy-DON concentration were measured. Warmer environment increased rectal and skin temperatures and respiration rates ($p < 0.001$ each) but did not affect blood parameters and the almost complete metabolization of DON into de-epoxy-DON. Altered growth conditions of the corn fed did not have single effects on sheep body temperature measures and differential blood count. Though the thermoregulatory activity of sheep was influenced by the thermal environment, the investigated cultivation factors did not indicate considerable impacts on the analysed parameters.

Keywords: Heat stress, CO₂ concentration, drought, sheep, deoxynivalenol

Introduction

Changing future climate will likely affect both livestock production and growth conditions of cereal and pasture plant species. The atmospheric CO₂ concentration has risen from a preindustrial level of about 280 parts per million (ppm) to a current value of approximately 390 ppm and is predicted to exceed 700 ppm by the end of the century. These processes are forecasted to be accompanied by further elevations of the global surface temperature in the range of 1.8 – 4°C until 2090-2099, depending on the future greenhouse emission scenario. Furthermore, increased drought is likely to occur in different regions throughout the world (IPCC 2007).

Rising future ambient temperatures will likely increase the incidence of heat stress especially in free-ranging farm animals such as sheep as heat stress is initiated if a combination of environmental conditions cause the effective temperature of the environment to be higher than the thermo-neutral zone of an animal (Bianca 1962). The exposure of sheep to heat stress evokes a series of drastic changes in the biological functions, which include decreases in feed intake, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretion and blood metabolites (Marai et al. 2007).

Such direct effects of changing environmental conditions on sheep will likely be accompanied by indirect impacts mediated by differing quantity and quality of feed. Rising atmospheric

CO₂ concentrations were found to stimulate yields but to reduce grain and forage protein concentrations of C₃ crops and to alter the contents of various essential minerals (Manderscheid et al. 2009; Porteous et al. 2009; Yang et al. 2007). Information about responses of C₄ plants such as corn (*Zea mays L.*) to elevated CO₂ is limited but of immense importance since corn silage is used prevalently as a main constituent of ruminant diets to meet the energy requirements of high-yielding animals. Substantial changes in diet formulation can affect the body temperature of both cattle (O'Kelly 1987) and sheep (Sudarman and Ito 2000) and thus may interact with rising ambient temperatures. The photosynthesis of C₄ crops is likely to be saturated at ambient CO₂ concentrations (Ghannoum 2009) and therefore the impact of increasing CO₂ may be limited. Corn photosynthesis, biomass and yield were reported to be unaffected under sufficient water supply using free air carbon dioxide enrichment (FACE) technology (Leakey et al. 2006) but were stimulated in periods of drought stress (Ghannoum 2009) potentially due to a decreased stomatal conductance. Therefore increased atmospheric CO₂ may reduce the adverse effects of drought on corn plant growth and grain yield which were described previously (Azeez et al. 1994; Efeoglu et al. 2009). Furthermore, both varying atmospheric CO₂ concentrations and water availability were reported to affect the growth of fungi from various genera in different media and to influence the production of several toxic secondary metabolites such as mycotoxins (Cairns-Fuller et al. 2005; Magan et al. 2011). The incidence of *Fusarium* toxins such as deoxynivalenol (DON) may increase in periods of drought since corn is known to be susceptible to infection during plant drought stress and accordingly the colonization of corn kernels by *Fusarium spp.* was reported to be higher during dry conditions (Arino and Bullerman 1994). Though in ruminants DON is converted almost completely into the less toxic metabolite de-epoxy-DON (Fink-Gremmels 2008; Seeling et al. 2006), the intake of high concentrations of DON may affect metabolism and immunological parameters of dairy cows (Korosteleva et al. 2007; Korosteleva et al. 2009). It is unclear, whether the capacity of the conversion of DON in ruminants may be impaired by increased ambient temperatures and associated heat stress.

The objective of the present study was to investigate the effects of the thermal environment on the metabolism of DON and thermoregulatory and blood parameters of sheep considering indirect influences possibly mediated by increased atmospheric CO₂ concentration and drought stress during the growth of whole corn plants fed as silage.

Materials and methods

The present study was a part of the experiment described by Lohölter et al. (2012).

Plant cultivation

Plants were cultivated in 2008 on an experimental field site of the Johann Heinrich von Thünen-Institute in Brunswick, Germany (52°18'N, 10°26'E). Agricultural measures of the 10 hectare field site were carried out according to local farm practices. Corn (*Zea mays L.*, variety “Romario”) was sown at May 9 with a plant density of 10 plants m⁻². Mineral nutrients were added according to local fertilizing practices based on soil analysis in springtime (Manderscheid et al. 2012). After plant emergence in mid of May a FACE system as described in detail by Lewin et al. (1992) was installed. Treatments included three rings with blowers and CO₂ enrichment to meet a target CO₂ concentration of 550 ppm during daylight hours (FACE) and three control rings operated under ambient air (Control), respectively. Mean seasonal CO₂ concentration in the Control treatment was 379 ppm. The rings had an outer diameter of 20 m and each ring was splitted into a well-watered (Well watered) and a dry semicircular subplot (Drought stress). In the well watered subplots water content in the upper 0.6 m soil profile was kept above 50% of maximum plant available soil water content by drip irrigation. Drought stress was achieved by the operation of rain shelters (Erbs et al. 2011). The dry subplots received 48% less water than the well watered subplots over the whole vegetation period (Manderscheid et al. 2012).

Whole corn plants were harvested manually on October 6 from every semicircle within an inner diameter of 16 m and chopped afterwards. Subsequently, ensiling was executed for at least 12 months in plastic barrels (O-TopTM Open Head Drums, Mauser AG, Bruehl, Germany) containing a volume of approximately 0.2 m³ each.

Experimental design

In a feeding experiment, the produced corn silages were fed to sheep which were subjected to three climatic treatments (Table 1). Accordingly, the three experimental factors were CO₂ concentration (FACE vs. Control) and irrigation (Well watered vs. Drought stress) during plant growth as well as the climatic conditions during the feeding experiment (Temperate: Temperature 14–16°C, relative humidity (RH)<50%, temperature humidity index (THI) 57–63; Mild heat: Temperature 24–26°C, RH<50%, THI 68–71; Severe heat: Temperature 34–36°C, RH<50%, THI 75–80).

Table 1 Climatic conditions during the feeding experiments (Means \pm standard error)

Treatment ^a	Temperature (°C)	RH (%)	THI ^b
T	15.1 \pm 0.01	39.6 \pm 0.1	58.8 \pm 0.02
MH	25.6 \pm 0.02	23.4 \pm 0.1	69.5 \pm 0.02
SH	35.1 \pm 0.01	17.8 \pm 0.1	78.2 \pm 0.03

^a: T, temperate; MH, mild heat; SH, severe heat

^b: Temperature humidity indices (THI) were calculated according to Hahn (1999): $THI = 0.8 \times td + RH \times (td - 14.4) + 46.4$

Animals and sampling

The feeding experiment was performed in temperature but not relative humidity controlled rooms of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Brunswick, Germany, following the guidelines for determining the digestibility of crude nutrients in ruminants (GfE 1991). Room dry bulb temperature (Td) and relative humidity were recorded every 10 minutes using data loggers (Tinytag Plus 2, TGP-4500, Gemini Data Loggers, Chichester, United Kingdom).

Adult castrated male sheep (German blackheaded mutton sheep) with an initial bodyweight of 100.1 \pm 7.4 kg were allocated randomly to experimental groups of 4 animals each. Both adaptation feeding and subsequent sampling periods were performed for 7 consecutive days. The animals were kept in individual boxes during adaptation and were transferred into metabolism cages for sampling. Individual abdominal fleece length was measured prior to each sampling period. All sheep had free access to water and were fed on 1 kg dry matter (DM) experimental corn silage and 20 g urea per day and animal, presented in 2 equal portions at 6.30 h and 13.30 h. All presented diets were consumed completely. Daily water usage was recorded manually. Representative samples of corn silages fed were pooled by daily aliquot retaining. Silage samples were oven-dried (60°C for 72 h) and then ground to pass through a 1 mm sieve for further analyses.

Rectal temperature, skin temperature and respiration rate were measured twice daily on day 3, 4, 5 and 6 of each sampling period between 9:00 and 11:00 h and between 15:00 and 17:00 h, respectively. Rectal temperature was determined by digital thermometry (Digitemp Servoprax E315, Servopax GmbH, Wesel, Germany). Skin temperature was measured at the left abdomen on a spot shaved prior to each sampling period using an infrared thermometer (Fluke 561, Fluke Corporation, Everett, WA, USA). Blood samples were obtained from the *Vena jugularis externa* directly after the last experimental morning feeding at 7.30 h. Whole blood and plasma (Heparin) samples were gained. Plasma was stored at -20°C for further analyses.

The effects of atmospheric CO₂ concentration and irrigation during plant growth on silage crude nutrient content and the concentration of DON were described previously (Lohölter et al. 2012). The crude nutrient content of silage DM was not affected by both treatments and is presented in Table 2. Both a significant effect of drought stress and an interaction of CO₂ and irrigation on silage DON concentration were observed resulting in the following DON contaminations of the corn silages fed (Means ± Standard Error, referring to DM): FACE/Well watered: 590±133 µg kg⁻¹, FACE/Drought stress: 897±60 µg kg⁻¹, Control/Well watered: 384±74 µg kg⁻¹, Control/Drought stress 1566±130 µg kg⁻¹.

Table 2 Crude nutrient content (g kg⁻¹ DM) and metabolizable energy (MJ kg⁻¹ DM) of experimental corn silages (Means ± standard error)

Treatment ^a		Parameter ^b						
CO ₂	I	CP	EE	CF	NDF	ADF	Starch	ME
FACE	WW	75.7±4.3	29.9±1.3	201±1	450±15	236±4	347±10	10.3±0.2
	DS	75.0±3.9	31.5±1.2	211±10	425±17	236±15	353±9	10.6±0.2
Control	WW	71.2±2.2	28.2±1.5	196±2	431±9	211±6	355±13	10.7±0.2
	DS	79.6±2.6	28.3±3.8	198±4	437±25	229±5	333±18	10.6±0.2

^a: Treatments were CO₂ concentration (CO₂, FACE vs. Control) and Irrigation (I, Well watered (WW) vs. Drought stress (DS)) during cultivation

^b: CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; ME = Metabolizable energy, calculation based on nutrient digestibilities measured with wethers (GfE 1991)

Analyses

Dry matter (DM), crude ash (Ash), crude fibre (CF), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch in corn silage samples were analyzed according to the protocols of the Association of German Agricultural Analysis and Research Centres (Naumann and Bassler 1993), whereat ADL, ADF and NDF were expressed without residual ash.

Total leukocytes were counted manually in stained whole blood samples using an improved Neubauer counting chamber (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and Türk's solution (VWR International GmbH, Darmstadt, Germany). Stained whole blood smears were generated on microscope slides to perform manual 200-cell differential counts.

Plasma samples were analysed for DON and de-epoxy-DON after incubation with 6000 U β-glucuronidase (Type H-2, min. 98800U/ml, Sigma, Steinheim, Germany) at pH 5.5 (Acetate buffer) and 37°C overnight. Samples were extracted on ChemElut columns (Varian, Darmstadt, Germany) and cleaned up with immunoaffinity columns (IAC) (DONtest, Vicam, Watertown, USA) before analysis by LC-ESI-MS/MS (liquid chromatography-electrospray ionization tandem mass spectrometry, API 4000 QTrap, Applied Biosystems, Darmstadt,

Germany, coupled with a 1200 series HPLC system, Aligent Technologies, Böblingen, Germany) as described by Döll et al. (2009). The limit of detection for both DON and de-epoxy-DON was 0.2 ng ml⁻¹. The mean recovery was 94% for DON and 97% for de-epoxy-DON, respectively. Analytical results were not corrected for recovery.

Calculations

Temperature humidity indices (THI) were calculated according to Hahn (1999):

$$\text{THI} = 0.8 \times \text{td} + \text{RH} \times (\text{td} - 14.4) + 46.4$$

where td represents the dry bulb temperature (°C) and RH (%) is the relative humidity expressed as a decimal.

The metabolization rate of DON was calculated following Keese et al. (2008) as the ratio of the De-epoxy-DON-concentration in plasma and the sum of both DON and De-epoxy-DON concentration in plasma and expressed as a percentage.

Daily DON intake was calculated as the ratio of daily DON intake and individual bodyweight.

Statistical analyses

Statistical analyses were performed using the software package SAS version 9.1. The evaluation of all investigated parameters was conducted by the MIXED procedure of SAS. CO₂ concentration and irrigation during corn growth and climatic conditions during feeding experiments were considered as fixed effects for all parameters except mycotoxin residues in plasma. Interactions between these factors were examined. The climatic conditions were defined as fixed effect for the analysis of plasma mycotoxins. The “random” statement was used for the individual animal effect to evaluate all parameters. Fleece length was tested as covariate and included if it improved the respective model. Time of measurement was tested as an additional covariate assessing rectal temperature, skin temperature and respiration rate to consider diurnal variation and was included if it improved the respective model. DON intake was used as an additional covariate for the evaluation of DON and de-epoxy-DON in plasma. The restricted maximum likelihood method (REML) was used to evaluate variances. Degrees of freedom were calculated by the Kenward-Roger method. To investigate

differences between least square means, the “PDIFF” option was used applying a Tukey-Kramer test for post-hoc analysis.

The dose-response relationship between DON intake and DON or de-epoxy-DON in plasma was considered by a simple linear regression analysis using STATISTICA software 10.

Differences were considered to be significant at $p < 0.05$. Trends were declared at $p < 0.1$.

Results

Thermoregulatory mechanisms

Elevated atmospheric CO₂ concentration and drought stress during corn growth did not affect rectal temperature, skin temperature, respiration rate and water usage of the experimental sheep (Table 3). In contrast, all investigated parameters were influenced by the thermal environment. Warmer climatic conditions induced increases in body temperatures, respiration rates and water usage ($p < 0.001$). Skin temperature data were characterized by a higher variation than rectal temperature. The rises of both rectal and skin temperature due to heat exposure seemed to be approximately linear. However, the differences between both body temperature measures decreased with progressing body heat load indicating an unequal slope. In contrast, respiration rate seemed to rise in an exponential manner and was subjected to a 3.5- to 4-fold increase between T and SH treatment. Though the interaction of irrigation and climatic conditions was significant for rectal temperature ($p < 0.001$), the numerical differences were minimal. An interaction of CO₂ concentration, irrigation and climatic conditions was observed for skin temperature ($p < 0.001$).

Table 3 Effects of the climatic conditions during feeding experiments as well as increased atmospheric CO₂ concentration and drought stress during corn growth on rectal and skin temperature, respiration rate and daily water usage of sheep (LSmeans \pm standard error)

Treatment ^a			Parameter ^b			
CC	CO ₂	I	Rectal temp	Skin temp	RR	Water usage
T	FACE	WW	38.5 \pm 0.1	28.6 \pm 0.3	26.5 \pm 3.6	1.6 \pm 1.3
MH			38.8 \pm 0.1	33.6 \pm 0.4	67.2 \pm 3.7	3.4 \pm 1.3
SH			39.3 \pm 0.1	37.6 \pm 0.5	124.0 \pm 4.8	4.0 \pm 1.4
T		DS	38.4 \pm 0.1	27.6 \pm 0.5	36.8 \pm 4.7	2.2 \pm 1.4
MH			38.9 \pm 0.1	33.2 \pm 0.4	73.4 \pm 3.3	3.8 \pm 1.3
SH			39.2 \pm 0.1	37.4 \pm 0.4	121.8 \pm 3.6	4.6 \pm 1.3
T	Control	WW	38.4 \pm 0.1	28.1 \pm 0.3	29.6 \pm 3.1	2.6 \pm 1.2
MH			38.7 \pm 0.1	34.6 \pm 0.3	63.3 \pm 3.1	3.7 \pm 1.3
SH			39.2 \pm 0.1	38.4 \pm 0.4	113.6 \pm 3.9	4.6 \pm 1.3
T		DS	38.4 \pm 0.1	28.6 \pm 0.4	33.9 \pm 4.6	2.4 \pm 1.3
MH			38.8 \pm 0.1	33.2 \pm 0.4	64.3 \pm 4.2	3.3 \pm 1.3
SH			39.0 \pm 0.1	36.7 \pm 0.4	121.8 \pm 4.4	4.6 \pm 1.3
p-value						
CC			<0.001	<0.001	<0.001	<0.001
CO ₂			0.441	0.338	0.152	0.903
I			0.374	0.173	0.436	0.804
CC \times CO ₂			0.397	0.443	0.130	0.501
CC \times I			<0.001	0.058	0.288	0.780
CO ₂ \times I			0.125	0.329	0.605	0.170
CC \times CO ₂ \times I			0.237	<0.001	0.182	0.910

^a: Treatments were climatic conditions during feeding experiments (CC; T, temperate; MH, mild heat; SH, severe heat) as well as CO₂ concentration (CO₂, FACE vs. Control) and Irrigation (I, Well watered (WW) vs. Drought stress (DS)) during cultivation

^b: Rectal temp, rectal temperature (°C); Skin temp, skin temperature (°C); RR, respiration rate (Breaths per minute); Water usage (kg/d)

Differential blood count

The total count of leukocytes and their distribution were generally not affected by the experimental treatments and were characterized by a considerable variation (Table 4). Furthermore, all interactions were found to be insignificant. Basophils (1.3 \pm 0.1, Means \pm Standard error), eosinophils (3.4 \pm 0.3) and monocytes (1.4 \pm 0.2) were within the reference ranges given by Kraft and Dürr (1999) but were not subjected to statistical analysis because of the low numbers of those cells. The total leukocyte count was observed to be partially lower than the reference range. In contrast, lymphocytes tended to exceed the expected percentage.

Table 4 Effects of the climatic conditions during feeding experiments as well as increased atmospheric CO₂ concentration and drought stress during corn growth on differential blood count of sheep (LSmeans ± standard error)

Treatment ^a			Parameter		
			Leukocytes, 10 ⁹ /L	Lymphocytes, %	Neutrophils, %
CC	CO ₂	I	4.2-6.2 ^b	40-65 ^b	20-45 ^b
T	FACE	WW	5.8±1.0	70.9±6.3	21.2±6.0
MH			6.0±1.1	56.6±7.1	36.8±6.6
SH			5.8±1.4	57.9±8.5	30.3±7.8
T		DS	2.0±1.2	75.3±7.7	20.2±7.1
MH			4.0±0.9	76.5±5.9	18.7±5.6
SH			3.6±1.0	69.4±6.0	27.1±5.8
T	Control	WW	5.0±0.8	67.3±5.2	25.5±5.0
MH			5.8±1.0	64.3±6.5	24.7±6.0
SH			4.6±1.1	61.1±7.2	27.6±6.6
T		DS	3.8±1.2	80.0±7.4	16.5±6.7
MH			3.4±1.1	73.1±6.8	21.2±6.3
SH			3.0±1.1	73.0±6.8	23.4±6.3
p-value					
CC			0.367	0.212	0.353
CO ₂			0.727	0.637	0.480
I			0.118	0.186	0.410
CC × CO ₂			0.392	0.924	0.783
CC × I			0.824	0.735	0.620
CO ₂ × I			0.309	0.887	0.758
CC × CO ₂ × I			0.370	0.438	0.309

^a: Treatments were climatic conditions during feeding experiments (CC; T, temperate; MH, mild heat; SH, severe heat) as well as CO₂ concentration (CO₂, FACE vs. Control) and Irrigation (I, Well watered (WW) vs. Drought stress (DS)) during cultivation

^b: Reference values according to Kraft and Dürr (1999)

Deoxynivalenol and its metabolite in plasma

The concentration of DON in the analysed plasma samples was not affected by the climatic conditions, though a numerical decrease was associated with warmer environment (Table 5). Plasma de-epoxy-DON was slightly increased during T treatment (p=0.057). The metabolization rate of DON was higher than 90 % and was not influenced by the climatic conditions (p=0.197), though it was found to be lowest during T treatment. The covariate daily individual DON intake had a significant effect on the concentration of de-epoxy-DON (p<0.001), but did not influence DON (p=0.225) and the metabolization rate of DON (p=0.803), respectively. The mean daily DON intake as determined by the varying contaminations of the corn silages was highest during T treatment (11.0±1.1 µg kg⁻¹ BW. Means ± standard error) compared to MH (7.5±1.2 µg kg⁻¹ BW) and SH (7.8±1.1 µg kg⁻¹ BW) and ranged from 2.4 to 19.3 µg kg⁻¹ BW. The concentration of DON was observed to be lower than 1 ng/ml plasma except for one sample. In contrast, the concentration of the metabolite

de-epoxy-DON strongly depended on the intake of DON and increased with rising oral DON exposure (Fig. 1).

Table 5 DON and de-epoxy-DON concentrations in plasma of sheep and metabolism rate as affected by the climatic conditions during feeding experiments (LSmeans \pm standard error. Values were pooled for treatments during growth of ensiled maize fed)

Parameter	Treatment**			p-value
	T	MH	SH	
DON concentration in plasma (ng ml ⁻¹)	0.37 \pm 0.07	0.28 \pm 0.07	0.22 \pm 0.07	0.368
De-epoxy-DON concentration in plasma (ng ml ⁻¹)	4.94 \pm 0.40	3.66 \pm 0.39	3.73 \pm 0.40	0.057
Metabolization rate* (%)	90.9 \pm 1.8	94.3 \pm 1.8	95.4 \pm 1.8	0.197

*: Metabolization rate = de-epoxy-DON concentration in plasma / (DON plus de-epoxy-DON concentration in plasma) x 100

** : Experimental treatments were temperate (T), mild heat (MH) and severe heat (SH)

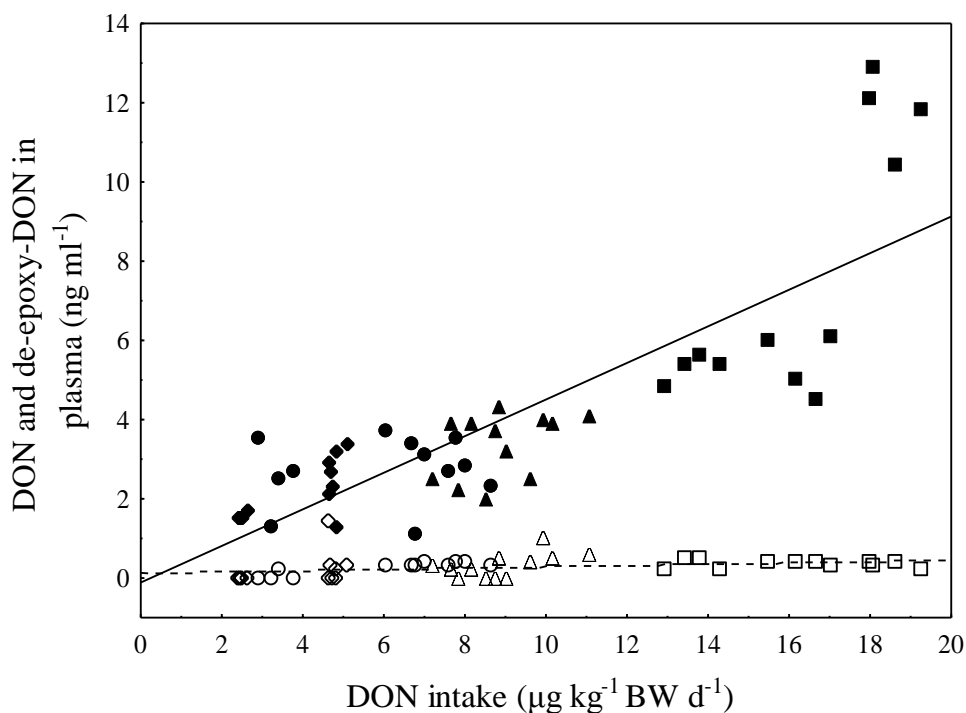


Fig. 1 Relationship between deoxynivalenol (DON) intake and DON ($y = 0.1321 + 0.0164x$, $r^2 = 0.09$, $p = 0.04$) or de-epoxy-DON ($y = -0.1139 + 0.4621x$, $r^2 = 0.69$, $p < 0.01$) concentration in plasma of sheep. Treatments during growth of the ensiled corn fed were highlighted (FACE/ well watered: \circ , \bullet ; FACE/ drought stress: Δ , \blacktriangle ; Control/ Well watered: \diamond , \blacklozenge ; Control/ Drought stress: \square , \blacksquare ; Open symbols represent DON, closed symbols represent de-epoxy-DON)

Discussion

The heat exchange between animal and environment depends on environmental variables such as ambient temperature, relative humidity, radiation and wind, diverse animal factors and the thermoregulatory mechanisms as well as circulatory adjustments (Nienaber et al. 1999). If

homeothermic animals are within the so-called thermo-neutral zone, heat production is minimal at normal rectal temperatures (Kadzere et al. 2002). In the present experiment, rectal temperatures were rising significantly due to warmer environment but did not exceed 39.3°C during SH treatment. That may be considered as a high physiological level that should not be problematic for adult animals during temporary exposure since rectal temperatures of sheep are characterized by a considerable variation between 38.3°C and 39.9°C under thermo-neutral conditions (Marai et al. 2007a) and can exceed 40°C if the animals are kept at 36°C (Llamaslamas and Combs 1990). The low RH of 17.8 % will likely have facilitated evaporative heat dissipation and therefore may have contributed to the relatively low rectal temperatures during SH treatment. The differences between rectal and skin temperature decreased with warmer environmental conditions and were lower than 2°C during SH treatment indicating an increased skin blood flow in order to dissipate excess body heat. In sheep, close positive correlations between ambient temperature and skin temperature were described (Monty et al. 1991) and the skin temperature measured at the trunk of unshorn pregnant Dorset ewes was observed to rise from 30.2°C to 37.2°C when the animals were kept at 25°C and 35°C, respectively (Hofman and Riegler 1977). In the present experiment, skin temperature increased from a higher initial level of approximately 33.5°C during MH treatment to a value exceeding 37°C during SH treatment. Furthermore, the respiration rate was found to increase exponentially resulting in a total 3.5 to 4-fold elevation between T and SH treatment. The process of rising respiratory energy dissipation due to an elevated body heat load was consistent with former literature. The proportion of respiratory heat loss accounts for approximately 20 % of the total body heat dissipation of sheep in a thermo-neutral environment of 12°C (Marai et al. 2007a) but increases up to 60 % if the animals are kept at 35°C (Thompson 1985).

In ruminants, the microbial activity during fermentation in the reticulorumen accounts for 3-8 % of the total heat production (Czerkawski 1980). Diet formulation may influence body heat production since feeding more concentrate at the expense of fibrous ingredients should reduce the heat increment (West 1999). For example, the level of nitrogen intake was found to affect vaginal and skin temperatures of sheep significantly (Sudarman and Ito 2000). However, such diet effects were caused by considerable alterations of the formulation of the diets and even substantial changes in diet composition do not necessarily affect the body temperature of sheep (Bhattacharya and Uwayjan 1975). Accordingly, the lack of effects of both increased atmospheric CO₂ concentration and drought stress during the growth of the corn silages fed

on the investigated thermoregulatory parameters was consistent since both factors did not cause significant alterations of the crude nutrient content of silage DM (Lohölter et al. 2012).

The differential blood count of sheep in the present experiment was generally not affected by the evaluated treatments. That coincides with a study of da Silva et al. (1992), where the exposure to high ambient temperatures of 25-46°C did neither affect the concentration of leucocytes, lymphocytes nor neutrophils in the blood of sheep of both sexes.

The exposure of monogastric animals to DON is well documented to result in adverse effects on health and performance (Dänicke et al. 2007; Goyarts et al. 2005). In contrast, ruminants are less susceptible to DON as in the rumen DON is converted almost completely into the less toxic metabolite de-epoxy-DON (Fink-Gremmels 2008; Seeling et al. 2006). In a study by Keese et al. (2008), lactating dairy cows were fed either a control diet with a daily DON intake between 15 and 20 $\mu\text{g kg}^{-1}$ BW or a DON contaminated diet associated with a considerably higher intake of 150-225 $\mu\text{g kg}^{-1}$ BW and day. The intake per kg BW of the control group was comparable to the daily intake in the present study which ranged between 2.4 and 19.3 $\mu\text{g kg}^{-1}$ BW. Low concentrations of unmetabolized DON were measured in the bovine serum samples of both control (Maximum: 2.0 ng ml^{-1}) and contaminated group (Maximum: 18.0 ng ml^{-1}) (Keese et al. 2008). That was congruent with the DON concentrations in the ovine plasma samples of the current experiment which were generally close to the limit of detection with a maximum of 1.4 ng ml^{-1} . Furthermore, the concentration of DON was not influenced by the daily DON intake and the climatic conditions during feeding, respectively. Thus neither the significant increase of DON in ensiled corn grown under drought stress conditions nor the reduction of silage DON contamination as induced by elevated atmospheric CO₂ in the plants affected by drought were reflected in the plasma concentration of DON. The metabolite de-epoxy-DON was the predominant compound found in plasma samples after oral DON exposure and was highly related to DON intake. That coincides with former literature since Seeling et al. (2006) investigated the effects of feeding *Fusarium* contaminated wheat on the biotransformation of DON in dairy cows and concluded that in the rumen DON is rapidly and almost completely (94-99 %) biotransformed to de-epoxy-DON. It needs to be elucidated whether the time between the consumption of the corn silages and blood sampling contributed to the low concentrations of plasma DON in the present study. However, similar results were reported for lactating cows in the experiment of Seeling et al. (2006). Offering one of four daily portions of contaminated concentrate containing 5.2 mg DON kg^{-1} DM two hours prior to blood sampling resulted in low serum concentrations of DON with a maximum of 5 ng ml^{-1} . In the current study, the de-epoxy-

DON concentration was found to be elevated during T treatment ($p=0.057$). However, the mean daily DON intake as determined by the varying contaminations within each treatment during plant growth was highest during T climate as well and will likely explain the increases because blood de-epoxy-DON strongly depended on the consumption of DON.

Though in ruminants DON is metabolized to a large extent, high dietary concentrations were reported to affect the ruminal utilization of protein (Dänicke et al. 2005) as well as metabolism and immunity (Korosteleva et al. 2007; Korosteleva et al. 2009) of dairy cows. Only few experiments were conducted in order to investigate toxic effects of DON in sheep (Eriksen and Pettersson 2004). For example, feed consumption, weight gain, feed efficiency and different blood parameters of lambs were not affected by feeding the animals with a diet containing $15.6 \text{ mg DON kg}^{-1}$ feed (Harvey et al. 1986). In the present study, the DON contaminations of the corn silages fed were generally lower than 2 mg kg^{-1} DM. As indicated by the high metabolization rate of DON, such low dietary concentrations should not be hazardous for adult sheep. Accordingly, the effects of the treatments during corn growth on the concentration of DON in the silages fed as described by Lohölter et al. (2012) were not reflected in effects on rectal or skin temperature, respiration rate and differential blood count.

Conclusions

In conclusion, the thermal environment did not affect differential blood count and metabolism of DON, respectively, though body temperature and respiration rate of adult castrated male sheep increased due to heat exposure. Furthermore, elevated atmospheric CO_2 concentration and drought stress during corn growth did not have single effects on the examined thermoregulatory parameters or differential blood count as both factors did not alter the crude nutrient content of silage DM. In sheep, the almost complete conversion of DON into the metabolite de-epoxy-DON was not related to the thermal environment but the concentration of de-epoxy-DON in plasma strongly depended on the intake of DON.

Acknowledgements

The assistance of the co-workers of the Institute of Animal Nutrition and the Experimental Station of the Friedrich-Loeffler-Institute in Brunswick, Germany in performing experiment and analysis as well as the provision of FACE corn samples by the staff of the Institute of Biodiversity, Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries in Brunswick, Germany and the analysis of blood gases and red

blood cells by the staff of the clinical chemical laboratory of the clinic for cattle in Hanover, Germany is gratefully acknowledged.

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Paper III

Evaluation of a device for continuous measurement of rumen pH and temperature considering localization of measurement and dietary concentrate proportion

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Livestock Science
Submitted

Abstract: Continuous rumen pH and temperature measurement may be useful tools for diverse diagnostic and research purposes including the detection of indications of subacute ruminal acidosis. The primary objective of the present study was to evaluate a commercially available device for continuous monitoring of rumen pH, temperature and pressure of cattle focussing on rumen pH determination and to test the effects of measurement localization as well as dietary concentrate proportion on rumen pH and temperature. Four rumen-fistulated cows were fed on two diets containing 0 and 40 % concentrate, respectively. Measurement was executed for 2 days per cow and diet. One probe was inserted each in the dorsal and ventral rumen sac to measure pH and temperature continuously. Manual temperature determination and removal of rumen fluid for subsequent manual pH measurement were performed postprandial in direct proximity to the probes at preset short term intervals. PH sensors were tested for drift. The pH sensor drift was inconsistent and in parts undirected with a considerable individual variation. A moderate correlation between manual and continuous measurement of pH ($r=0.59$, $p<0.001$) and temperature ($r=0.46$, $p<0.001$) was calculated. The Bland-Altman comparison of both methods indicated moderate agreement that may as well be due to a deficient accuracy of manual pH measurement. A bias effect of probe pH determination with a pH overestimation in the range of low rumen pH below 6.0 and an underestimation of higher rumen pH above 6.5 was observed. Rumen pH was not affected by the localization of measurement, but by diet and time after feeding. Significant effects of localization, diet and time and an interaction of localization and diet on rumen temperature were found. In conclusion, the evaluated technique was promising. However indications of inaccuracy of probe pH measurement suggested the need of further improvement.

Keywords: continuous rumen pH measurement, rumen temperature, concentrate proportion

1. Introduction

Subacute ruminal acidosis (SARA) is a metabolic disorder affecting rumen fermentation and functionality, animal health and productivity of dairy cows with a considerable prevalence in European herds (Kleen et al., 2009; Morgante, 2007). It may be induced by the consumption of diets containing high amounts of easily fermentable carbohydrates, particularly in combination with a rumen environment insufficiently adapted to such diets as frequently occurring in post-partum periods (Kleen et al., 2003). Feeding high amounts of grain results in increased production of short chain fatty acids (Bauman, 1971) and decreased rumen pH,

whereas especially in early lactation the rumen mucosal papillae are short with a small surface for the absorption of short chain fatty acids.

Various thresholds of rumen pH have been discussed to indicate the onset of SARA. Garrett et al. (1999) suggested a critical value of 5.5 and discussed the determination of pH in rumen fluid from group subsamples as a potential tool to detect SARA in dairy cow herds. In a recent study, the onset of SARA was supposed to be characterized by a rumen pH below 5.6 for at least 3 hours per day (Gozho et al., 2005). The validity of results obtained by pH measurement in rumen fluid gained either by means of rumenocentesis, via oro-ruminal probes or through a rumen cannula as frequently executed in research is discussed to be limited. Restricted times of sampling, sample and animal number as well as sampling sites in the rumen or saliva contamination may affect the significance of obtained results. Furthermore the mentioned methods can hardly be applied by farmers. Enemark (2008) discussed the continuous monitoring of rumen pH instead of spot sampling as a promising measure to contribute to SARA diagnosis. One opportunity to realize a continuous pH measurement may be the use of indwelling rumen probes, which would allow the animal to move freely and undisturbed and would offer the benefit of sampling rumen pH at programmed intervals and thus give the chance to closely follow the course of rumen pH as influenced by different feeding regimes (Enemark, 2008). After the beginning of continuous rumen pH determination by means of intraruminal devices (Dado and Allen, 1993) various probes were evaluated for the application both in small ruminants (Penner et al., 2003) and cattle (Enemark et al., 2003; Penner et al., 2006; Phillips et al., 2010). Effects of the sites of sampling on pH of withdrawn rumen fluid were reported, mean pH values at the cranial-dorsal rumen were slightly but not significantly lower than those at the cranial-ventral rumen (Duffield, 2004; Li et al., 2009). Dietary concentrate proportion is well known to affect rumen pH (AlZahal et al., 2009; Mishra et al., 1970), though small alterations of the forage : concentrate ratio in dairy cow diets do not necessarily influence mean rumen pH (Maekawa et al., 2002). Information about interactions of the localization of pH measurement in the rumen and the amount of concentrate fed is rare but may be valuable for the interpretation of pH data determined by indwelling rumen probes.

Recently, Kaur et al. (2010) tested commercially available devices for rumen pH, pressure and temperature measurement with the ability of telemetric data transfer (KB 1101 bolus, Kahne Limited, New Zealand). They observed a weak relationship between bolus and manual pH measurement in withdrawn rumen fluid and a steadily increasing pH sensor drift. Bolus pH determination was performed via ISFET (ion-selective field-effect transistor) sensors,

which may show long-term drift and low performance in comparison to glass electrodes (Oelssner, 2005). Meanwhile, a renewed successor of these probes is available using a glass membrane sensor for pH measurement. Though an improved accuracy and agreement with manual rumen pH determination may be expected, reliable information about the actual performance of that transformed probe is required. Furthermore, rumen temperature measurement may aid in the detection of SARA since a close inverse correlation with rumen pH was reported (AlZahal et al., 2008). Forestomach temperature was found to be strongly correlated with rectal temperature (Bewley et al., 2008; Burns et al., 2002) and may also be a useful diagnostic parameter for the detection of estrus, heat stress or infectious diseases in dairy cows (Fordham et al., 1988; Kadzere et al., 2002; Martello et al., 2010). The aim of the present study was to evaluate new commercially available devices for pH, temperature and pressure measurement in the rumen of cattle primarily focussing on the examination of pH measurement and to test potential effects of the localization of measurement in the rumen as well as dietary concentrate proportion on rumen pH and temperature.

2. Materials and Methods

2.1 Animals and feeding

The present study was performed at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health in Braunschweig, Germany, in compliance with the European Union Guidelines concerning the protection of experimental animals. Four non-lactating German Holstein cows with a mean initial bodyweight of 614 ± 76 kg equipped with large rubber cannulas in the dorsal rumen sac were used in the experiment, which was divided into two successive periods. Due to illness one animal had to be replaced by a lactating cow before adaptation feeding of diet 2 was started. The animals were kept in a tethered barn with individual troughs and free access to water. Two diets were fed successively after three weeks of adaptation each. Feeding was performed *ad libitum* twice daily at 05:15 and 15:15 h. Daily individual dry matter intake was recorded to calculate organic matter intake. Diet 1 was composed of 60 % maize silage and 40 % grass silage on a dry matter (DM) basis. Diet 2 was designed as a Total Mixed Ration (TMR) containing 36 % maize silage, 24 % grass silage and 40 % concentrate. Concentrate was composed of 50.0 % wheat grain, 26.8 % soybean meal, 20.8 % corn grain and 2.4 % mineral and vitamin premix. Feed samples were collected daily to produce an aggregate

sample for analysis of the chemical composition. Diet crude nutrient contents were analyzed according to the suggestions of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Naumann and Bassler, 1993). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to Goering and Van Soest (1970) and expressed without residual ash. The chemical composition of the diets is presented in Table 1.

Table 1: Chemical composition (g/ kg DM) and energy content (MJ/ kg DM) of experimental diets

Parameter	Diet 1	Diet 2
Organic matter	948	948
Crude protein	97	144
Ether extract	35	33
Crude fibre	227	149
ADF	245	164
NDF	447	333
ME ¹	10.4	11.6
NE _L ¹	6.2	7.1

¹Tabular values were used to estimate silage and concentrate digestibility (DLG, 1997). Metabolizable Energy (ME) and Net Energy Lactation (NE_L) were calculated as following (GfE, 2001): ME [MJ/kg] = 0.0312* DEE [g/kg] + 0.0136* DCF [g/kg] + 0.0147* (DOM - DEE - DCF) [g/kg] + 0.00234* CP [g/kg], where DEE = digestible ether extract; DCF = digestible crude fibre; DOM = digestible organic matter.

2.2. Rumen probes

The experiment was performed using two rumen probes (KB 3/04 bolus, Kahne Limited, New Zealand). The boluses were a successor of the technique described by Kaur et al. (2010) and were constructed to measure rumen pH, temperature and pressure in an adjustable frequency of 10 to 59 seconds or 1 to 255 minutes. The probes were constructed as a copolymer barrel of 145 mm length and 27 mm diameter with wings of altogether 185 mm attached to the tapered top. For pH determination a glass membrane pH sensor was incorporated in the bottom of the boluses. Pre- and post-use storage of the pH sensor was performed as recommended in a 3 molar potassium chloride solution. Kahne Data Processing System V 5.1 software was used for the calibration of the boluses and to download and export data for evaluation. The pH sensors were calibrated according to Kaur et al. (2010) in a water bath of 40 ± 0.1 °C in standard buffer solutions with a pH of 7.0 (first) and 4.0 (second), respectively (ZMK-Analytik-GmbH, Bitterfeld-Wolfen, Germany). A Kahne KR 2001 transceiver was connected to a computer by USB cable to transfer the calibration and setting instructions. Both temperature and pressure sensor were integrated in the probe enclosure. No

manufacturer information about construction and functionality of the temperature sensor were available. Measured data were stored in an integrated memory card for later download with a maximum storage capacity of 11.955 data points per bolus, according to manufacturer. Data could be transferred to a computer in real time simultaneously. Logged bolus data transmission was initialized on demand using a handheld trigger device (Kahne Wand KW1, frequency 134.2 KHz). Data were received by a KR 2002 receiver (frequency 433.9 MHz) including antenna with a range of up to 30 meters, according to manufacturer.

2.3. Sampling

Bolus and manual measurement was performed simultaneously for two consecutive days per cow and diet. Manual rumen pH and temperature as well as rectal temperature were determined 15, 45, 75, 135, 195, 255, 315, 375, 435 and 495 minutes after morning feeding. Two boluses were set to measure every 5 minutes. The probes were fixed to a coated cord tied to the inner cannula and weighed down by a galvanised iron weight of approximately 500 g in such a way to place one bolus in the dorsal and ventral rumen sac, respectively. The dorsal bolus was immersed approximately 10 cm in the rumen content at pre feeding level in the morning. Bolus data were downloaded using the trigger device. To minimize the impact of short term variation bolus rumen pH and bolus rumen temperature were calculated by taking the arithmetic mean of all recorded values within ± 15 minutes from preset manual measurement times. To investigate pH sensor drift boluses were removed at the end of both 8 days periods followed by pH measurement in unused standard buffer solutions at 40 °C as utilized for calibration. This procedure was followed by bolus recalibration. Temperature sensors were not subjected to drift examination as their drift was reported to be negligible in a former study conducted by Kaur et al. (2010). Pressure data were not included in the evaluation due to expected data falsification by the rumen cannula and the necessity of opening it during the experiment.

Manual temperature measurement was performed via digital thermometer (Digitemp Servoprax E315, Servopax GmbH, Wesel, Germany) rectally and in the rumen in direct proximity of the two boluses. For manual pH measurement rumen content was withdrawn from the localizations of manual temperature determination. Obtained samples were squeezed immediately through a close meshed nylon net followed by pH measurement in the gained fluid using a pH meter (WTW pH 530 BCB, LAT Labor- und Analysenbedarf, Garbsen, Germany).

2.4. Statistical analyses

Statistical analyses were performed utilizing the software package SAS version 9.1 (SAS Institute Inc., 2004). Pearson correlation coefficients between rumen pH, rumen temperature and rectal temperature data were calculated using the procedure “CORR”. Linear regression analysis was executed by means of the “REG” procedure to compare bolus and manual pH measurement. The method described by Bland and Altman (1986) was used to assess the agreement between both techniques, differences between bolus and manual pH were plotted against the arithmetic mean for the pairs at each measurement point. The bias as the mean difference (d) including 95% confidence interval (CI) and the standard deviation of the differences (s) were calculated. The upper and lower limits of agreement were defined as $d \pm 1.96 s$ and used to summarize the level of agreement between both methods.

The procedure “MIXED” was applied to analyze rumen pH and temperature data. Diet, localization in the rumen, method and time were considered as fixed effects. Interactions between these variables were investigated. Rumen temperature and rumen pH were included as covariates assessing rumen pH and temperature, respectively. The “random” statement was utilized for the individual cow effect. The restricted maximum likelihood method (REML) was used to evaluate variances. Degrees of freedom were calculated by the Kenward-Roger method. To investigate differences between least square means, the “PDIFF” option was used applying a Tukey-Kramer test for post-hoc analysis. Values used to quantify the effects of the mentioned variables were presented as LS means. Differences were considered to be significant at $p < 0.05$.

3. Results

3.1. General results

The mean daily organic matter intake per cow was 9.6 kg for diet 1 and 17.3 kg for diet 2. One bolus had to be replaced after feeding diet 1 due to technical disturbances which occurred after sampling. The operation of trigger device, receiver and software was easy to handle and appropriate for the download of data under the given experimental conditions, though the trigger had to be used in direct proximity of the animals. The receiver was able to receive data continuously and directly after the record of each data point from a distance of approximately 5 meters, whereat the manufacturer`s data of a range of up to 30 meters was not verified. A

total of 315 paired samples were available each for the comparison of pH and temperature data obtained by bolus and manual measurement, respectively.

The drift of the pH sensors after both 8 days periods resulted in a mean bias of 0.04 ± 0.12 (Mean \pm s.d.) in pH 4 buffer solution and -0.02 ± 0.15 in pH 7 buffer solution. One sensor showed a minimal positive drift in pH 4 buffer solution (Diet 1: 0.02, diet 2: 0.01) but slightly negative drift in pH 7 buffer solution (Diet 1: -0.03, diet 2: -0.08). However the other 2 sensors drift was either positive (Diet 1: pH 4: 0.19, pH 7: 0.20) or negative (Diet 2: pH 4: -0.04, pH 7: -0.16). Due to the partially undirected pH drift bolus pH data were not subjected to drift correction.

Slightly negative overall Pearson correlation coefficients between rumen pH and rumen temperature were calculated for both bolus ($r=-0.11$, $p=0.052$) and manual ($r=-0.25$, $p<0.001$) measurement. A closer inverse relationship was found between rumen pH at both localizations of measurement and dorsal rumen temperature compared with ventral rumen temperature (Table 2). The correlations between pH values and rectal temperature (Bolus: $r=-0.06$, $p=0.323$. Manual: $r=-0.14$, $p=0.002$, respectively) and between rumen and rectal temperature (Bolus: $r=0.15$, $p=0.09$. Manual: $r=0.11$, $p=0.053$, respectively) were not existent or minimal.

Table 2: Correlation coefficients of rumen pH and rumen temperature among methods and localizations of measurement

				Temperature			
				Bolus		Manual	
				Ventral	Dorsal	Ventral	Dorsal
pH	Bolus	Ventral	r	0.07	-0.35	-0.01	-0.26
			p	0.385	<0.001	0.936	0.001
		Dorsal	r	0.06	-0.20	0.01	-0.16
			p	0.463	0.012	0.895	0.040
	Manual	Ventral	r	0.05	-0.43	-0.09	-0.37
			p	0.553	<0.001	0.272	<0.001
		Dorsal	r	0.01	-0.30	-0.11	-0.36
			p	0.935	<0.001	0.165	<0.001

3.2. Comparison of methods

The mean (\pm s.d.) total rumen pH was 6.39 ± 0.38 for bolus and 6.31 ± 0.57 for manual measurement. The number of recorded data points with a pH below 6.0 were in total $n=50$ for bolus and $n=144$ for manual reading. The correlation coefficients between the evaluated methods were $r=0.59$ ($p<0.001$) for rumen pH and $r=0.46$ ($p<0.001$) for rumen temperature. A

closer correlation was found between ventral bolus pH and both ventral and dorsal manual pH ($r=0.80$, $p<0.001$ and $r=0.74$, $p<0.001$, respectively) than between dorsal bolus pH and ventral and dorsal manual pH ($r=0.51$, $p<0.001$ and $r=0.45$, $p<0.001$, respectively). The intra method correlation between pH determination at the ventral and dorsal rumen sac, respectively, was $r=0.59$ ($p<0.001$) for bolus and $r=0.85$ ($p<0.001$) for manual sampling.

Performing linear regression analysis the relationship between bolus and manual pH measurement was characterized by a considerable variation around the regression line with a relatively low coefficient of determination of $r^2=0.3436$ (Fig. 1). The Bland-Altman comparison indicated a bias effect of bolus pH measurement with the tendency of pH overestimation in the range of low rumen pH below 6.0 and a pH underestimation at rumen pH above 6.5 (Fig. 2). The estimated limits of agreement between bolus and manual pH measurement were -0.83 to 0.97.

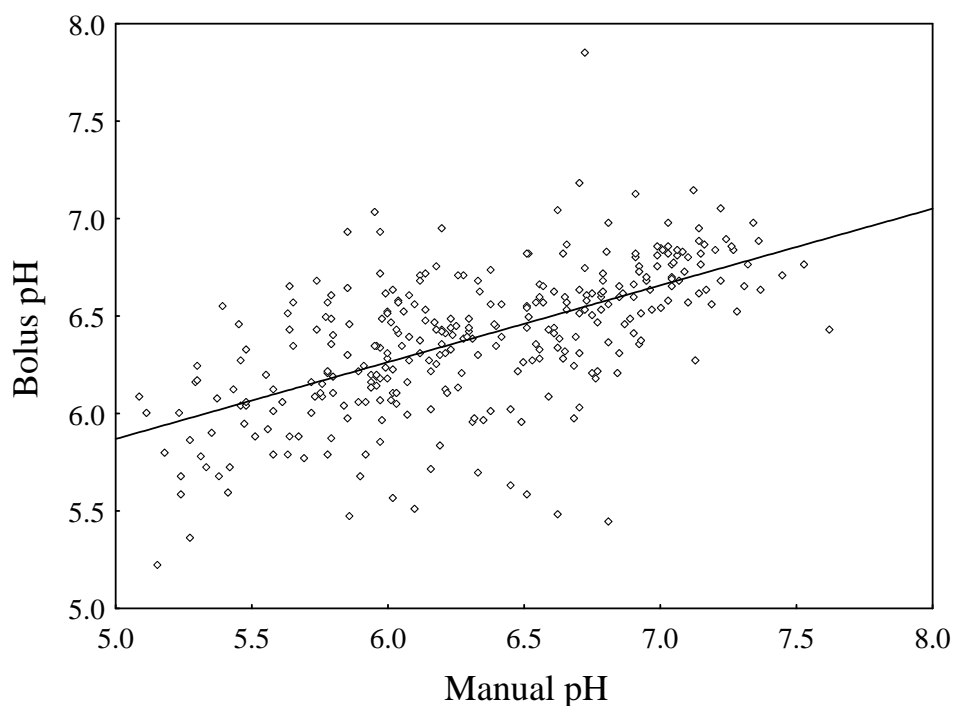


Fig. 1: Relationship between manual and bolus pH measurement. $y = 0.3938x + 3.9011$, $r^2 = 0.3436$, $N = 315$.

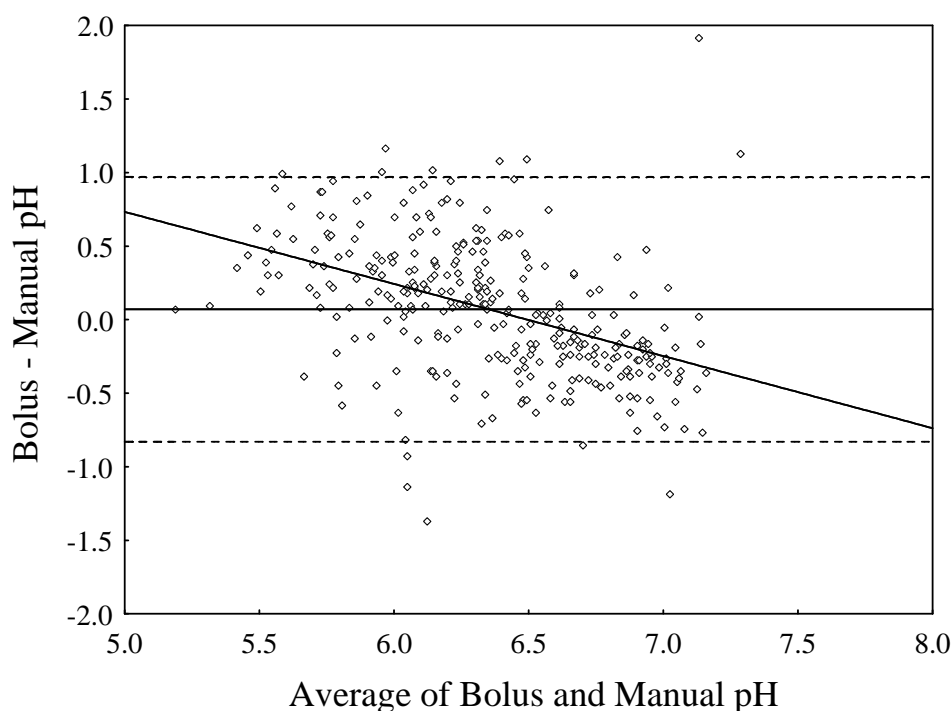


Fig. 2: Differences of bolus and manual rumen pH measurement versus their mean. N = 315. Bias: 0.07, 95 % confidence interval: 0.03 to 0.11. Dotted lines show limits of agreement. A best fit line has been added to show change in bias with pH.

3.3. Effects of diet, localization and time

A significant diet effect on rumen pH was observed (Diet 1: 6.61. Diet 2: 6.16. $p < 0.001$, Fig. 3). The localization of measurement in the rumen did not influence pH values (Dorsal: 6.39. Ventral: 6.37. $p > 0.05$), whereas the method of pH determination had a significant effect ($p = 0.004$). Rumen pH was affected by time after feeding ($p < 0.001$, Fig. 3). It decreased postprandial and recovered approximately to the initial value within the sampling period of 495 minutes. Though the interaction of diet and localization was significant ($p = 0.014$), the nominal effects were marginal. Dorsal and ventral rumen pH were nearly equal feeding diet 1 (Dorsal: 6.58, ventral: 6.63) and differed only slightly feeding diet 2 (Dorsal: 6.20, ventral: 6.12). No interactions were found between diet and time ($p > 0.05$) and localization and time ($p > 0.05$), respectively.

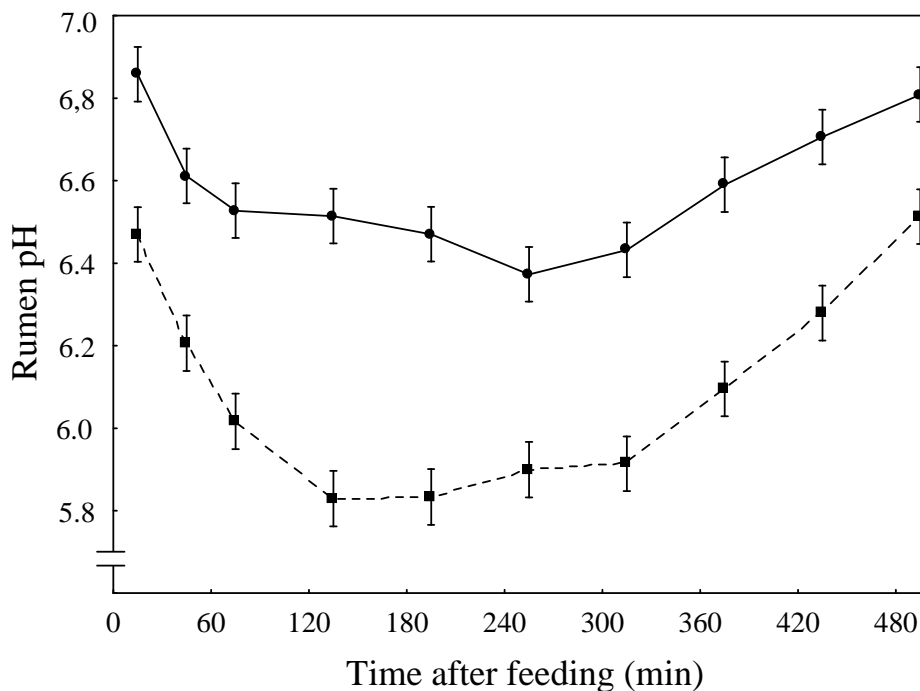


Fig. 3: Rumen pH depending on time after feeding (min) and diet (diet 1: ●, diet 2: ■). Ventral and dorsal pH values were pooled and presented as means \pm standard error.

Rumen temperature was influenced by diet and increased due to feeding low fibre but high energy (Diet 1: 38.9 °C, Diet 2: 39.3 °C. $p < 0.001$). The localization of measurement had a significant effect on rumen temperature ($p < 0.001$). Ventral and dorsal temperature were almost equal feeding diet 1 (Ventral: 39.0 °C, dorsal: 38.9 °C), however a temperature gradient was observed for diet 2 (Ventral 39.0 °C, dorsal: 39.7 °C. Localization x diet: $p < 0.001$). The applied methods did not affect temperature values ($p = 0.115$). A time effect on rumen temperature ($p < 0.001$) was investigated. The significant interaction between diet and time was characterized by a time dependent decline of rumen temperature feeding diet 1 with the nadir after 75 minutes and a full recovery within the sampling period ($p < 0.001$, Fig. 4). Localization and time did not interact significantly ($p > 0.05$).

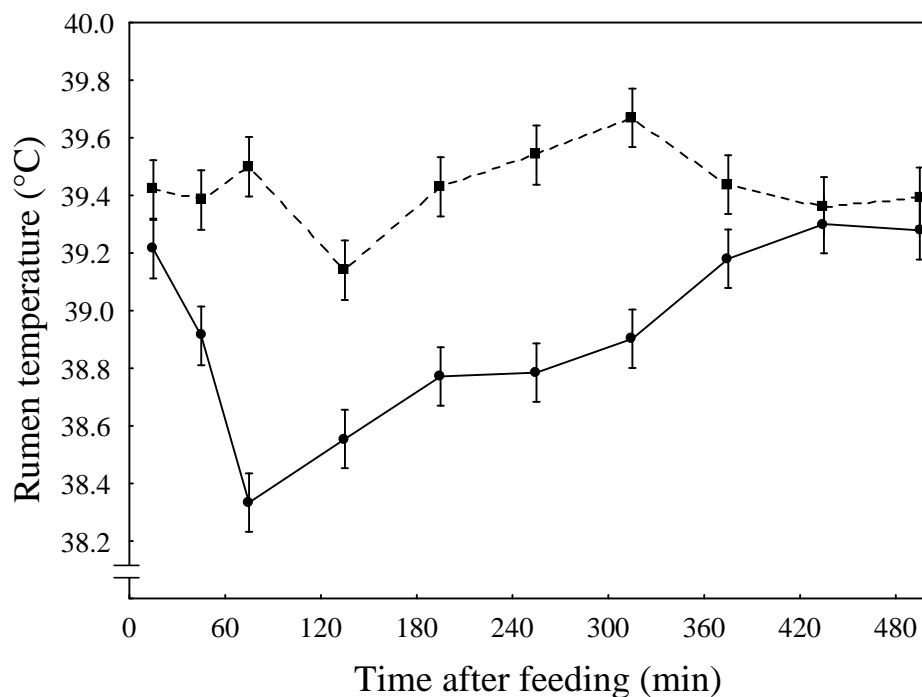


Fig. 4: Rumen temperature depending on time after feeding (min) and diet (diet 1: ●, diet 2: ■). Ventral and dorsal pH values were pooled and presented as means \pm standard error.

4. Discussion

A close relationship between rumen pH and temperature as indicated by AlZahal et al. (2009) would be expected to be inverse and to arise especially during intensive postprandial fermentation reflecting both decreasing rumen pH but increasing rumen temperature. In the present study, the correlations between rumen pH and temperature were considerably lower than those reported in earlier experiments. An inverse relationship of $r=-0.39$ between rumen pH obtained by manual determination in fluid withdrawn from the ventral rumen sac of sheep and bolus temperature measured in vivo was found by Kaur et al. (2010). AlZahal et al. (2008) observed a correlation of $r=-0.46$ between ventral rumen pH and temperature utilizing an indwelling electrode measuring both parameters simultaneously in lactating dairy cows, whereas the direct proximity of both sensors may have contributed to the close relationship. The authors developed an equation for the prediction of rumen pH from rumen temperature and thus discussed the potential of rumen temperature for the detection of SARA in cattle. In the present experiment, rumen pH measured via both methods was distinctly closer correlated to dorsal than to ventral temperature. Thus, under the present conditions, the potential contribution of rumen temperature measurement to the prediction of rumen pH and the use as an indication of SARA may depend on the site of intraruminal temperature determination.

A close correlation ($r=0.65$) between reticulum temperature measured by an indwelling probe and rectal temperature of intact dairy cows was observed by Bewley et al. (2008) and based on a large amount of paired samples which were taken during several seasons. Burns et al. (2002) used the same technique in cows near the occurrence of oestrus and reported a relationship of $r=0.50$ between reticular and rectal temperature. The restricted postprandial sampling times may have contributed to the low relationship of rumen and rectal temperature in the present trial. Though after feeding a time effect was observed for rumen temperature, rectal temperature may have been less affected causing a low correlation. Uncertainty exists whether an outflow of heat through the perforating rumen cannula has contributed to the low relationship of the two parameters. However a close correlation between rumen and rectal temperature ($r=0.92$) was reported for rumen-fistulated lactating cows in a former study using a prototype rumen bolus (Sievers et al., 2004).

Observations concerning sensor drift of indwelling devices for rumen pH measurement were diverse. Penner et al. (2006) reported partially undirected but not significant pH drift after 72 hours using an encapsulated electrode for pH measurement in the ventral rumen sac of dairy cows. In a former study, Enemark et al. (2003) utilized devices which were initially developed for marine animals and observed a slightly positive electrode drift after 10 days of continuous application in the reticulum of cows. In a study of Kaur et al. (2010) a predecessor generation of probes for intraruminal pH, temperature and pressure measurement produced by the same manufacturer was evaluated utilizing fistulated sheep in 10 day periods. Bolus pH sensor drift was visually apparent after 48 h and increased steadily from that time. The technical comparability of both probe series may be limited due to constructional differences, as the former boluses were equipped with ISFET sensors for pH measurement, which may exhibit long-term drift and low performance in comparison to glass electrodes (Oelssner et al., 2005). The inconsistent and partially undirected pH sensor drift in the present study and the wide range reported for sensors in earlier experiments suggested the need to assess occurrence and direction of pH sensor drift individually and depending on application time. Therefore further investigations of direction, amount and time-dependence of pH sensor drift seemed to be recommended prior to longer-term application of the evaluated boluses in intact animals.

Differing correlations between pH measurement via intraruminal probes and manual determination were reported in earlier studies. Duffield et al. (2004) observed varying but mainly weak relationships between pH measurement via a device placed in the ventral sac of the rumen of cows and manual pH determination in the second 200 ml of rumen fluid gained by a tube-like probe through a rumen cannula from different intraruminal sites (Cranial-

ventral: $r=0.25$, Caudal-ventral: $r=0.24$, Central: $r=0.58$, Cranial-dorsal: $r=0.53$). A rather low correlation ($r=0.46$) between pH readings gained by the former probe generation produced by the same manufacturer as the boluses used in the present study and manual measurement was investigated by Kaur et al. (2010). The closer correlation between bolus and manual pH data in the present study may be due to the better performance of the used glass membrane pH sensors and the potentially wider distance of probe and manual sampling in the former experiment. Other workers have proved closer correlations of $r=0.85$ (Dado and Allen, 1993), $r=0.88$ (AlZahal et al., 2007), $r=0.88$ as well as $r=0.98$ (Penner et al., 2006) and $r=0.98$ (Phillips et al., 2010) between various indwelling probes and in vitro pH measurement in withdrawn rumen fluid samples.

The minor correlations between dorsal bolus pH and manual pH readings and the low intra method relationship between bolus pH measurement at the dorsal and ventral rumen sac, respectively, may be due to methodological reasons. The probe used to measure dorsal rumen pH was fixed to a cord to be immersed in the rumen content for approximately 10 cm at pre morning feeding level and may have protruded into the rumen gas phase for several short times during the sampling periods and thus may have produced a distortion of dorsal bolus pH data.

Though in the present trial the different standard deviations of pH values determined by both methods of rumen pH measurement indicated an unequal variation, the mean bolus pH was only slightly higher than the mean manual pH. That is consistent with results of Kaur et al. (2010), who found the probe pH to be 0.05 to 0.21 pH units higher than the manual pH measured by a pH meter, depending on diet. Contradictory findings were observed in earlier studies, where manual pH values were reported to be higher in rumen fluid removed through a cannula than the pH measured in vivo in the rumen (Dado and Allen, 1993) or the reticulum (Enemark et al., 2003) of dairy cows. The distance between the probe location and the site of rumen fluid sampling was discussed as a possible explanation for the differences in pH (Enemark et al., 2003).

The significance of the Pearson correlation coefficient as a tool of method comparison is limited as it is a measure of the linearity between two variables, not of the agreement between them. No indication of how the plotted data deviate from the line $y = x$ can be derived (Lin, 1992) and data which seem to be in poor agreement can produce high correlations (Bland and Altman, 1986). Furthermore it may be difficult to assess differences between methods by a simple plot of the results of one method against those of the other. The method described by Bland and Altman involved plotting the difference (y-axis) of the paired measurements

against their arithmetic mean (x-axis). The true value is unknown and the mean is the best available estimate (Bland and Altman, 1986). In the present study a deficient accuracy of manual pH determination cannot be excluded and may be caused by inherent bias like temperature effects (Meinrath and Spitzer, 2000). Such potentially restricted accuracy of manual pH measurement may have been responsible for the limited agreement between both methods. The positive bias of 0.07 in the Bland-Altman analysis would suggest the probes typically gave slightly higher results than the standard manual method. However the trend line indicated a lack of bias consistency with a pH overestimation in the range of rather low rumen pH below 6.0 and a pH underestimation at higher rumen pH above 6.5. This is congruent with the lower number of total data points of rumen pH below 6.0 detected by bolus measurement (n=50) in comparison with the manual technique (n=144) and may be misleading especially in interpreting rumen pH values to obtain indications of SARA. The calculated limits of agreement were - 0.83 to 0.97 indicating rather low agreement within the given experimental design. This range is expected to include 95 % of the values and its magnitude can be utilized to assess the utility of an alternative method (MacFarlane et al., 2010). Bland and Altman (1986) suggested that the acceptable range of the limits of agreement should be based on the clinical impact of the results within this range. Though it would be difficult to define suitable limits of agreement for rumen pH measurement, the determination of rumen pH requires a high accuracy to ensure reliable results for both research and diagnostic purposes.

The evaluation of the accuracy of continuous measurement via indwelling rumen probes using aggregated bolus data may be adequate for a comparison with spot sampling techniques same as the described manual method. Actually such method comparison did not consider the option of continuous measurement being the primarily advantage of the investigated boluses. According to Gozho et al. (2005), SARA in cattle should be defined as a depression of rumen pH below 5.6 for 3 or more hours per day. In intact animals, such intervals are hardly detectable with spot sampling techniques.

Rumen pH decreased significantly due to feeding concentrate as previously reported for steers (Jaakkola and Huhtanen, 1993; Owens et al., 2008) and cows fed high amounts of grain (Mishra et al., 1970). Besides the effects of dietary concentrate, the feeding of diet 2 was characterized by a higher OM intake level which may have contributed to the calculated diet effect on rumen pH. The localization of measurement in the rumen did not influence pH readings significantly ($p>0.05$). Though a considerable pH gradient would be expected to emerge especially between reticulum and rumen, earlier experiments partially proved effects of the site of measurement in the rumen on mean pH values (Duffield et al., 2004; Li et al.,

2009). Despite the absence of such a pH gradient between dorsal and ventral rumen sac in the present experiment, its possible emergence should be taken into consideration for potential on-farm and research application of the evaluated boluses or similar unfixed devices for intraruminal usage, as the probes may move through the reticulorumen if used in intact animals without certainty of the exact site of measurement.

In the current study rumen temperature was affected by diet, however a rise in mean rumen temperature associated with the feeding of concentrate was primarily observed at the dorsal rumen. Similar to the evaluation of diet effects on rumen pH, the higher intake level of diet 2 may have increased the described effect on rumen temperature. AlZahal et al. (2009) reported a significant increase of rumen temperature due to feeding high amounts of grain in comparison to a mixed hay diet. Such effects may depend on the amount of concentrate fed as in a study of Gasteiner et al. (2009) differences in rumen temperature of steers were not significant between a 100 % hay and a 50 % concentrate diet. The postprandial development of a temperature gradient in the rumen of dairy cows with an increased dorsal temperature due to feeding high-concentrate diets was coherent as, first, concentrate would be expected to be subjected to a faster ruminal degradation than forage and, secondly, the materials in the top stratum of the rumen digesta are recently ingested and are subjected to a higher fermentative activity than those contained in the middle and bottom strata of the rumen (Martin et al., 1999; Tafaj et al., 2004).

5. Conclusion

The evaluated devices showed a moderate linear relationship and agreement of pH measurement with the applied manual method, a fact that may as well be due to a deficient accuracy of manual pH determination. Varying and partially undirected but low pH sensor drift was observed. Indications for an inconsistent bias of bolus pH determination were found. Effects of the site of measurement in the rumen were not observed for pH but for temperature, may interact with the diet fed and should be taken into consideration for a potential use of indwelling devices. Though the described indications of inaccuracy of bolus pH measurement suggested the need of further improvement, the technique was promising especially due to the option of continuous intraruminal measurement.

Acknowledgements

The authors would like to thank the Niedersächsisches Ministerium für Wissenschaft und Kultur for financial support. Furthermore, the assistance of the co-workers of the Institute of Animal Nutrition and the Experimental Station of the Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany in performing the experiment and analysis as well as the Clinic for Cattle of the University of Veterinary Medicine Hannover and Mr. Pat Fernley from Kahne Limited, New Zealand, is gratefully acknowledged.

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Paper IV

Effects of niacin supplementation and dietary concentrate proportion on body temperature, ruminal pH and milk performance of primiparous dairy cows

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Archives of Animal Nutrition

Submitted

Abstract

The objective of this study was to investigate the effects of niacin and dietary concentrate proportion on body temperature, ruminal pH and milk production of dairy cows. In a 2×2 factorial design, 20 primiparous Holstein cows (179 ± 12 days in milk) were assigned to four dietary treatments aimed to receive either 0 or 24 g niacin and 30% (Low) or 60% (High) concentrate with the rest being a partial mixed ration composed of 60% corn and 40% grass silage (On a dry matter basis). Ambient temperature and relative humidity were determined and combined by the calculation of temperature humidity index (THI). Respiration rates, rectal, skin and subcutaneous temperatures were measured. Milk production and composition were determined. Ruminal pH and temperature were recorded in a frequency of 5 minutes using wireless devices for continuous intraruminal measurement (Boluses). PH values were corrected for pH sensor drift. The climatic conditions varied considerably but temporarily indicated mild heat stress. Niacin did not affect skin, rectal and subcutaneous temperatures but tended to increase respiration rates. High concentrate reduced skin temperatures at rump, thigh and neck by 0.1 to 0.3 °C. Due to technical disturbances, not all bolus data could be subjected to statistical evaluation. However, both niacin and high concentrate influenced mean ruminal pH. High concentrate increased the time spent with a pH below 5.6 and ruminal temperatures (0.2 to 0.3 °C). Niacin and high concentrate enhanced milk, protein and lactose yield but reduced milk fat and protein content. Milk fat yield was slightly reduced by high concentrate but increased due to niacin supplementation. In conclusion, niacin did not affect body temperature but stimulated milk performance. High concentrate partially influenced body temperatures and had beneficial effects on milk production.

Keywords: niacin, concentrate, body temperature, milk production

1. Introduction

The global surface temperature has increased within the last century (0.74 ± 0.18 °C) and will likely be elevated in the range of 1.8–4 °C until 2090-2099, depending on the prospective greenhouse emission scenario (IPCC 2007). Rising ambient temperatures will probably increase the incidence of heat stress in livestock which is initiated if a combination of environmental conditions cause the effective temperature of the environment to be higher than the thermo-neutral zone of an animal (Bianca 1962). Heat stress is well known to decrease dry matter intake and milk performance of high-yielding dairy cows, which are particularly susceptible to body heat load (Kadzere et al. 2002; West 2003; Bohmanova et al. 2007).

Elevated dissipation of excess body heat by rising peripheral vasomotor activity could ameliorate the adverse effects of heat stress and thus contribute to the maintenance of health and productivity.

In humans, the B-vitamin niacin or nicotinic acid can induce flushing (Andersson et al. 1977), which is primarily characterized by cutaneous vasodilation. This process results to a considerable extent from the niacin mediated activation of the G protein-coupled receptor 109A (GPR109A) in epidermal Langerhans cells (Benyo et al. 2005; Benyo et al. 2006), leading to the production of prostaglandins, which act on receptors in the capillaries (Kamanna et al. 2009). Previous studies revealed varying vasomotor and skin temperature responses of heat stressed dairy cows to dietary niacin supplementation. DiCostanzo et al. (1997) reported reductions in skin temperatures of lactating Holstein cows consuming 12 and 24 g niacin per day. However, increased consumption was not necessarily related to decreased skin temperatures because a daily supplementation of 36 g did not have significant effects. Furthermore, feeding 12 g encapsulated niacin enhanced evaporative heat loss of dairy cows during periods of peak heat and was associated with reductions of vaginal and rectal temperatures in the range of 0.2 to 0.4 °C during mild heat though skin temperatures were not affected (Zimelman et al. 2010). However, both studies used multiparous animals and it is unclear, whether congruent effects are to be expected for primiparous cows which typically have lower dry matter intakes (DMI) and milk yields (Ray et al. 1992).

Besides such thermoregulatory effects niacin can alter the microbial populations in the rumen and these changes may affect the patterns of ruminal fermentation (Niehoff et al. 2009a). For example, additive niacin was reported to increase the total numbers of rumen protozoa, especially *Entodinia* (Kumar and Dass 2005). Moreover, the concentration of short chain fatty acids (SCFA) may be altered due to supplemental niacin (Kumar and Dass 2005). In ruminants, the microbial activity during fermentation in the reticulorumen can account for 3-8% of the total heat production (Czerkawski 1980) and altered amounts of SCFA may influence body heat increment (West 1999).

Moreover, high dietary proportions of concentrate may reduce the negative effects of warm environmental conditions on the productivity of dairy cows as low fiber diets were observed to be associated with lower heat production (Reynolds et al. 1991) and the large amounts of easily fermentable carbohydrates fed in typical high concentrate diets should minimize the production of body heat (West 1999).

Therefore the objective of the present study was to investigate the effects of niacin supplementation and concentrate proportion in consideration of the thermal environment on body temperatures, ruminal pH and milk production of primiparous dairy cows.

2. Materials and Methods

2.1 Experimental design, animals and feeding

The study was performed in 2010 at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), in Brunswick, Germany, was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany (File Number 33.14-42502-04-085/09) and was conducted according to the European Community regulations concerning the protection of experimental animals. The experiment was part of a more comprehensive feeding trial executed to investigate a higher number of animals, was started one day ante partum and continued during the complete lactation (Unpublished data). The presented examinations were started in mid-april and lasted until drying off at the beginning of august to test the effects of the assessed dietary treatments under heat stress conditions.

In a 2×2 factorial design, 20 lactating primiparous German Holstein cows producing 16 ± 3.7 kg milk per day balanced for bodyweight (597 ± 47 kg) and stage of lactation (179 ± 12 days in milk (DIM)) were assigned to four dietary treatments. On a dry matter (DM) basis, the four diets offered were aimed to be composed of 30% concentrate and 70% partial mixed ration (PMR, 60% corn and 40% grass silage) (Group 1), 30% concentrate and 70% PMR supplemented with 24 g niacin/d and animal (Group 2), 60% concentrate and 40% PMR (Group 3) and 60% concentrate and 40% PMR supplemented with 24 g niacin/d and animal (Group 4). The niacin was powdered, contained at least 99.5 % nicotinic acid (Lonza Ltd, Basel, Switzerland) and was included in the pelletized concentrate. Except for niacin, all rations were formulated to meet the requirements of the German Society of Nutrition Physiology (2001). Water was offered for *ad libitum* intake. The cows were kept in group pens in a free stall barn equipped with slatted floors and cubicles covered with rubber mattresses. The PMR was offered in self-feeding stations (TYPE RIC, Insentec, B.V., Marknesse, The Netherlands), which were re-filled daily at approximately 10.00 h. All animals were marked with ear transponders to record daily water and feed intake (DMI). Concentrate was provided via computerized concentrate feeding stations (Insentec, B.V., Marknesse, The Netherlands). Though feed was aimed to be provided for *ad libitum* intake, the available amount of concentrate and PMR was adjusted individually twice weekly basing

on the current respective DMI of both components in order to realize the intended dietary forage to concentrate ratios in all feeding groups.

Table 1. Components and chemical composition of concentrates and partial mixed ration (Means \pm SD).

Variable	Concentrate				PMR [#]
	Group 1	Group 2	Group 3	Group 4	
Component (%)					
Wheat grain	50	50	50	50	
Soybean meal	26	26	26.8	26.8	
Corn grain	20	20	20.8	20.8	
Mineral premix*	4		2.4		
Mineral premix including supplemental niacin		4		2.4	
Niacin in concentrate (g/kg DM)		3.52		1.76	
Nutrients (g/kg DM)					
Crude ash	63	62	51	49	58
Crude protein	217	219	219	224	106
Ether extract	30	29	30	30	34
Crude fiber	33	34	34	34	220
NDF	128	130	185	156	440
ADF	47	47	47	47	241
Energy ⁺ (MJ/kg)					
NE _L	8.2	8.2	8.3	8.3	6.4

Notes: [#]: Partial mixed ration (60 % maize silage, 40 % grass silage on DM basis); ^{*}: Per kg mineral feed: 140 g Ca; 120 g Na; 70 g P; 40 g Mg; 6 g Zn; 5.4 g Mn; 1 g Cu; 100 mg I; 40 mg Se; 5 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D₃; 1500 mg vitamin E; ⁺: Calculation based on nutrient digestibilities measured with wethers for silages (GfE 1991) and tabulated values for concentrate (DLG 1997); NE_L = Net energy lactation.

2.2 Sample collection

Representative concentrate samples were taken once, corn and grass silage samples twice a week and were pooled over four weeks. Milking was performed twice daily at 5.30h and 14.30h. Individual milk yield was recorded by automatic milk counters and bodyweight was recorded automatically when the animals left the milking parlor. Milk samples were taken twice weekly and were stored at 4 °C until analysis of composition.

2.3 Chemical analyses

Dry matter (DM), crude ash (Ash), crude fiber (CF), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analysed according to the protocols of the Association of German Agricultural Analysis and Research Centres (Naumann and Bassler 1993), whereat ADF and NDF were expressed without residual ash.

Milk samples were analyzed for fat, protein and lactose using an infrared milk analyzer (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark).

2.4 Temperature measurements

The determination of respiration rate (RR) and body temperature was conducted at four preset days per week. Measurements were performed at 8.00 h or 16.00 h at two days each to consider diurnal variation. After morning or afternoon milking, respectively, all cows were fixed in a feed fence in the free stall barn. After completed fixation the measurements were initiated after a delay of 15 min to avoid data falsification mediated by potentially different previous animal activity. Respiration rates were determined by twofold visual counting of flank movements for 15 and 30 seconds and calculated as the mean of both values per minute. Rectal temperature (Rectal T) was recorded via digital thermometry (Digitemp Servopax E315, Servopax GmbH, Wesel, Germany). Skin temperature (Skin T) measurement was conducted using an infrared thermometer (Fluke 561, Fluke Corporation, Everett, WA, USA) on shaved spots at both dextral and sinistral rump, dextral and sinistral thigh and sinistral neck. Subcutaneous temperature (Subcutaneous T) was obtained by an implanted microchip of 1.2 × 0.1 cm which was constructed as a passive radio frequency identification unit (LifeChip, Destron Fearing, South Saint Paul, USA). The microchip was preloaded into a sterile syringe by the manufacturer and was aseptically inserted subdermally in the sinistral neck of each animal. Temperature measurement and subsequent information communication were initiated using the energy of the radio signal transmitted by a Reader (Pocket Reader EX, Destron Fearing, South Saint Paul, USA) using a frequency of 143.2 kHz, according to manufacturer. The reader had to be applied in direct proximity of the microchip and displayed both unique chip number and temperature.

Barn dry bulb temperature (Td) and relative humidity (RH) were recorded in a frequency of 10 minutes using two data loggers (Tinytag Plus 2, TGP-4500, Gemini Data Loggers, Chichester, United Kingdom). One data logger each was placed in the free stall barn and at the feed fence used for animal fixation, respectively. Td and RH were combined by the calculation of the temperature-humidity-index (THI) according to Hahn (1999).

2.5 Rumen probes

Ruminal pH and temperature were determined using probes designed for continuous intraruminal measurement (KB 3/04 bolus, Kahne Limited, New Zealand). The boluses were constructed to determine ruminal pH and temperature in an adjustable frequency and were built as a copolymer barrel of 145 mm length and 27 mm diameter with wings of altogether 185 mm attached to the tapered top. A glass membrane pH sensor was incorporated in the bottom of the probes. Pre-use storage of the pH sensor was performed as recommended in a 3

molar potassium chloride solution. The temperature sensor was integrated in the probe enclosure. Associated software was applied for the calibration of the boluses and to download and export data (Kahne Data Processing System V 5.1). The pH sensors were calibrated as described by Kaur et al. (2010) in a water bath of 40 ± 0.1 °C in standard buffer solutions with a pH of 7.0 (first) and 4.0 (second), respectively (ZMK-Analytik-GmbH, Bitterfeld-Wolfen, Germany). A transceiver (Kahne KR 2001, Kahne Limited, New Zealand) was connected to a computer by USB cable to transfer the calibration and setting instructions. Measured data were stored in an integrated memory card. The probes were set to measure every five minutes, inserted through the oesophagus using a balling gun and persisted in the reticulorumen of all investigated cows throughout the experiment. In group 3, one cow was not equipped with a rumen probe. Logged bolus data transmission was initialized on demand using a handheld trigger device operating with a frequency of 134.2 kHz (Kahne Wand KW1, Kahne Limited, New Zealand). Data were captured by a receiver with a frequency of 433.9 MHz (KR 2002, Kahne Limited New Zealand) including antenna with a range of up to 30 meters, according to manufacturer.

2.6 PH drift correction

An additional experiment was conducted for 70 days to test the occurrence of time dependent pH sensor drift. Four lactating German Holstein cows equipped with large rubber cannulas in the dorsal rumen sac were kept in a tethered barn with individual troughs and had free access to water. Therefore the animals were fed twice daily on a ration consisting of 30% concentrate, 42% corn silage and 28% grass silage, referring to DM. The concentrate consisted of 27% wheat grain, 20% rapeseed meal, 10% oat grain, 10% barley grain, 5% soybean meal, 5% dried sugar beet pulp, 2% mineral and vitamin premix, 0.4% calcium carbonate and 0.1% sodium chloride. The contents of minerals and vitamins per kg premix were as follows: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6 g Zn, 5.4 g Ma, 1 g Cu, 100 mg I, 40 mg Se, 24 mg Co, 1.000.000 International Units (IU) vitamin A, 100.000 IU vitamin D₃, 1.500 mg vitamin E, 90 mg pantothenic acid.

Four boluses were calibrated as described in the previous section and were set to measure every 15 minutes. The probes were fixed to a cord of approximately 50 cm which was tied to the inner cannula. One probe was inserted in the rumen of each cow. In the first week, the boluses were removed daily in a frequency of 24 hours. From week 2 on the removal was conducted every 7 days. After each removal, the boluses were cleaned thoroughly with distilled water. Subsequently, pH measurement was conducted at 40 ± 0.1 °C in four standard

buffer solutions (ZMK-Analytik-GmbH, Bitterfeld-Wolfen, Germany) with a pH of 4.0, 5.0, 6.0 and 7.0, respectively. This procedure was performed twice consecutively. The data over this 70 days period were plotted to generate a simple linear regression equation. All ruminal pH values determined in the main experiment were then corrected by the calculated linear rate of increase in pH (0.0042) at the respective day of measurement.

2.7 Calculations

Temperature humidity indices (THI) were calculated according to Hahn (1999):

$$\text{THI} = 0.8 \cdot \text{td} + \text{RH} \cdot (\text{td} - 14.4) + 46.4$$

where td represents the dry bulb temperature (° C) and RH is the relative humidity expressed as a decimal.

Minutes per day of ruminal pH below 5.6, 5.8 and 6.0, respectively, were determined by multiplying the individual daily percentage of values below the respective threshold with the total number of minutes per day to avoid data falsification by potentially missing values.

Fat-corrected milk (FCM) was estimated as following:

$$\text{FCM [kg/d]} = ((\text{milk fat [\%]} \times 0.15) + 0.4) \cdot \text{milk yield [kg/d]} \text{ (Gaines 1928)}$$

Ruminal pH data were corrected by the calculated linear rate of increase using the following equation:

$$\text{Ruminal pH} = \text{Measured ruminal pH} - (0.0042 \cdot \text{Respective number of days after bolus insertion})$$

2.8 Statistics

Statistical analyses were performed by the PROC MIXED procedure of the software package SAS version 9.1 (SAS Institute Inc., 2004). Bolus data were only subjected to statistical evaluation if pH or temperature values were available for complete days to consider potential diurnal variation. Three different models were used for the evaluation of ruminal pH and temperature data (Model 1), respiration rate, rectal, skin and subcutaneous temperatures (Model 2) and milk yield, protein, fat, lactose and dry matter intake (Model 3). Dietary concentrate proportion and niacin supplementation were considered as fixed effects in all models and the interactions between these variables were investigated. In model 1, THI on a daily basis and DMI were included as covariates. Time of day was included as an additional

covariate for the evaluation of mean ruminal pH and temperature. For model 2, THI were divided in nine classes, with the first class being $\text{THI} < 68$. Subsequent classes were defined as one class for each 2 THI points thereafter until the last class which was $\text{THI} > 81$. Measurements conducted at THI class one were not included to test the effects of the fixed effects exclusively under heat stress conditions. In model 2, THI class, time of day, DMI and bodyweight were considered as covariates. In model 3, THI, DIM, DMI and bodyweight were considered as covariates but were not included if the respective parameter was evaluated as dependent variable. The individual cow effects resulting from the frequent measurements in the course of the experiment were considered by the repeated procedure in all models. The restricted maximum likelihood method (REML) was used to evaluate variances. Degrees of freedom were calculated by the Kenward-Roger method. Pearson correlation coefficients were calculated using the PROC CORR procedure. To investigate differences between least square means, the “PDIF” option was used applying a Tukey-Kramer test for post-hoc analysis. Values used to quantify the effects of the mentioned variables were presented as LS means. Differences were considered to be significant at $p < 0.05$.

3. Results

3.1 General results

The climatic conditions at the days of body temperature measurement are presented in Fig. 1 and were characterized by a considerable variation with a mean THI of 66 ± 7.8 (Variation: 50-84) as calculated from ambient temperature (20 ± 5.7 °C, Variation: 9-35 °C) and RH ($74 \pm 17.2\%$, Variation: 37-100%).

The realized intake of concentrate and PMR met the aimed proportions in the different feeding groups to a large extent (Group 1: $30 \pm 4\%$ Concentrate, $70 \pm 5\%$ PMR; Group 2: $30 \pm 5\%$ Concentrate, $70 \pm 5\%$ PMR; Group 3: $57 \pm 7\%$ Concentrate, $43 \pm 7\%$ PMR; Group 4: $56 \pm 8\%$ Concentrate, $44 \pm 8\%$ PMR; referring to DM). In contrast, the calculated daily intake of supplemental niacin was below the intended quantity of 24 g per animal in the respective groups (Group 2: 20.5 ± 3.0 g; Group 4: 17.9 ± 4.8 g).

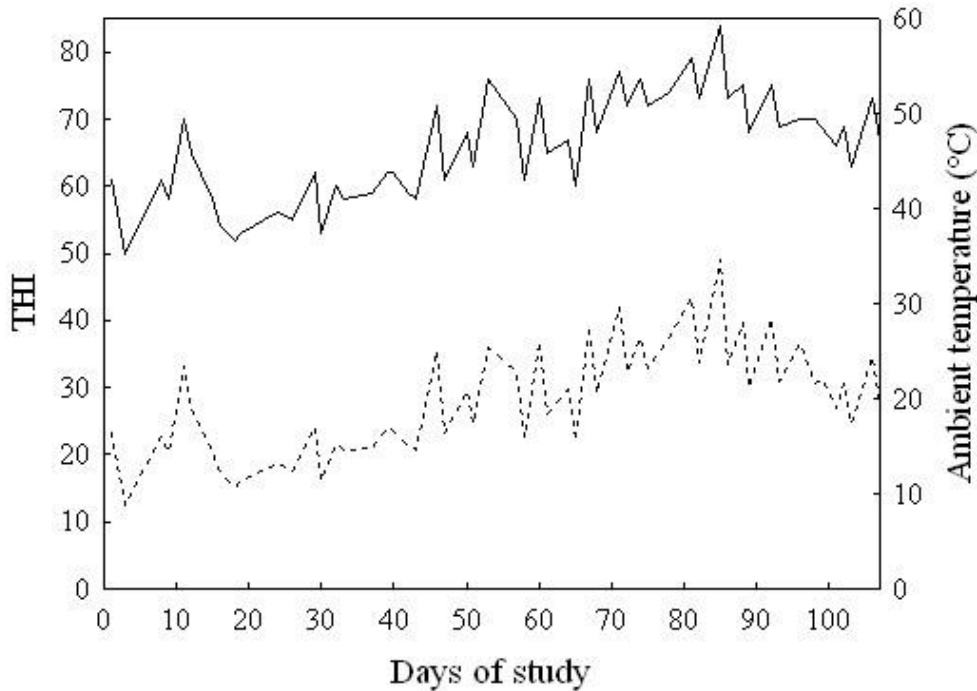


Figure 1. Temperature humidity index (THI, Solid line) and ambient temperature (Dotted line) at the days of body temperature measurement.

3.2 Ruminant pH and temperature

Though a continuous measurement of ruminal pH and temperature was intended throughout the complete experiment, technical disturbances caused losses of bolus data. However, a total of 57,884 ruminal pH (Measured at 24 days) and 16,010 ruminal temperature data (Measured at 17 days) could be subjected to statistical evaluation. The results obtained in the additional experiment conducted to investigate the occurrence of pH sensor drift indicated a time dependent positive drift. All pH values determined in the main experiment were corrected for the drift at the respective day of measurement after the initialization of pH recording.

Rumen pH and temperature data were observed to have a weak but significant negative relationship ($r=-0.13$, $p>0.001$). Ruminal pH was affected by niacin supplementation, dietary concentrate proportion and the interaction of both variables ($p<0.001$, Table 2). The total time of ruminal pH spent below 6.0 and 5.8, respectively, was not affected by dietary treatments ($p>0.05$). However, the interactions of niacin supplementation and concentrate proportion were significant for both parameters evaluated as a percentage of time below the respective threshold. In total, ruminal pH was found to be below 6.0 for approximately 700 minutes per day and below 5.8 for up to 500 minutes per day.

Table 2. Effects of dietary concentrate proportion (%) and niacin supplementation on rumen pH and rumen temperature of dairy cows (LSMeans±SE)

Variable	Treatment						P-value
	30 % Concentrate			60 % Concentrate			
	Control (Group 1, n=5)	Niacin (Group 2, n=5)	Control (Group 3, n=4)	Niacin (Group 4, n=5)	Concentrate	Niacin Concentrate× Niacin	
Ruminal pH	6.00±0.003	5.91±0.004	5.89±0.004	5.92±0.004	<0.001	<0.001	<0.001
pH<6.0 (Min/d)	632±29	709±34	715±31	699±30	0.267	0.317	0.130
pH<5.8 (Min/d)	410±27	493±32	500±29	495±28	0.138	0.168	0.121
pH<5.6 (Min/d)	234±23	292±27	313±25	324±23	0.032	0.148	0.332
Percent of time pH<6.0 (%)	44±2	49±2	52±2	48±2	0.161	0.601	0.045
Percent of time pH<5.8 (%)	29±2	34±2	36±2	34±2	0.085	0.352	0.049
Percent of time pH<5.6 (%)	16±2	20±2	23±2	22±2	0.020	0.299	0.177
Ruminal Temperature (°C)	39.6±0.01	39.5±0.01	39.8±0.02	39.8±0.04	<0.001	0.777	0.046

In contrast, time of ruminal pH spent below 5.6 expressed in minutes per day ($p=0.032$) or as a percentage ($p=0.020$) was increased by feeding high concentrate diets and was observed to exceed 300 minutes per day in the groups aimed to consume 60% concentrate. Ruminal temperature was not influenced by the supplementation of niacin but increased by 0.2 to 0.3 °C due to feeding high amounts of concentrate ($p<0.001$). Moreover, an interaction between dietary niacin and concentrate was observed for ruminal temperature ($p=0.046$).

The patterns of diurnal variation in ruminal pH were to a large extent comparable among the different feeding groups (Fig. 2). PH generally decreased between 0 and 2h and was afterwards subjected to gradual increases reaching a peak at the time of PMR re-filling at approximately 10h. Subsequently, pH values declined again and remained on a relatively low level until they increased again in the late evening. However, ruminal pH showed a different progression in feeding group 3 because it further decreased after 18.00 h.

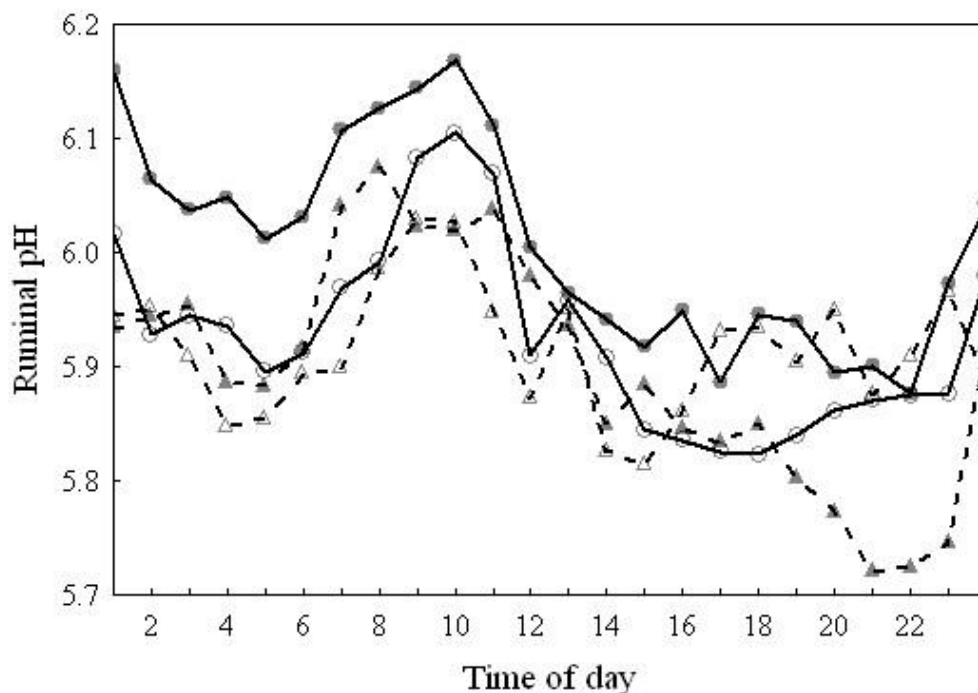


Figure 2. Diet effects on diurnal variation in ruminal pH of dairy cows (● group 1, ○ group 2, ▲ group 3, △ group 4. Values are presented as means per hour).

An example of individual one-day courses of ruminal pH and temperature of two selected cows during warm environmental conditions (THI=73) is given in Fig. 3. The ruminal pH of both presented animals was generally characterized by a considerable short-term variation and the individual differences in pH were visually apparent. Ruminal temperature was observed to be on a relatively high and stable level until approximately 10 h in the morning. However, it

was repeatedly subjected to abrupt declines which could mainly be assigned to drinking events.

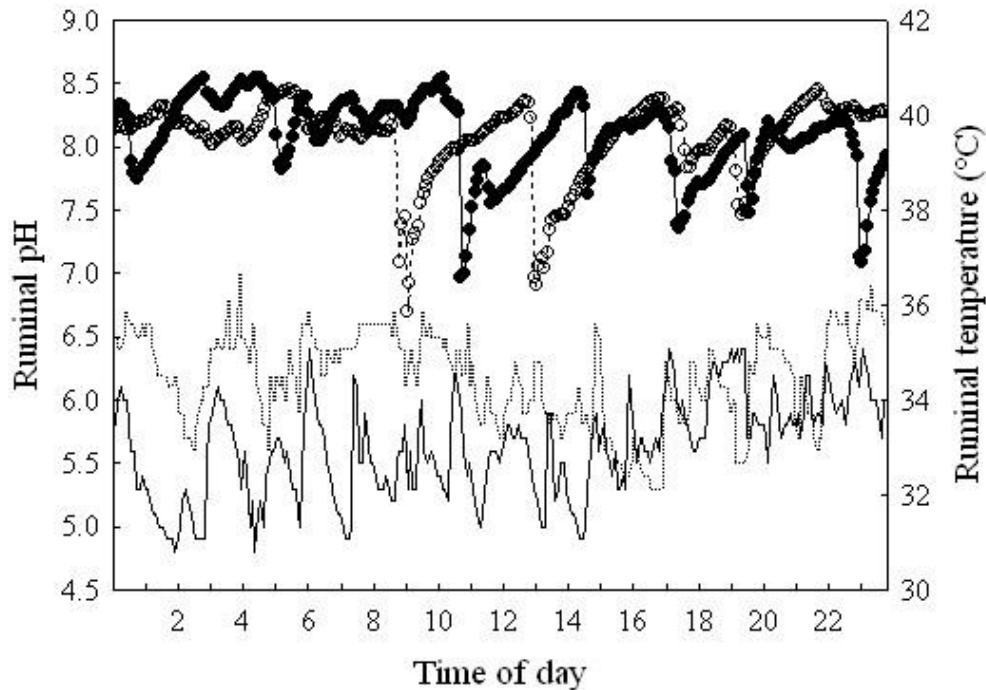


Figure 3. Example of one-day variation in ruminal pH and temperature of two dairy cows at warm environmental conditions (Temperature humidity index = 73). The presented animals were the individuals with the highest (PH: Dotted lines; Temperature: Empty symbols \circ) and the lowest mean pH (PH: Solid lines; Temperature: Closed symbols \bullet) of all animals throughout the experiment.

3.3 Body temperature

The supplementation of niacin did not affect the investigated measures of body temperature during periods of $\text{THI} \geq 68$ ($p > 0.05$, Table 3). Feeding high proportions of concentrate significantly reduced skin temperatures measured at sinistral rump ($p < 0.001$), sinistral thigh ($p = 0.007$), dextral thigh ($p = 0.003$) and neck ($p = 0.038$), respectively, in the range of 0.1 to 0.4 °C. Rectal temperatures tended to decrease due to high concentrate ($p = 0.050$), though the changes were below 0.2 °C. Respiration rates were not significantly altered by the tested dietary treatments, but tended to increase due to additive niacin ($p = 0.097$). Mean rectal temperatures were found to be approximately 38.5 °C, whereas subcutaneous temperatures were almost 37 °C and skin temperatures did not exceed 36.5 °C. Positive correlations were observed among all body temperature and respiration rate data and the calculated THI (Table 4).

Table 3. Effects of dietary concentrate proportion (%) and niacin supplementation on skin, rectal and subcutaneous temperatures as well as respiration rates of dairy cows at THI¹≥68 (LSMeans±SE)

Variable ²	Treatment						P-value
	30 % Concentrate			60 % Concentrate			
	Control (Group 1, n=5)	Niacin (Group 2, n=5)	Control (Group 3, n=4)	Niacin (Group 4, n=5)	Concentrate	Niacin	
Skin T (°C)							
Rump sinistral	36.4±0.07	36.4±0.08	36.1±0.08	36.0±0.08	<0.001	0.342	0.500
Rump dextral	36.3±0.07	36.3±0.07	36.2±0.07	36.1±0.07	0.114	0.831	0.335
Thigh sinistral	36.1±0.08	36.0±0.08	35.8±0.08	35.7±0.08	0.007	0.188	0.883
Thigh dextral	36.0±0.08	36.1±0.09	35.7±0.09	35.8±0.09	0.003	0.624	0.831
Neck	36.3±0.07	36.3±0.07	36.2±0.07	36.2±0.07	0.038	0.928	0.972
Rectal T (°C)	38.6±0.05	38.5±0.05	38.5±0.05	38.4±0.05	0.050	0.258	0.628
Subcutaneous T (°C)	36.9±0.06	36.8±0.06	36.8±0.06	36.8±0.06	0.199	0.452	0.115
RR (Bpm)	48.1±1.89	51.2±1.95	45.5±2.08	48.6±1.94	0.240	0.097	0.984

¹ Temperature humidity index.

² Skin T, skin temperature; Rectal T, rectal temperature; Subcutaneous T, subcutaneous temperature; RR, respiration rate (Bpm, breaths per minute).

Skin temperatures determined at different body sites were interrelated in the range of $r=0.69$ to $r=0.89$ and were closely correlated to both subcutaneous temperature ($r=0.66$ to $r=0.74$) and THI ($r=0.70$ to $r=0.78$). In contrast, the correlation coefficients between rectal temperatures and respiration rates, respectively, and skin as well as subcutaneous temperatures and THI were less close ($r=0.43$ to $r=0.62$).

Table 4. Correlation coefficients between THI¹ and body temperature measures (°C) as well as respiration rates of dairy cows

Parameter ²	Skin T				Neck	Rectal T	Sub T	RR (BPM)	THI
	Rump sinistral	Rump dextral	Thigh sinistral	Thigh dextral					
Skin T									
Rump sinistral	/	0.77**	0.78**	0.74**	0.69**	0.47**	0.66**	0.50**	0.70**
Rump dextral	0.77**	/	0.81**	0.83**	0.78**	0.49**	0.72**	0.53**	0.78**
Thigh sinistral	0.78**	0.81**	/	0.89**	0.80**	0.52**	0.74**	0.57**	0.76**
Thigh dextral	0.74**	0.83**	0.89**	/	0.78**	0.50**	0.72**	0.56**	0.74**
Neck	0.69**	0.78**	0.80**	0.78**	/	0.46**	0.74**	0.53**	0.76**
Rectal T	0.47**	0.49**	0.52**	0.50**	0.46**	/	0.44**	0.52**	0.43**
Sub T	0.66**	0.72**	0.74**	0.72**	0.74**	0.44**	/	0.52**	0.70**
RR (Bpm)	0.50**	0.53**	0.57**	0.56**	0.53**	0.52**	0.52**	/	0.62**
THI	0.70**	0.78**	0.76**	0.74**	0.76**	0.43**	0.70**	0.62**	/

¹ Temperature humidity index.

² Skin T, skin temperature; Rectal T, rectal temperature; Sub T, subcutaneous temperature; RR, respiration rate (Bpm, breaths per minute).

** $P<0.01$.

3.4 Milk performance and feed intake

DMI was lower in the groups fed on high concentrate ($p<0.001$, Table 5). Both the supplementation of niacin and feeding 60% concentrate increased milk yield ($p<0.001$). However, the interaction between both factors revealed relatively higher elevations of milk yield due to niacin supplementation if the animals were fed on high percentages of concentrate ($p<0.001$). This cumulative effect resulted in a total difference of approximately 7.7 kg milk per day and animal between feeding group 1 (21.9 kg) and feeding group 4 (29.6 kg). In contrast, FCM was not affected by dietary concentrate but increased by more than 2 kg due to additional niacin ($p<0.001$). Milk fat percent was decreased by both niacin supplementation and high concentrate and the interaction showed larger reductions due to niacin addition if the animals were fed on high concentrate ($p<0.001$ each).

Table 5. Effects of dietary concentrate proportion (%) and niacin supplementation on milk performance of dairy cows (LSMeans±SE)

Variable	Treatment							P-value	
	30 % Concentrate		60 % Concentrate			Concentrate	Niacin		Concentrate× Niacin
	Control (Group 1, n=5)	Niacin (Group 2, n=5)	Control (Group 3, n=4)	Niacin (Group 4, n=5)	Concentrate× Niacin				
DMI (kg d ⁻¹)	17.0±0.3	17.7±0.3	15.9±0.2	15.0±0.3	<0.001	0.531	0.002		
Milk yield (kg d ⁻¹)	21.9±0.2	25.6±0.2	24.3±0.2	29.6±0.2	<0.001	<0.001	<0.001		
Milk fat (%)	4.64±0.07	4.55±0.07	4.16±0.07	3.38±0.08	<0.001	<0.001	<0.001		
Milk fat yield (kg d ⁻¹)	1.01±0.02	1.14±0.02	1.02±0.02	1.01±0.02	0.003	<0.001	<0.001		
Milk protein (%)	3.49±0.03	3.36±0.03	3.63±0.03	3.45±0.03	<0.001	<0.001	0.425		
Milk protein yield (kg d ⁻¹)	0.76±0.01	0.86±0.02	0.89±0.01	1.05±0.02	<0.001	<0.001	0.025		
Milk lactose (%)	4.78±0.04	4.88±0.04	4.85±0.03	4.87±0.04	0.383	0.070	0.274		
Milk lactose yield (kg d ⁻¹)	1.05±0.02	1.25±0.02	4.85±0.03	1.48±0.02	<0.001	<0.001	0.027		
FCM (kg d ⁻¹)	24.0±0.4	27.2±0.4	24.9±0.3	27.2±0.4	0.229	<0.001	0.216		

Total milk fat yield was affected by the supplementation of niacin and high dietary concentrate. Milk protein percent was reduced by supplemental niacin but increased due to high concentrate proportions ($p < 0.001$). Nevertheless, the total yield of milk protein was significantly elevated by added niacin (More than 100 g per day and animal) and high concentrate (More than 150 g per day and animal), respectively. Lactose yield was increased by both supplemental niacin ($p < 0.001$) and high concentrate ($p < 0.001$), respectively. Same as for milk yield, the interactions between concentrate proportion and niacin on both protein ($p = 0.025$) and lactose ($p = 0.027$) yield were in the direction of higher positive responses to niacin if the animals were fed on high concentrate.

4. Discussion

The climatic conditions in the course of the present experiment covered a relatively wide range of THI with a considerable day to day variation. However, at different days THI were on a level indicating the development of mild heat stress because heat stress may be initiated if THI exceeds a value of 71 (Armstrong 1994).

The intended dietary concentrate proportions were met to a large extent but varied within the limits of the performed feeding scheme. The temporary restriction of the individual access to PMR in order to realize the aimed concentrate to forage ratios will likely explain the negative effect of high dietary concentrate on total DMI because feeding more concentrate at the expense of forage otherwise should have increased the voluntary feed intake of dairy cows (Friggens et al. 1998; Shingfield et al. 2002; Kennedy et al. 2008).

The observed effects of niacin on ruminal pH were below 0.1 pH units and the relatively high number of ruminal pH measurements will likely have contributed to the calculated statistical significance. Accordingly, in previous experiments the supplementation of niacin did not substantially affect the pH measured either in vitro in a fermenter (Horner et al. 1988) or in rumen fluid of lactating Holstein cows (MadisonAnderson et al. 1997), respectively. Niacin slightly reduced ruminal pH in the animals fed on low concentrate, a process that was not confirmed for the high concentrate diets. This observation may be mediated indirectly by stimulating effects of niacin on rumen protozoa such as *Entodinia* (Erickson et al., 1990; Kumar and Dass 2005). *Entodinia* contribute to the degradation of fibrous constituents and are able to consume starch granula (Erickson et al. 1991). Elevated SCFA concentrations due to increased protozoal fiber degradation may have contributed to the decreased ruminal pH values of the animals fed on low concentrate. These niacin-associated reductions in pH may have been compensated by an increased protozoal inclusion of starch and thus altered

fermentation patterns of the higher amounts of starch available if the animals were fed on high concentrate.

Both the decreased mean ruminal pH and the extended time spent below pH 5.6 due to feeding high concentrate are consistent because it is known that increased dietary concentrate may reduce the ruminal pH of ruminants (Mishra et al. 1970; Jaakkola and Huhtanen 1993; Owens et al. 2008). However, the time of ruminal pH below specific low thresholds is not necessarily elevated even by high amounts of concentrate (Nocek et al. 2002; Maekawa et al. 2002). Prolonged periods with a depressed ruminal pH may indicate subacute ruminal acidosis (SARA) in cattle (Kleen et al. 2003; Morgante et al. 2007; Kleen et al. 2009). Various thresholds have been proposed to identify the onset of SARA. For example, Garrett et al. (1999) discussed a critical value of pH 5.5. Gozho et al. (2005) suggested the onset of SARA to be associated with a ruminal pH below 5.6 for at least 3 hours a day. In the present experiment, the daily interval of ruminal pH below 5.6 was found to be considerably higher than 3 hours independent of dietary treatment and exceeded 5 hours in the groups fed on high concentrate. Accordingly, the exclusive interpretation of the measured ruminal pH may indicate the presence of SARA. However, SARA as a ruminal disorder is usually associated with further clinical signs such as diarrhea, loss of body condition and laminitis (Nocek 1997) but these symptoms were not observed to occur frequently in the current study.

Feeding high proportions of concentrate was accompanied by elevations of mean ruminal temperatures in the range of 0.2 to 0.3 °C. Such increased ruminal temperatures were in accordance with results obtained by AlZahal et al. (2009), who reported rises in mean ruminal temperature (Approximately 0.7 °C) due to feeding a high concentrate TMR compared to a control TMR. Such effects may depend on the total amount of concentrate fed because Gasteiner et al. (2009) did not induce differences in ruminal temperature by feeding steers a 100% hay or a 50% concentrate diet, respectively.

Increases of ruminal temperatures but simultaneous reductions of both skin and rectal temperatures of dairy cows during periods of heat stress due to high concentrate diets are not necessarily contradictory. First, the fermentation in the rumen accounts for only 3-8% of the total heat production (Czerkawski 1980). Secondly, feeding high fiber diets was reported to result in greater metabolic and total heat production that may be associated with increased rectal and skin temperatures because, for example, feeding cows on 100, 75 or 50% alfalfa with the rest being a mixture of corn and soybean meal was associated with a heat production of 688, 647 and 620 kcal per megacalorie ME (Coppock et al. 1964; Reynolds et al. 1991). Moreover, in an early work of Stott and Moody (1960) lactating dairy cows subjected to high

ambient temperatures and fed on high fiber diets primarily composed of alfalfa hay were reported to have higher body temperatures and respiration rates than cows fed on a highly digestible low fiber diet. Such rising dietary heat increments due to high fiber rations may arise because the metabolism of acetate is associated with a higher heat production than the metabolism of propionate (West et al. 1999). MacRae and Loble (1982) suggested that the main reason for the larger thermal losses due to feeding roughage may be a different ability to use excess acetate. In the same study it was hypothesized that the greater amounts of propionate gained from high dietary concentrate should supply enough NADPH to enable acetate to be converted into body fat whereas high fiber diets are related to larger amounts of acetate and smaller propionate production leading to a metabolic excess of acetate that has to be eliminated as heat.

The closer positive correlations between THI and respiration rates as well as skin temperatures ($r=0.62$ to 0.78) than between THI and rectal temperatures ($r=0.43$) were consistent. If homeothermic animals are within the so-called thermo-neutral zone, rectal temperatures are normal at minimal heat production (Kadzere et al. 2002). If warm climatic conditions induce the need to dissipate excess body heat to avoid hyperthermia, the loss of heat via both the mammalian skin and respiratory moisture are pathways for the thermal exchange between animal and environment and were thus observed to be elevated in lactating cows in hot periods (Marai et al. 2007; Dikmen et al. 2008). Accordingly, respiration rates and skin temperatures are likely to increase in a warm environment in order to maintain thermal homeostasis before a response of core body or rectal temperatures is inevitable.

In humans, niacin was reported to induce flushing, a strong cutaneous vasodilation (Andersson et al. 1977). The vasodilative effect of niacin is likely related to the production of prostaglandins following the niacin mediated activation of the G protein-coupled receptor 109A (Benyo et al. 2005; Benyo et al. 2006; Kamanna et al. 2009). During mild heat stress, the supplementation of niacin did not affect skin, subcutaneous or rectal temperatures in the present study. Accordingly no indications for an elevated skin blood flow and a potential alleviation of heat stress via increased dissipation of excess body heat were obtained. Current information about impacts of niacin supplementation on vasodilation and body temperatures of heat stressed dairy cows is rare and primarily bases on two experiments. Zimelman et al. (2010) did not report differences between skin temperatures of dairy cows assigned either to a niacin treatment with a daily supplementation of 12 g or a control group under thermo-neutral and mild heat stress conditions, respectively. However, evaporative heat loss was higher in the niacin fed cows and both vaginal and rectal temperatures were reduced by 0.2 to 0.4 °C

during mild heat stress. The authors suggested that these observations were associated with a niacin-induced cutaneous vasodilation. Though not significant, the cows fed on supplemental niacin had higher respiration rates, a trend that was observed in the present study as well. The lack of measurable niacin effects on skin temperatures remains to be elucidated but seems not to be related to the investigated thermal environments or the total amount of additive niacin fed. DiCostanzo et al. (1997) reported reduced skin temperatures at tail and rump of dairy cows in the range of 0.3 to 0.4 °C due to a supplementation of 12 g niacin per day and animal in a period of mild heat stress. However, increasing the addition of niacin to 24 g in a second period of severe heat stress was not related to further reductions of skin temperatures as they were only reduced at the tail (0.3 °C) and a daily amount of 36 g niacin was not related to any reduction of skin temperatures in a period with thermo-neutral conditions.

The observed positive effects of dietary niacin on milk yields were only partially reflected in previous literature as lower levels of niacin in the range of 6 to 12 g did either not affect (MadisonAnderson et al. 1997; Niehoff et al. 2009b) or increase milk yields by 2.2 to 2.5 kg (Cervantes et al. 1996; Drackley et al. 1998). Ottou et al. (1995) suggested that positive lactational responses are mainly to be expected for cows in early lactation when the animals are in a negative energy balance. However, niacin does not necessarily elevate milk production during early lactation (Driver et al. 1990) and the hypothesis could not be examined in the present experiment because the investigations were initiated when the animals were in mid-lactation. Moreover, the number of lactation seems not to be a pivotal factor affecting the response to dietary niacin because Muller et al. (1986) used a large quantity of animals and milk yield was improved for both first lactation and older cows by 0.7 to 0.9 kg.

Experimental results concerning the effects of niacin on milk protein were characterized by a high variability (Niehoff et al. 2009a). For example, Drackley et al. (1998) reported decreased protein contents but a trend towards increased total protein yield. The authors attributed the reduced protein contents to dilutive effects because milk yield was increased. These findings were in accordance with the current study where niacin decreased milk protein content but increased protein yield in the range of 0.1 to 0.15 kg per day. Erickson et al. (1992) suggested two possible explanations for the responses of milk protein to niacin. First, the amino acid uptake by the mammary gland might be elevated because niacin may increase plasma insulin (Horner et al. 1986) and intravenous insulin was shown to increase milk protein content (Molento et al. 2002). Secondly, niacin supplementation was reported to enhance the microbial protein synthesis (Horner et al. 1988).

Both milk fat percent and yield were not altered in different trials investigating the effects of niacin (Driver et al. 1990; Aschemann et al. 2012). However, Muller et al. (1986) reported unaffected milk fat percentages but improved fat yields and the greatest daily increases approximating 0.08 kg were found for high-yielding cows. In the present study, milk fat yield was slightly elevated but milk fat percent decreased, a fact that may be attributed to dilutive responses considering the increases in milk yield. A similar tendency towards reduced milk fat percent due to niacin was reported by Cervantes et al. (1996). Niehoff et al. (2009a) suggested that changes in milk fat following dietary niacin may be due to effects at the ruminal level but this thesis needs to be verified because most research on the effects of niacin in the rumen focused primarily on the rumen and milk measurements were rare.

In accordance with the present results, increased levels of concentrate generally enhanced milk yields of dairy cows and were found to stimulate protein yields in previous studies (Agnew et al. 1996; O'Mara et al. 1998). However, FCM yield was not altered in the present study. Milk fat percent decreased due to high dietary concentrate, an observation that is well established because reduced forage to concentrate ratios are usually but not necessarily associated with decreases in milk fat content (Sutton 1989).

5. Conclusions

Based on the present results, the potential of supplemental niacin to contribute to an alleviation of heat stress in lactating primiparous dairy cows is likely to be limited. Furthermore, the effects on ruminal pH and temperature were marginal or not significant. High concentrate diets were associated with decreased skin temperatures and may thus reduce the negative effects of body heat increment during warm environmental conditions. However, the applicability of concentrate is restricted as high proportions were demonstrated to decrease mean ruminal pH and the duration of pH spent below 5.6, respectively, increasing the probability of the development of SARA. Both niacin and high dietary concentrate slightly altered milk fat yield and increased milk, protein and lactose yield. Milk constituents were rather decreased or not affected.

Acknowledgements

The study was supported by the Ministry for Science and Culture of Lower Saxony within the network KLIFF – climate impact and adaptation research in Lower Saxony. Furthermore, the assistance of the co-workers of the Institute of Animal Nutrition and the Experimental Station

of the Friedrich-Loeffler-Institute in Brunswick, Germany in performing experiment and analysis is gratefully acknowledged.

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General discussion

Current and future climate change will likely induce massive and long-lasting environmental modifications that may considerably alter the conditions of global feed and food production in a world characterized by both growing human population and food demand. The exact magnitudes of the prospective alterations are unknown and strongly depend on the future greenhouse gas emission scenario (Eby et al., 2009). In general, the probably most important climatic changes in relation to agriculture are increased atmospheric CO₂ concentrations, elevated ambient temperatures and altered precipitation patterns.

It is unknown whether rising atmospheric carbon dioxide may directly affect the feed value of maize silage via altered contents of nutrients or mycotoxin contamination and if CO₂ induced ameliorations of the negative effects of future drought on nutrient formation are to be expected due to decreases in plant water loss as described previously (Leakey et al., 2006; Markelz et al., 2011). Hence, the effects of an atmospheric CO₂ concentration of 550 ppm as expected by the middle of the 21st century and drought stress on feed value and contents of chosen mycotoxins of ensiled maize were investigated in a FACE experiment and the digestibility of the generated silages was tested in sheep at different climatic conditions (**Paper I**). In addition, the effects of enriched CO₂, drought and varying thermal environments on thermoregulation and blood parameters of sheep were investigated and it was tested whether differing climatic conditions may affect the metabolization of DON in the ovine rumen (**Paper II**).

Increasing future incidence and severity of heat stress are likely to have a variety of adverse effects on health and performance of dairy cows (Nienaber et al., 1999; Kadzere et al., 2002). High proportions of dietary concentrate and the supplementation of niacin to dairy cow rations were suggested to potentially reduce the negative impact of heat by means of decreased heat increment (concentrate) or improved dissipation of heat via increases in skin blood flow due to vasodilatative properties (niacin) (West, 2003; Zimbelman et al., 2010). Therefore, the effects of varying concentrate proportions and supplemental niacin on skin and core body temperatures, respiration rates and milk performance of primiparous dairy cows were assessed (**Paper IV**). In ruminants, the applicability of high concentrate diets is generally limited as they can substantially decrease ruminal pH and lead to the development of SARA (Kleen et al., 2003). Innovative technology such as wireless probes for continuous measurement of ruminal pH and temperature may help to detect SARA in cattle (Enemark et al., 2003; AlZahal et al., 2008; Kaur et al., 2010). New devices for an ongoing *in vivo*

determination of both parameters were evaluated in dairy cows using method comparison techniques in consideration of the effects of different dietary concentrate proportions, the localization of measurement in the rumen and a potential occurrence of pH sensor drift (**Paper III-IV**).

1 Feed value of maize silage

1.1 General aspects

Feeding ensiled maize plants that were grown in a fictitious future atmosphere to ruminants which were exposed to different thermal environments including warm conditions that were likely to induce heat stress to simulate future climatic conditions was an innovative approach conducted to contribute to an estimation of the impact of climate change of agricultural production. The absence of negative interactions between elevated CO₂ concentrations during maize growth and warm climatic conditions during feeding of the generated silages on nutrient content and digestibility of future maize silage may be considered a positive aspect. However, the expected future climatic alterations are likely to interact in a very complex manner that could not be simulated completely within the present investigations. For example, severe future increases in summer heat will not only directly impact livestock but its occurrence during maize growth is known to disrupt kernel growth and endosperm starch biosynthesis and could thus have serious negative effects on yield, nutrient content and digestibility of maize silage (Cheikh and Jones, 1995; Monjardino et al., 2005). Though it is difficult to induce experimental variations in ambient temperature in field studies such as FACE trials, such investigations should be considered in future research to cover the complete range of possible effects of climatic changes on future feed and food production.

1.2 Nutrients

The photosynthesis of C₄ plants such as maize should theoretically be saturated at current atmospheric CO₂ concentrations (Ghannoum et al., 2000; Ghannoum, 2009) and therefore future increases in carbon dioxide should not stimulate maize yield or affect the feed value of ensiled whole maize plants in terms of altered nutrient contents. Generally, it has to be kept in mind that the past rises in atmospheric CO₂ that have taken place since the beginning of the industrialization in the 19th century (Initial concentration: Approximately 280 ppm) sum up to a total elevation of nearly 40 % leading to a current concentration of approximately 385 to 390 ppm (Forster et al., 2007) and that C₄ photosynthesis was likely not CO₂-saturated at

concentrations below 350 to 370 ppm as shown in **Figure 3**. Thus this past increases in carbon dioxide will probably have stimulated the basal photosynthesis of C₄ plants that were grown under natural field conditions in comparison to the previous atmospheric concentration and this photosynthetic stimulation may have been accompanied by qualitative reductions as reported for C₃ crops in terms of protein (Manderscheid et al., 1995; Högy and Fangmeier, 2008; Manderscheid et al., 2010).

The present results were in principle consistent with this theoretical background because the cultivation of maize in an atmosphere containing 550 ppm CO₂ did not influence the concentration of crude protein, ether extract, starch or different fibre fractions such as NDF, ADF and ADL in the produced silages in comparison to a control treatment of approximately 380 ppm CO₂ (**Paper I**). In contrast, the protein contents of grassland and different C₃ crops such as wheat or barley were not only decreased by carbon dioxide enrichment but the quality of protein for both human nutrition purposes or feeding of monogastric livestock species such as pigs was considerably impaired due to decreases in essential amino acids (Manderscheid et al., 1995; Wu et al., 2004; Milchunas et al., 2005; Högy and Fangmeier, 2008). These observations were confirmed in a recent study of Porteous et al. (2009) and the authors concluded that for wheat increased future yields are likely to be accompanied by reductions in the quality of both grain and straw in terms of depleted crude protein and protein to energy ratio, respectively, and that this may lead to decreases in the total supply of protein that is useable for livestock feeding. In the present experiment, the amino acid sequence of maize protein was not determined and thus it cannot be excluded that alterations may have been induced by CO₂ enrichment. However, this should not negate the validity of the current results as, first, whole plant maize silage is predominantly fed to ruminants for which the source of N is known to be a less important factor than for monogastric species due to the production of high-quality microbial protein in the forestomach and, secondly, maize silage usually contains relatively low amounts of crude protein that do not exceed 10 % of silage DM and therefore ensiled maize is primarily used as a supplier of energy in ruminant diets (Jetana, et al., 2000; Kirkland et al., 2005; Keady et al., 2008). Hence potential CO₂-induced changes in the amino acid sequence of maize protein may be more important if other maize products such as grain were used, for example, in pig or poultry feeding.

The described decreases in plant protein of various C₃ species were mainly due to an accumulation of carbohydrates that was induced by a photosynthetic stimulation due to atmospheric carbon dioxide enrichment (Korner, 2000). In several studies, this accumulation was reported to manifest in increased starch concentrations in wheat grain using both FACE

or growth-chamber technology (Fangmeier et al., 1999; Wu et al., 2004; Porteous et al., 2009). Grain comprise approximately 70 % carbohydrates and almost all carbohydrates are present as starch with less than 3 % other compounds such as sucrose, glucose or fructose (Abouguen and Dappolon, 1973; Boyacioglu and Dappolonia, 1994). However, experimental results were inconsistent as such CO₂ effects on the concentration of starch were not necessarily observed in wheat grain (Högy et al., 2009) or were not significant in some experiments as reviewed by Högy and Fangmeier (2008). In maize silage, starch may be considered the most important compound with regard to its feed value that often exceeds 30 % of DM and thus substantially contributes to the supply of dietary energy (Khan et al., 2009). The ruminal degradability of maize starch is known to be relatively low, though it was observed to be increased by ensiling, and bypass starch that escapes the fermentation in the rumen is to a large extent subjected to enzymatic digestion in the intestine, a process that was discussed to be energetically more efficient than ruminal fermentation and absorption of SCFA (Huntington, 1997; Philippeau and Michalet-Doreau, 1998; Garnsworthy et al., 2009). As presented in **Paper I**, the concentration of starch in the investigated maize silages was found to be generally higher than 30 % and was not affected by CO₂ enrichment. Thus, increased future carbon dioxide will likely not increase the starch content of ensiled maize or affect its potential future supply of feed energy.

The dry subplots in the present FACE experiment received 48 % less water than the well watered subplots and drought treatment depleted maize grain yields by 35 % in the CO₂ Control treatment and by 8 % in the FACE plots (Manderscheid et al., 2012). However, drought did not affect the nutrient content of the investigated maize silages and no interactions between CO₂ and drought were observed. Consequently, in terms of crude nutrients there were no indications of, first, direct drought effects and, secondly, of a potential alleviation of negative effects of restricted water availability by a decreased stomatal conductance and plant water loss due to enriched CO₂ as described for maize in previous literature (Leakey et al., 2006). That was surprising especially in consideration of the distinctly lower drought-associated losses in grain yields if maize was grown at elevated carbon dioxide and the reduced content of DM in experimental silage fresh matter that demonstrated the presence of a drought effect (**Paper I**). These results can lead to the conclusion that the water deficit may have been large enough to decrease yields but probably was too low to result in measurable changes of the investigated nutrients in whole plant silage.

1.3 Digestibility

In general, the use of results from digestibility trials with sheep for the evaluation of feed for other ruminants such as cattle may be restricted but can provide useful information if characteristic interspecies differences are considered and when the available amounts of feed are limited as in the present investigations. Mertens and Ely (1982) suggested that sheep have a better capacity to digest high quality feed but cattle have a higher ability to digest low quality feed and that the crossover point was at a DM digestibility of 660 g/kg. Experimental results comprise a considerable variation and differences in apparent digestibility of feedstuff between cattle and sheep may depend on feeding level or turnover rate (Bines et al., 1988; Mulligan et al., 2001). However, Aerts et al. (1984) evaluated a large number of digestibility trials involving 26 maize silages and concluded that sheep and cattle did not differ systematically in digesting maize silages. Consequently, the present results may give transferable indications for the estimation of the effects of increased atmospheric CO₂ concentrations and drought during plant growth on the digestibility of maize silage in cattle as well.

The lack of effects of elevated atmospheric CO₂ on the digestibility of maize silage CF, ADF, NDF, starch and OM was consistent with the absence of CO₂ effects on the concentration of crude nutrients. Information about the *in vivo* digestibility of plants that were grown in high CO₂ atmospheres is very rare, though several experiments were conducted in order to assess the *in vitro* digestibility of different plant species (**Table 3**). These results were found to be relatively inhomogeneous. However, with the exception of one experiment the *in vitro* digestibility of DM or OM of the investigated C₃ and C₄ grasses and crops was either not affected or reduced by elevated CO₂ during growth. All decreases in digestibility were induced by higher carbon dioxide concentrations (640 to 720 ppm) than those used in **Paper I** and **Paper II** and these effects were not limited to C₃ species. For example, the DM digestibility of the C₄ grass *Bouteloua gracilis* was found to be reduced by enriched CO₂ as well (Fritschi et al., 1999; Carter et al., 1999; Morgan et al., 2004; Milchunas et al., 2005). Against this background the lack of negative CO₂ effects on OM and nutrient digestibility of the investigated ensiled whole maize plants may be regarded as a positive aspect. However, the repeated detection of decreases in the *in vitro* digestibility of C₃ and C₄ species highlights the importance of further research to evaluate the impact of high atmospheric CO₂ concentrations that considerably exceed 550 ppm as expected by the end of the century.

Table 3. Effects of atmospheric CO₂ enrichment on the digestibility of various plants by different authors

Reference	Enrichment facility ¹	CO ₂ -concentration	Plant species	Determination of digestibility	Studied fraction ²
Akin et al. (1994)	FACE	550 ppm	Sudangrass (<i>Sorghum bicolor</i> L.)	<i>In vitro</i>	DM: n.s.
Bertrand et al. (2007)	Growth chambers	800 ppm	Alfalfa (<i>Medicago sativa</i> L.)	<i>In vitro</i>	DM: n.s.
Carter et al. (1999)	Growth chambers	700 ppm	Birds-Foot trefoil (<i>Lotus corniculatus</i> L.)	<i>In vitro</i>	Leave DM: ↓ Stem DM: n.s.
Fritschi et al. (1999)	Greenhouses	640 ppm	Rhizoma peanut (<i>Arachis galabra</i> L.) Bahia grass (<i>Paspalum notatum</i>)	<i>In vitro</i>	OM: ↓ OM: n.s.
Lilley et al. (2001)	Field tunnels	690 ppm	Subterranean clover (<i>Trifolium subterraneum</i> L.) Phalaris (<i>Phalaris aquatica</i> L.)	<i>In vitro</i>	DM: n.s. DM: n.s.
Milchunas et al. (2005)	Open-top chambers	720 ppm	Shortgrass steppe: 45 % <i>Bouteloua gracilis</i> (C ₄ -grass) 25 % <i>Stipa comata</i> (C ₃ -grass) 18 % <i>Pascopyrum smithii</i> (C ₃ -grass)	<i>In vitro</i>	DM: ↓
Morgan et al. (2004)	Open-top chambers	720 ppm	<i>Bouteloua gracilis</i> (C ₄ -grass) <i>Pascopyrum smithii</i> (C ₃ -grass) <i>Stipa comata</i> (C ₃ -grass)	<i>In vitro</i>	DM: ↓ DM: ↓ DM: ↓
Muntifering et al. (2006)	FACE	516–533 ppm	Red clover (<i>Trifolium pratense</i> L.) White clover (<i>Trifolium repens</i> L.)	<i>In vitro</i>	DM: n.s. Cell walls: n.s. DM: n.s. Cell walls: n.s.
Paper I	FACE	550 ppm	Maize (<i>Zea mays</i> L.)	<i>In vivo</i>	CF: n.s. ADF, NDF: n.s. Starch: n.s. OM: n.s. DM: ↑
Picon-Cochard et al. (2003)	FACE	600 ppm	C ₃ grassland community	<i>In vitro</i>	DM: ↑

¹ : FACE, free air carbon dioxide enrichment

² : n.s., not significant; ADF, acid detergent fibre; NDF, neutral detergent fibre; CF, crude fibre; DM, dry matter; OM, organic matter

The climatic conditions during feeding were related to the most marked effects on the digestibility of ADF, NDF and OM of maize silage (**Paper I**). The coldest climate induced the lowest digestibilities of ADF, NDF and, by trend, CF, though the positive effects of heat on fibre digestibilities were not reflected in OM digestibility indicating compensations via altered digestibilities of other nutrients. In general, increases in nutrient or diet digestibility due to heat exposure during feeding may improve the feed utilization in ruminants. That may be of particular importance if heat stress induces reductions in DM intake as illustrated for cows in **Table 2**. Higher digestibilities of plant cell walls during warm environmental conditions may compensate for a part of the usually reduced intake of feed energy that takes place in periods of increased expenditure of energy due to the dissipation of excess body heat as shown in **Figure 5**.

Similar effects of the climatic conditions during feeding on the digestibility of ruminant diets were described in previous experiments but the largest part of the relevant fundamental research was conducted between 1970 and 1985. In general, changing ambient temperatures can induce alterations in feed efficiency in sheep (Marai et al., 2007). Christopherson and Kennedy (1983) reviewed the effects of the thermal environment on digestion in ruminants and suggested that an energy deficit of the animal modulates gut function as, for example, Graham et al. (1982) reported differences in gastrointestinal weights of cold- and warm-acclimated sheep fed approximately at maintenance level. Furthermore, the increases in the digestibility of DM and fibre in cattle and sheep diets at high ambient temperatures are related to decreases in the rate of passage of ingesta through the gastrointestinal tract via reduced motility and probably rumination activity (Christopherson and Kennedy, 1983). The retention time of specific markers in the gastrointestinal tract of steers that were fed on forage based diets was increased from 36.6 to 43.2 h and the longer retention time enhanced the digestibility of DM, ADF and NDF (Warren et al., 1974). In several studies with sheep it was demonstrated that the longer retention times of markers in the digestive tract of animals kept in warm environments were primarily due to increased retention times in the reticulorumen, the main localization of fibre degradation via microbial fermentation in ruminants, and that post-ruminal retention time was either not or inconsistently affected (Westra and Christopherson, 1976; Kennedy et al., 1977; Kennedy and Milligan, 1978; Kennedy et al., 1982). The improved cell wall digestibility in the current experiment (**Paper I**) due to heat exposure was accompanied by increases in rectal and skin temperatures, respiration rates and water usage that occurred mainly within the physiological variation of sheep (**Paper II**). However, the shifts in body temperatures and respiratory activity indicated the presence of

heat effects and the need to dissipate excess body heat. Therefore the retention time of ingesta in the forestomach was likely increased as well during periods of mild and severe heat leading to an improved ruminal fermentation of maize cell walls as reported in previous literature.

In ruminants, variations in consumed amount and temperature of drinking water may potentially contribute to effects of the thermal environment during feeding on the digestibility of plant cell walls as observed in **Paper I** but were not considered in most experiments that evaluated the impact of the climatic conditions on diet digestibility. The temperature of drinking water in most commercial and experimental farms is likely to strongly depend on ambient temperature if there is no device operated to adapt to shifts in ambient temperature by means of cooling or heating. Hence, the cooling effect of drinking water should be higher during cold environmental conditions and the potential reduction of ruminal temperatures may increase during cool periods. It was reported that the intake of high amounts of water that is differing in temperature can considerably affect reticuloruminal temperatures and therefore the conditions of microbial fermentation may be temporarily influenced as well. For example, Bewley et al. (2008b) investigated the impact of a forced intake of 25 kg hot (34.3 °C), warm (18.2 °C) or cold (5.1 °C) water on reticular temperatures of dairy cows and reported substantial declines with mean maximum drops of 2.2, 6.9 and 8.5 °C, respectively. Even after 3 hours, reticular temperatures did not consistently return to baseline. Similar results were reported for sheep (Brod et al., 1982). Though a single consumption of 25 kg water should not occur frequently in well-managed dairy cow herds, even distinctly smaller amounts of drinking water can considerably reduce the temperature in the forestomach by more than 4 °C within a few minutes and the recovery can last several hours as shown in **Figure 6**. The total intakes of drinking water of the investigated cows numbered 711 and 716 at the presented days were 33.5 and 57.1 kg in a warm environment (a) but amounted 53.5 and 69.0 kg (b) in thermo-neutral conditions. Day two (b) followed a warm period with $THI \geq 71$ for several days and this previous heat increment including the respective losses of water may explain the higher water intakes during colder ambient temperatures. As indicated in **Figure 6**, almost all large reductions in ruminal temperatures were related to single consumptions of more than 5 kg drinking water. Moreover, the abrupt decreases in ruminal temperatures seemed to occur more frequently at the day with thermo-neutral conditions (b). This observation may be related to both colder drinking water temperatures and the higher total water intakes in comparison to the other investigated daily profile (a).

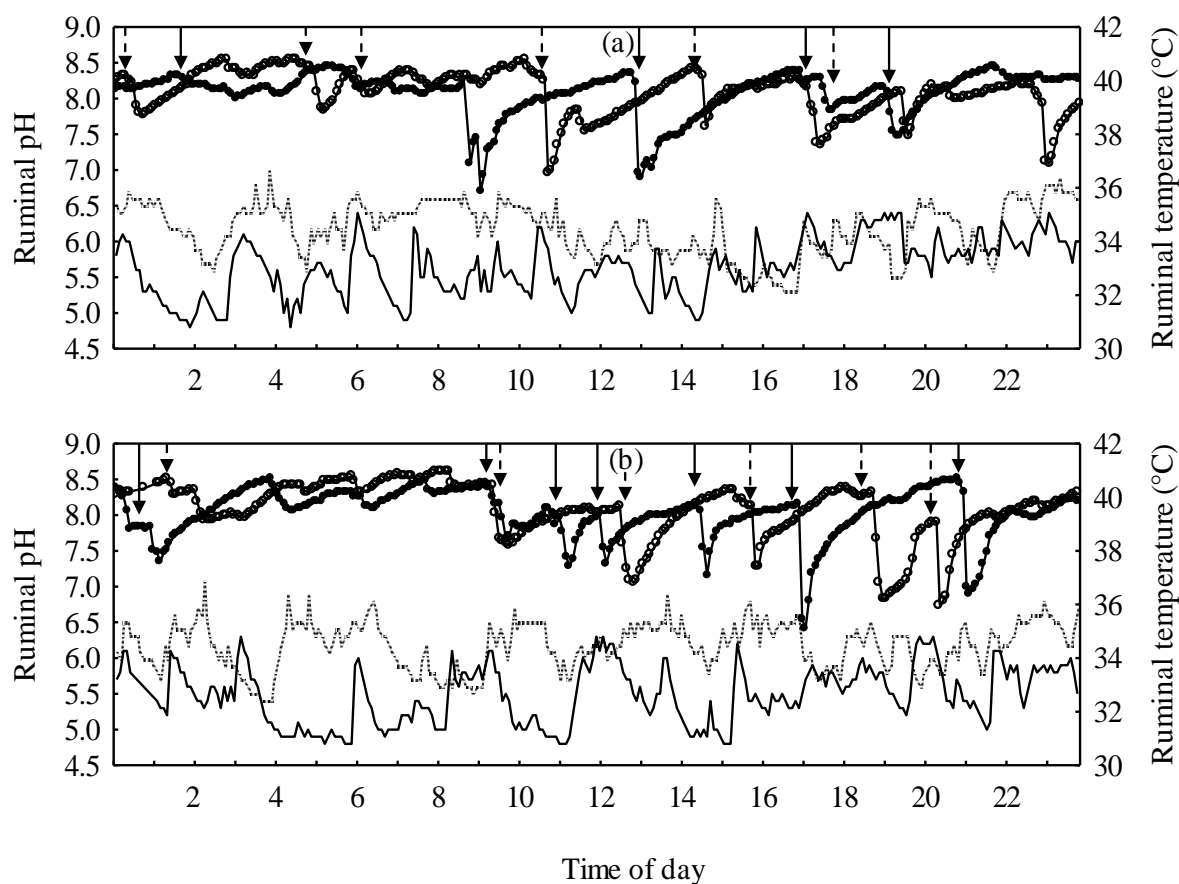


Figure 6: Example of one-day variation in ruminal pH and temperature of two dairy cows at warm (a, THI=73) and thermo-neutral (b, THI=64) conditions (Based on data published in **Paper IV**. Frequency of measurements: 5 min). The animals were chosen on the basis of mean overall pH and the presented individuals were the cows with the highest (Cow 711. Dotted lines: pH, filled symbols: temperature) and lowest mean pH (Cow 716. Solid lines: pH, empty symbols: temperature) of all investigated animals during the experiment. Arrows indicate the onset of drinking events with an intake of ≥ 5 kg water

The microbial population in the rumen should be adapted to a range of temperatures and it needs to be elucidated, which magnitude in ruminal temperature changes may cause the ruminal environment to become suboptimal for certain microbe species. Recently, Uyeno et al. (2010) investigated the molecular diversity of ruminal bacteria in Holstein heifers which were exposed to 20, 28 and 33 °C and found that the relative proportions of the genera *Clostridium* and *Streptococcus* increased but *Fibrobacter* decreased due to exposing the animals to high ambient temperatures. However, these results were not related to drinking water intake and temperature. In a previous study, Gengler et al. (1970) assessed the effects of different ambient and hot ruminal temperatures on the concentrations of SCFA in non-lactating fistulated Holstein cows and concluded that lower concentrations of SCFA were found at high ambient temperatures but these effects were not due to alterations in ruminal

temperatures. Low ruminal temperatures as typically induced by the intake of high amounts of cold drinking water were not evaluated in the cited experiment and therefore the validity of the results was limited to the effects of high temperatures in the rumen. In sheep, there were no significant differences in the *in vitro* gas production by rumen fluid gained from animals that were exposed to warm or cold environments (Kennedy et al., 1982). Further research is required to determine whether consumption and temperature of drinking water may affect the microbial populations in the forestomach or the digestibility of ruminant diets.

1.4 DON in maize silage and its metabolization in the rumen

The probably most pronounced effects of the investigated treatments during growth of the evaluated maize plants were a considerable drought-associated increase in the concentration of DON in the generated silages and a reduction of this rises by cultivating the plants in a high CO₂ atmosphere of 550 ppm as reported in **Paper I**. As shown graphically in **Figure 7**, enriched carbon dioxide did not directly affect the concentration of silage DON. That is in accordance with previous literature as it was suggested that even distinctly higher CO₂ concentrations up to 1000 ppm as expected by the end of the century should not affect mycotoxigenetic fungi (Magan et al., 2011). Thus the CO₂-induced reduction of DON in maize silages made of plants that were grown under conditions of drought may be rather related to a decreased plant water loss than to direct carbon dioxide effects. Reduced water losses could have diminished the susceptibility of maize to fungal infection as reported previously because the dry subplots in the FACE experiment received 48 % less water than the well watered plots and high atmospheric CO₂ is known to reduce the stomatal conductance of maize (Arino and Bullerman, 1994; Leakey et al., 2006; Manderscheid et al. 2012). This indirect CO₂-related decrease of DON in ensiled maize which was grown under conditions of restricted water availability obviously represents a positive implication of the prognosticated future atmospheric alterations. However, it remains unclear whether such a reducing effect will be able to completely compensate for the probably increasing future incidence of *Fusarium* fungi infections and hence DON contamination of maize due to an elevated occurrence of drought in diverse regions and if the overall effects of climate change on occurrence and concentration of mycotoxins in maize will be adverse or not. Future increases in ambient temperatures were not investigated in the present experiment but may as well play an important role in assessing conceivable climate change effects on, for example, DON in maize plants as they exhibit the potential to modify growth and mycotoxin production of different *Fusarium* species (Waalwijk et al., 2003; Jennings et al., 2004; Giorni

et al., 2007). Very few studies were performed in order to investigate interactions between atmospheric CO₂ concentrations below 1000 ppm, a_w and differing ambient temperatures but such experiments could provide valuable information in predicting possible climate change effects on fungal contamination and mycotoxin production that would not necessarily be limited to *Fusarium* species (Paterson and Lima, 2010; Magan et al., 2011).

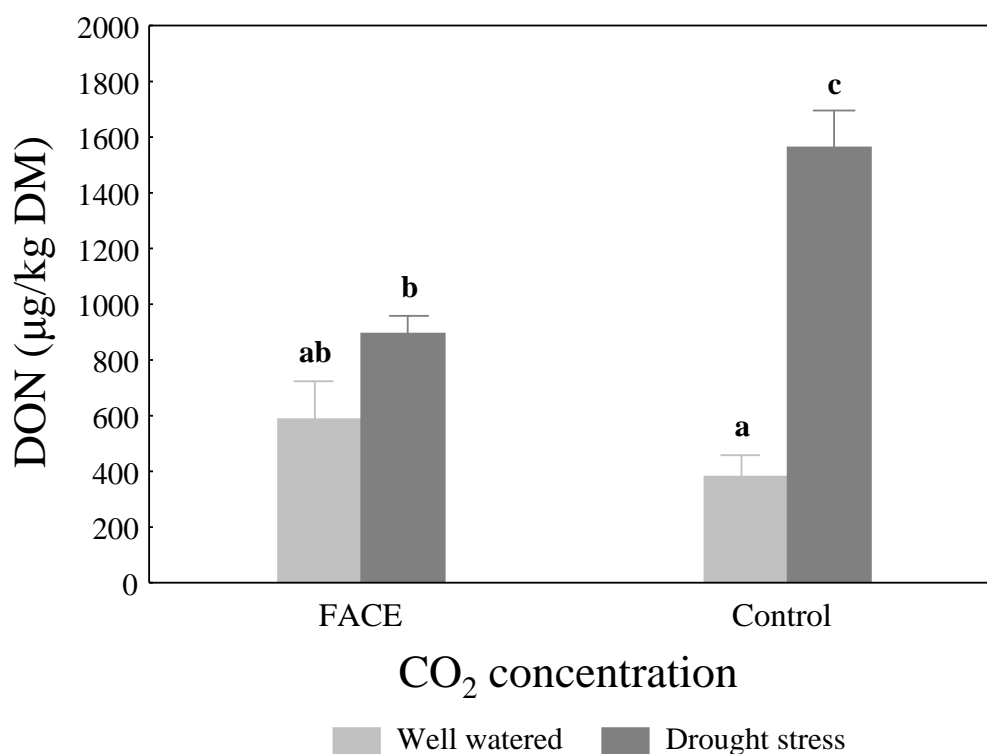


Figure 7: Interaction of atmospheric CO₂ concentration and irrigation during plant growth on the concentration of DON in whole plant maize silages (Means ± SE)

During feeding of the ensiled maize plants, the metabolization of DON in the rumen into the less toxic metabolite de-epoxy-DON was almost completely as it generally exceeded 90 % and only negligible concentrations of DON were detected in the investigated samples of ovine plasma (**Paper II**). These observations were generally in agreement with experimental results reported for ruminants in several former studies where the ruminal metabolization of DON to de-epoxy-DON was demonstrated to be nearly complete (Seeling et al., 2006; Fink-Gremmels, 2008; Keese et al., 2008). In the present investigation, the plasma concentration of de-epoxy-DON strongly depended on dietary DON intake in relation to bodyweight. However, the varying concentrations of DON due to the assessed CO₂ and irrigation treatments during plant growth were not reflected in corresponding concentrations of DON in

plasma. It can be concluded that exposing the animals to warm or hot environmental conditions did not impair the known detoxification potential of the rumen (Fink-Gremmels, 2008) as neither the concentration of DON in the assessed plasma samples nor the metabolization of DON into de-epoxy-DON were affected by the climatic conditions. Hence the present results did not give indications for negative interactive effects of increasing future ambient temperatures during feeding and the metabolization of dietary DON that may vary due to a potentially altered contamination of maize exposed to drought and increased atmospheric carbon dioxide. Accordingly, the differing concentrations of silage DON were not reflected in corresponding effects of atmospheric CO₂ and drought during maize cultivation on nutrient or OM digestibility of the produced silages (**Paper I**) or body temperature, respiration rate, water usage and blood parameters of sheep as presented in **Paper II**.

2 Heat stress and nutritional alleviation

2.1 Dissipation of body heat

If homoiotherm livestock species are kept within the thermoneutral zone, heat production is minimal at normal and relatively constant core body temperatures and rectal temperatures may serve as a practical indicator of core body temperatures (Marai et al., 2007). The UCT is referred to as the ambient temperature that induces the need to dissipate excess body heat due to rises of core body temperature and the UCT may therefore indicate the onset of heat stress (Kadzere, et al., 2002). In the current experiment, the exposure of castrated male sheep to warm environmental conditions caused the rectal temperatures to rise reaching approximately 38.8 °C at mild heat and about 39.2 °C at severe heat treatment in comparison to the initial mean of 38.4 °C during thermoneutral conditions (**Paper II**). According to the reference values for adult sheep (38.4 to 40.0 °C) given by Hörnicke (1987), the increases were within a physiological range that should theoretically not indicate the presence of noxious heat stress. In contrast, the rises of skin temperatures and respiration rates due to an increasing thermal load were considerably more pronounced as shown in **Figure 8**. That is in accordance with the results reported in **Paper IV** because the thermal environment was closer related to skin temperatures and respiration rates ($r= 0.62$ to 0.78) than to rectal temperatures ($r=0.43$) of the investigated dairy cows. The mammalian skin is a central pathway for the exchange of heat between animal and environment and the observed rises in skin temperatures are likely to be the result of adjustments of skin blood flow in order to eliminate excess body heat via

conductive and convective mechanisms (Silanikove, 2000). The evaporative dissipation of heat is generally more efficient in hot and dry than in wet climates. In the course of the balance experiment, RH was generally below 50 % and decreased with higher ambient temperatures (**Paper I and II**). Therefore RH should not have limited respiratory heat loss substantially. The potential elimination of body heat via sweating is restricted in unshorn sheep due to the presence of a wool coat and hence the importance of the respiratory dissipation of heat increases with rising ambient temperatures and a decreasing temperature gradient between animal and environment, respectively. Respiratory heat loss accounts for up to 60 % if sheep are kept at 35 °C (Marai et al., 2007). This process was reflected in the current study because the total rises in breaths per minute between the exposure of sheep to ambient temperatures of 15 and 25 °C were found to be approximately 35 but the rises between ambient temperatures of 25 and 35 °C exceeded 50 breaths per minute (**Paper II**).

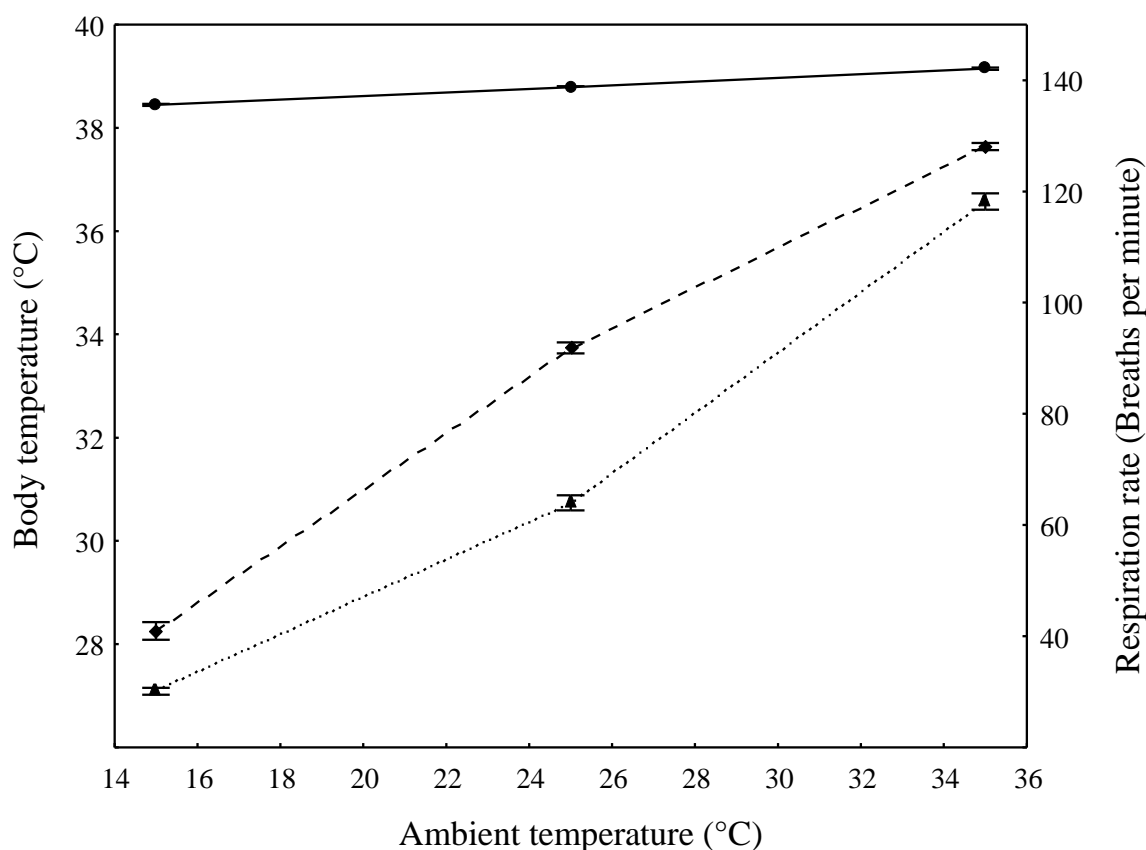


Figure 8: Effects of the thermal environment on rectal temperatures (°C; —; ●), skin temperatures (°C; - - -; ■) and respiration rates (Breaths per minute; ···; ▲) of sheep (Based on data published in **Paper II**. Means ± SE): Values were pooled for CO₂ and irrigation treatments during growth of ensiled maize fed.

2.2 Concentrate

The processes that are associated with maintenance, digestion, metabolism and activity generate heat and it was reported that the loss of heat of a 600 kg cow yielding 40 kg of 4 % fat milk accounted for approximately 31 % of the total energy consumption (Coppock, 1985; West, 1999). Though the production of heat is advantageous during cold environmental conditions and enables the animal to maintain core body temperatures, the dissipation of such large amounts of heat is a physiological burden during warm and hot periods. Increasing the proportion of concentrate in diets fed to heat stressed animals provides two advantages. First, reductions in voluntary feed intake and milk yield as summarized in **Table 2** can be compensated to a certain extent by increases in the content of dietary energy and nutrients (Beede and Collier, 1986). Secondly, feeding high concentrate at the expense of roughage ingredients was reported to reduce the heat production associated with feed consumption (Coppock, 1985; Orskov and Macleod, 1990; Reynolds et al., 1991). These findings were in accordance with the results reported in **Paper IV** as increased proportions of dietary concentrate (60 % in comparison to 30 %, referring to DM) in rations of heat stressed primiparous dairy cows were accompanied by reductions in skin temperatures in the range of 0.1 to 0.4 °C and tended to decrease rectal temperatures by 0.1 to 0.2 °C. West (1999) suggested that the heat increment of high fibre diets exceeds the heat increment of rations high in concentrate because the metabolism of acetate is related to a higher heat production than the metabolism of propionate. This may be due to unequal abilities to use excess acetate. The larger amounts of propionate associated with feeding high concentrate may provide sufficient reduction equivalents to enable acetate to be converted into body fat but high fibre rations lead to a higher production of acetate but lower propionate formation and excess metabolic acetate has to be eliminated as heat (MacRae and Lobley, 1982).

Besides economic factors, the applicability of high concentrate rations in ruminant feeding is primarily restricted for two reasons. Elevations in the production of SCFA may induce SARA and though the supplementation of concentrate usually increases the voluntary feed intake of dairy cows, very low forage to concentrate ratios may depress feed consumption (Friggens et al., 1998; O'Mara et al., 1998; Andersen et al., 2003; Krause and Oetzel, 2006). Prolonged periods of low ruminal pH may be useful indicators of SARA as according to Gozho et al. (2005) the onset of SARA may be indentified by ruminal pH below 5.6 for at least 3 hours per day. In the present experiment, ruminal pH was found to be lower than 5.6 for more than 3 hours in the cows fed on 30 % concentrate and for more than 5 hours in the animals fed on 60 % concentrate (**Paper IV**). These values were likely to be relatively reliable and should at

least not overestimate ruminal pH as they were obtained after correcting for the observed time-dependent positive drift of pH-sensors (**Paper IV**). Moreover, performing a Bland-Altman comparison to manual pH measurement in rumen fluid *in vitro* revealed that the boluses tended to overestimate low rumen pH below 6.0 indicating that the actual pH may have been even lower (**Paper III**). However, the animals did not show enhanced clinical signs of SARA such as diarrhea, loss of body condition, depressions in voluntary feed intake or laminitis and both DMI and milk yield were within a normal magnitude for primiparous Holstein cows (Nocek, 1997; Kleen et al., 2003; Le Cozler et al., 2009; Janovick and Drackley, 2010). These observations suggested that the exclusive interpretation of ruminal pH may possibly lead to an insufficient detection of SARA and that clinical examinations imperatively have to be integrated in an expedient diagnosis. Within this context, the usefulness of pH values measured by means of spot sampling techniques such as rumenocentesis or the application of oro-ruminal probes has to be strongly questioned. As shown in **Figure 6** and in **Paper IV** ruminal pH is subjected to a considerable short term variation and this variability was visually apparent even after aggregating ruminal pH values of individual animals to means per hour (**Figure 9**). Furthermore, ruminal pH showed a substantial postprandial decline irrespective of dietary concentrate percentage (**Paper III**). Therefore the interpretation of results obtained by spot sampling techniques requires precise information about recent intakes of water and amount and type of feed as considerable diet effects on ruminal pH were verified (**Paper III-IV**).

An elevation of the proportion of dietary concentrate can reduce heat production and body temperatures of heat stressed dairy cows and increase the energy density of rations in periods of heat-induced decreases of DMI and milk performance. Thus higher percentages of concentrate may represent a useful nutritional measure to reduce the negative impacts of heat on the productivity of dairy cows. However, the applicable percentage of concentrate is limited due to inherent physiological restrictions.

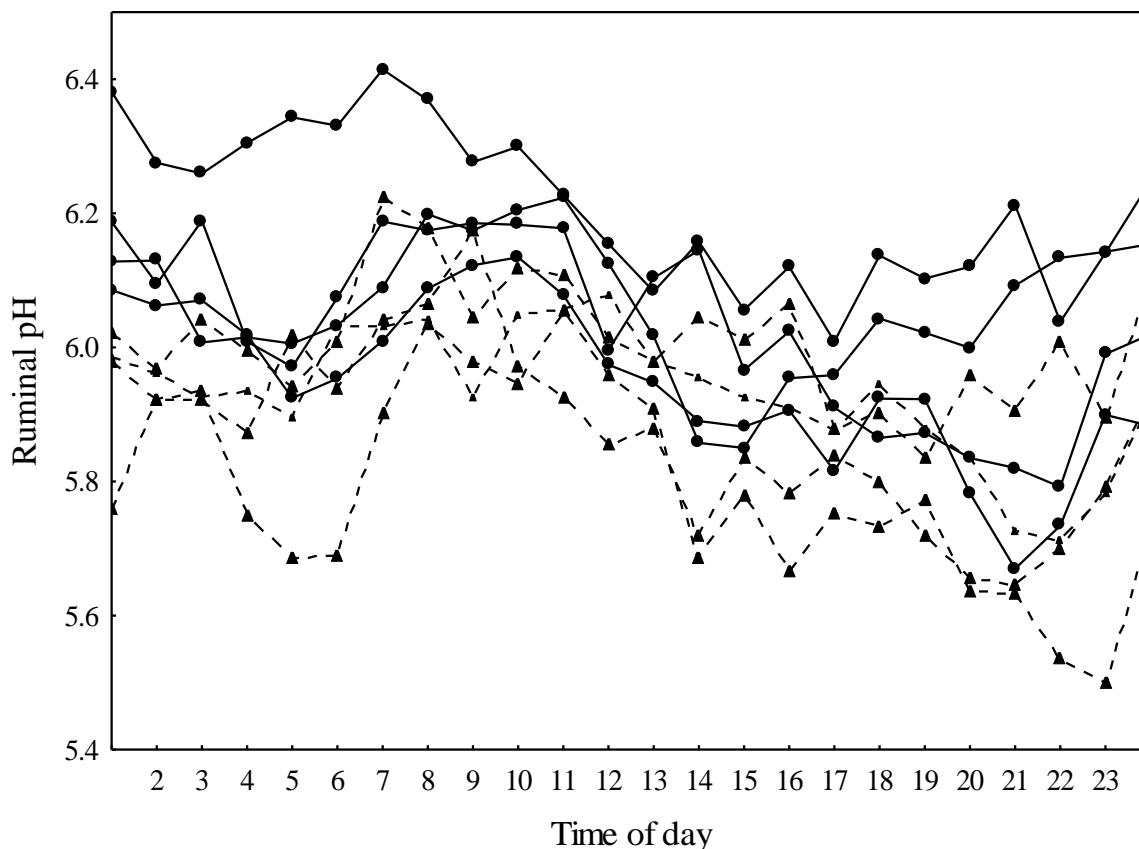


Figure 9: Effects of dietary concentrate proportion on individual diurnal variation in ruminal pH of primiparous dairy cows (Based on data published in **Paper IV**. Values are presented as means per hour. ●, solid line, 30 % concentrate, 70 % roughage composed of 60 % maize silage and 40 % grass silage; ▲, dotted line, 60 % concentrate, 40 % roughage composed of 60 % maize silage and 40 % grass silage).

2.3 Niacin

In humans, the therapeutic use of niacin is well known to induce flushing, a strong cutaneous vasodilatation (Benyo et al., 2005; Kamanna et al., 2009). It was hypothesized that in dairy cows this vasodilative effect may be used in a beneficial manner as the supplementation of niacin could improve the dissipation of excess body heat via an increased skin blood flow.

Two pilot studies demonstrated that additional niacin can reduce skin and rectal temperatures of heat stressed lactating Holstein cows but the results were contradictory as such effects were not observed consistently throughout the tested dietary and environmental treatments and higher doses of niacin were not necessarily accompanied by respective reductions of body temperatures (DiCostanzo et al., 1997; Zimelman et al., 2010). In the current investigation, the mean daily supplementation of 17.9 g and 20.5 g niacin to diets of primiparous dairy cows did not influence ruminal, skin, subcutaneous and rectal temperatures during periods of $\text{THI} \geq 68$ indicating the presence of heat stress (**Paper IV**). This lack of niacin effects on the investigated thermoregulatory parameters was likely not due to a too low amount of

supplemented niacin because Di Costanzo et al. (1997) reported that the daily addition of 12 and 24 g niacin per animal partially induced reductions in skin temperatures during mild to severe heat stress but supplementing 36 g niacin in a period predominantly characterized by thermo-neutral conditions did not affect rectal or skin temperatures and respiration rates.

The reason for the discrepancies between the results obtained in the present experiment and the findings published by Di Costanzo et al. (1997) and Zimbelman et al. (2010) needs to be clarified. Dissimilarity in the experimental feeding schemes and in the time of body temperature measurement after the intake of supplemental niacin may have generated unequal concentrations of niacin in blood. This parameter generally varies in a relatively wide range, probably due to differences in the analyzed blood fractions and difficulties in vitamin analysis (Niehoff et al., 2009a). The ruminal disappearance of niacin was reported to exceed 98 % (Santschi et al., 2005a). However, no absorption should take place in the rumen and most of the niacin is incorporated into the bacterial fraction (Erickson et al., 1991; Santschi et al., 2005b). Dietary addition was reported to increase the amount of niacin reaching the duodenum and the duodenal absorption of niacin was found to be approximately 80 % (Riddell et al., 1985; Zinn et al., 1987; Santschi et al., 2005a). Accordingly, Campbell et al. (1994) reported postprandial increases in the concentration of nicotinic acid in duodenal fluid 1 to 6 hours after feed consumption with the peak after 2 hours feeding 12 g niacin per day in two equal portions. Rises in blood nicotinamide within similar postprandial time spans were described by Cervantes et al. (1996) and Niehoff et al. (2009b).

In the study of Di Costanzo et al. (1997), the cows were fed a TMR with or without supplemental niacin twice daily at 8.00 h and at 15.00 h, respectively. Body temperature measurements were initiated directly after both feeding events suggesting that, though not determined, blood niacin was likely elevated during both periods of temperature detection. In the present experiment, the supplemented niacin was integrated in the concentrate which was offered separately via computerized feeding stations and the amount of individually available concentrate was adjusted in order to meet predefined dietary forage to concentrate ratios (**Paper IV**). Therefore the intake of concentrate was theoretically possible throughout the main part of the day and it seems questionable that relatively large amounts of dietary niacin were consistently consumed shortly before the initiation of body temperature determination at 8.00 h or 16.00 h, respectively, because the cows were fixed for temperature detection directly after milking. However, once serum nicotinamide was determined at 8.00 h and the supplementation of niacin was shown to induce significant increases of the vitamer in serum as shown in **Figure 10**. These rises may have been too low to manifest in vasodilatative

responses that triggered reductions of body temperatures as Zimbelman et al. (2010) reported that the observed niacin effects on rectal and vaginal temperatures were related to distinctly higher concentrations of total plasma niacin in the range of 1.3 to 1.8 $\mu\text{g/ml}$.

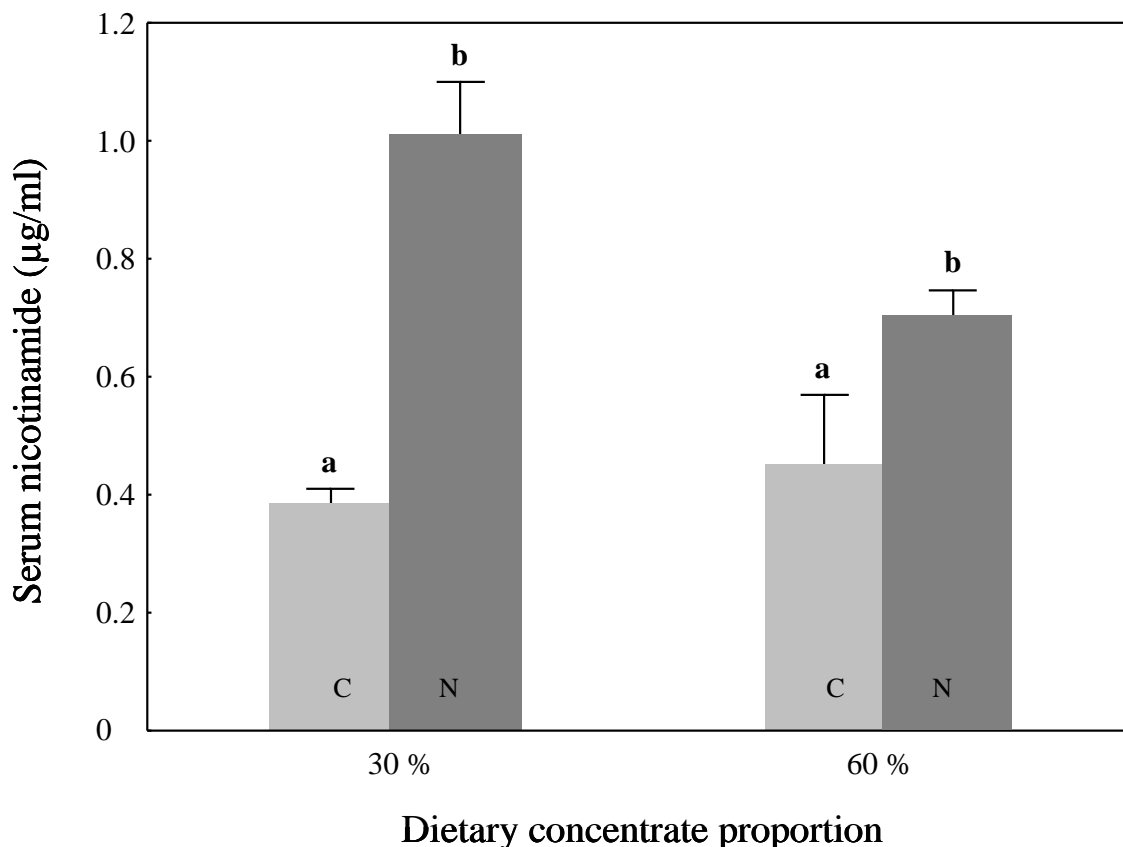


Figure 10: Effects of dietary concentrate proportion (30 % vs. 60 %, referring to DM) and niacin supplementation (N, niacin; C, control) on the concentration of nicotinamide in serum of primiparous dairy cows (Unpublished data based on **Paper IV**. Means \pm SE). Nicotinamide in serum was determined via HPLC as described by Niehoff et al. (2009b). Statistical evaluation was performed according to model 3 as described in **Paper IV** exclusive the covariates

It needs to be elucidated whether single consumptions of high doses of niacin prior to the determination of body temperatures may be associated with stronger vasodilatative responses than observed in the present experiment that could generate alterations in body temperatures of heat stressed dairy cows. However, the practical use of such single administrations to reduce the negative impact of heat stress is likely to be limited if a potential amelioration only takes place within restricted time spans due to postprandial peaks in blood niacin as reported previously (Campbell et al., 1994). Moreover, limiting the intake of supplemental niacin to a few predefined portions per day could lead to a reduction of the positive effects of niacin on milk yield and milk protein production as described in **Paper IV**. The continuous intake of

niacin throughout the day via concentrate feeding stations may have been one reason for the relatively high stimulation of milk, FCM, protein and lactose yield in the present experiment that partially exceeded the effects observed in past literature if niacin was fed once or twice daily (MadisonAnderson et al., 1997; Niehoff et al., 2009b).

The lack of thermoregulatory niacin effects in the present experiment and the inconsistent results reported in previous studies suggested that niacin could potentially contribute to an alleviation of heat stress in dairy cows but the relief is likely to be limited to certain environmental and nutritional preconditions. More information about optimal dose and time of supplementation is strongly required before the administration of niacin as a tool for the alleviation of heat stress can be recommended.

3 Continuous measurement of ruminal pH and temperature

The assessment of the devices constructed for continuous intraruminal pH, temperature and pressure measurement primarily focused on ruminal pH and was performed in order to evaluate the presence of three main confounders that could impair pH measurement: The accuracy of the pH sensors indicated by the relationship to traditional manual pH determination (1), the occurrence of pH sensor drift (2) and impacts of the localization of measurement in the rumen (3).

First, the linear relationship of $r=0.59$ between bolus and manual pH measurement and the considerable variation around the regression line represented a rather moderate correlation (**Paper III**). However, this relationship indicated a better agreement compared to Kaur et al. (2010) who reported a less close correlation of $r=0.46$. In contrast, previous experiments demonstrated distinctly closer positive correlations between manual and automated intraruminal pH determination (Penner et al., 2006; Phillips et al., 2010). However, the differences in pH in the present experiment were not clearly attributable to one method and may thus have been due to an insufficient accuracy of manual pH determination as well. Performing a Bland-Altman comparison revealed bias effects with a tendency of pH overestimation of low ruminal pH below 6.0 but a pH underestimation of rumen pH higher than 6.5. Both observations may limit the validity of bolus pH measurement and in this context the relatively long time spent below specific thresholds such as pH 5.6 in dependence on dietary concentrate proportion as reported in **Paper IV** may actually have been longer. In particular the overestimation of rather low ruminal pH below 6.0 in the critical range for SARA detection may restrict the usefulness of bolus pH measurement in detecting ruminal

acidosis in cattle and imperatively has to be considered in the interpretation of such results gained by the evaluated boluses.

Secondly, pH sensor drift was found to be undirected in the first 8 days of *in vivo* measurement after calibration (**Paper III**) but prolonged investigation showed the occurrence of a time-dependent positive drift that can be corrected for by the linear rate of increase (**Paper IV**). In contrast, Kaur et al. (2010) reported an earlier onset of positive pH sensor drift after 48 hours followed by a relatively constant subsequent increase. These observations and the need to correct for pH drift obviously complicate the practical applicability of the evaluated boluses for the detection of ruminal pH and SARA.

Thirdly, as shown in **Paper III**, the site of measurement in the rumen does not necessarily impact mean ruminal pH. Nevertheless, the emergence of pH gradients within the rumen were partially reported in previous studies and unfixed boluses which move freely within the reticulorumen could be temporarily transferred into the reticulum where the ingesta can have higher pH than in the rumen due to greater saliva and water contamination (Duffield et al., 2004; Li et al., 2009). Therefore the interpretation of single values can only give limited indications for the presence of SARA and the main advantage of the evaluated boluses, the continuity of measurement, should be used especially in SARA detection to create mean values which integrate a larger amount of data to minimize the potential falsification.

The successful application of the technique required the consideration of specific constraints that could affect reliability and durability of measurement. For example, the operation of boluses, software, receiver and trigger device was relatively uncomplicated and served to generate, download and export data for further analysis but its usage needed a specific introduction (**Paper III**). Moreover, the calibration of the pH sensors required standard buffer solutions and a water bath to ensure constant temperatures and these prerequisites are likely to limit the applicability of the complete technique to research institutions or larger farms and companies with respective resources and knowledge. The trigger had to be used in direct proximity to the animals and therefore its use is restricted if the cows are able to move in free stall barns. Initiating the transfer of recorded data from boluses that are used *in vivo* may result in data loss if a larger quantity of cows is fixed in direct vicinity because accidentally triggering more than one bolus was observed to possibly cause inconsistent data transmission. Furthermore, the trigger used a frequency of 134.2 KHz that is prevalently applied in radiofrequency identification (RFID) technology for farm purposes like the record of estrus, milk performance and feeding behaviour (Erasmus and Jansen, 1999; Samad et al., 2010). If the animals pass a signal with a similar frequency in the milking parlor or feeding stations,

unintended data transmission may be triggered and data loss can occur. However, losing data can be prevented by a specific setting of the software that only enables the trigger download within predefined intervals.

The evaluated bolus technology represents a promising tool for the ongoing determination of ruminal pH and temperature in cattle, may serve as a diagnostic method and can contribute to an improved understanding of ruminal fermentation processes in particular due to the option of continuous measurement as shown in **Figure 6** and **Figure 9**. However, indications for a limited accuracy of pH measurement and time dependent pH sensor drift were found and indicated the need of further improvement. The emergence of pH-gradients within the reticulorumen should be taken into consideration and a detailed introduction and training prior to the application *in vivo* is strongly recommended.

CONCLUSIONS

The future feed value of maize silage is likely to remain relatively unaffected if the growing plants are exposed to restricted levels of drought and elevated atmospheric CO₂ concentrations of 550 ppm as expected to arise within the 21st century. As the quality of various C₃ crops was found to be considerably impaired by rising CO₂, the absence of such effects on the C₄ plant maize may be considered a positive aspect.

The concentration of DON in ensiled maize may increase in periods of restricted water availability during plant cultivation but such rises could be reduced almost to base level by enriched atmospheric CO₂.

Both the evaluated increased atmospheric CO₂ concentration and drought during maize growth will likely not influence thermoregulatory and blood parameters of sheep fed the generated silages. Heat exposure will probably not impair the almost complete ruminal metabolization of varying levels of dietary DON into the less toxic metabolite de-epoxy-DON.

High amounts of dietary concentrate can reduce body temperatures of heat stressed dairy cows and may therefore contribute to an amelioration of the known negative impacts of heat on lactating cattle. Increasing the proportion of concentrate in dairy cow diets to 60 % can stimulate milk and protein yield but may reduce milk fat and protein percentages leading to a lack of effects on FCM yield in comparison to a 30 % concentrate ration. However, the applicability of high concentrate diets is limited as they induce substantial decreases of ruminal pH indicating the risk of SARA development.

In contrast to previous experiments, it was shown that the supplementation of relatively high doses of niacin, a feed additive potentially able to alter skin blood flow, does not necessarily influence skin, subcutaneous or rectal temperatures of heat stressed lactating dairy cows. As a consequence, the capability of niacin to facilitate the dissipation of excess body heat via an increased skin vasodilatation is likely to be limited to restricted postprandial time intervals. However, supplemental niacin can stimulate milk, FCM, protein and lactose yields of primiparous dairy cows, though milk fat, protein and lactose percent are either decreased or not affected.

The investigated wireless boluses designed for *in vivo* application in bovines represent a promising technology for an ongoing determination of ruminal pH and temperature in cattle and can serve to improve the knowledge about the patterns of ruminal fermentation and to indicate SARA in particular due the option of continuous intraruminal measurement. However, a limited accuracy of the pH sensor, the occurrence of time dependent positive pH drift and possibly emerging pH-gradients within the reticulorumen may falsify the obtained results and imperatively have to be considered in their interpretation.

SUMMARY

Considerable and lasting climatic changes were projected to occur within the next decades. Some of the probably most important alterations in relation to agriculture are increases in atmospheric CO₂ concentrations and global surface temperatures as well as modifications of precipitation patterns that are likely to induce a higher incidence of summer drought in middle Europe.

Elevated atmospheric CO₂ concentrations are known to stimulate photosynthesis and yields of C₃ crops but were reported to substantially reduce their feed quality not only by altered proportions of protein. C₄ photosynthesis should theoretically be saturated at current CO₂ but the actual responses of feed value and mycotoxin contamination of C₄ plants such as maize are unknown. Uncertainty exists whether interactions with drought during cultivation and increased ambient temperatures during feeding of the generated maize silages are to be expected.

Rising ambient temperatures are likely to increase incidence and severity of heat stress that is well known to impair health and productivity of livestock. In ruminants, potential dietary countermeasures against heat stress may be elevations in the proportion of concentrate and the supplementation of niacin which is known to have vasodilatative properties and was described to reduce body temperatures of heat stressed dairy cows. However, the applicability of high concentrate diets is limited as they were reported to reduce ruminal pH and to induce SARA. Innovative devices (boli) for a continuous measurement of ruminal pH and temperature may contribute to the diagnosis of ruminal acidosis.

Hence, the effects of an increased atmospheric CO₂ concentration and drought during maize growth on feed value, mycotoxin contamination and digestibility of maize silage were to be investigated in consideration of the climatic conditions during feeding. Moreover, the implications on thermoregulatory and blood parameters of sheep and the ruminal metabolization of DON should be assessed. The impact of supplemental niacin and high dietary concentrate on thermoregulation and milk performance of heat stressed dairy cows were to be tested. New devices for intraruminal pH and temperature measurement were aimed to be evaluated considering different localizations of measurement in the rumen and varying proportions of dietary concentrate.

For these purposes, a FACE (Free Air Carbon dioxide Enrichment) facility was operated in a maize field to produce an enriched CO₂ concentration of 550 ppm. Drought was induced by the exclusion of precipitation in one half of all experimental plots. Whole plants were

harvested, ensiled and analyzed for their contents of nutrients and chosen mycotoxins. In a balance experiment on sheep, nutrient digestibilities, blood and thermoregulatory parameters and the concentration of DON and de-epoxy-DON in plasma were determined for three climatic treatments (temperate; mild heat; severe heat).

Moreover, four non-lactating rumen fistulated cows were fed on 0 and 40 % concentrate, respectively. One bolus was inserted each in dorsal and ventral rumen sac to continuously measure ruminal pH and temperature. For method comparison, postprandial manual temperature measurement and removal of rumen fluid for manual pH determination were conducted in direct proximity to the boluses in preset short term intervals.

In addition, 20 primiparous cows were allocated to four dietary treatments and were aimed to receive either 0 or 24 g niacin and 30 % or 60 % concentrate. The effects on body temperatures and respiration rates were investigated during periods likely to induce heat stress. Milk production as well as ruminal pH and temperature were assessed. In a concomitant experiment, the rumen boluses were tested for the occurrence of pH sensor drift using four rumen fistulated cows.

Elevated atmospheric CO₂ and drought during maize growth did not alter the crude nutrient content of silage DM or nutrient digestibilities indicating a mainly unaffected future feed value of ensiled whole maize plants. However, drought induced significant increases in the concentration of DON summing up to more than 1500 µg/ kg DM. These drought-associated rises were reduced almost to base level by growing the plants in a high CO₂ atmosphere, an observation that may have been due to a reduced plant water loss leading to decreases in the susceptibility to fungal infection. The climatic conditions during feeding had the most pronounced effects on nutrient digestibility and the lowest digestibilities of ADF, NDF and, by trend, CF, were observed during the coldest climatic treatment. This may have been caused by increases in the retention time of ingesta in the reticulorumen during warm periods. The investigated blood parameters, body temperatures and respiration rates of sheep remained mainly unaffected by elevated atmospheric carbon dioxide and drought during the cultivation of the silages fed. Heat exposure induced increases in thermoregulatory activity within the physiological limits of sheep. The ruminal metabolization of varying concentrations of dietary DON into the less toxic metabolite de-epoxy-DON was found to be higher than 90 % and was not impaired by exposing the animals to hot environments. Accordingly, the concentrations of DON in ovine plasma were predominantly lower than 1 ng/ml.

The performed Bland-Altman comparison and the linear relationship between bolus and manual pH measurement ($r=0.59$) indicated moderate agreement between both methods of pH

determination, a fact that may as well be due to deficient accuracy of manual pH detection. A bias effect of bolus pH measurement with an overestimation of ruminal pH below 6.0 and an underestimation of ruminal pH higher than 6.5 was observed. In addition, the occurrence of a time-dependent positive pH sensor drift indicated the need of further improvement.

Rising proportions of dietary concentrate decreased ruminal pH values but increased ruminal temperatures. The localization of measurement did not affect ruminal pH but ruminal temperature. Feeding high concentrate diets may contribute to an amelioration of the negative effects of heat as they reduced skin temperatures of heat stressed dairy cows by 0.2 to 0.3 °C indicating a reduced dietary heat increment. The time spent with a ruminal pH below 5.6 was significantly increased to more than 5 hours per day by feeding high concentrate leading to a rising risk of SARA. Elevations in the proportion of dietary concentrate to 60 % stimulated milk and protein yield but reduced milk fat and protein percent resulting in an unaffected FCM yield in comparison to a 30 % concentrate diet. The potential of additional niacin to alleviate heat stress via increased skin vasodilatation is likely to be limited as the supplementation did not affect body temperatures during heat exposure. Milk, FCM, protein and lactose yields were elevated by additional niacin, though milk fat, protein and lactose percent were either decreased or not affected.

It can be concluded that the future feed value of maize silage will likely remain mainly unaffected by elevated atmospheric CO₂ concentrations of 550 ppm and drought during plant growth. However, the concentration of DON may increase in periods of drought but can be reduced by enriched atmospheric carbon dioxide. In sheep, the almost complete ruminal metabolization of different levels of dietary DON into de-epoxy-DON should not be impaired by hot climatic conditions during feeding. High concentrate diets could contribute to an alleviation of heat stress and can induce beneficial effects on milk production. However, the applicability of high concentrate rations is limited by considerable decreases in ruminal pH that can be detected by promising ruminal boluses. The potential of supplemental niacin to reduce the impacts of heat on dairy cows is limited. However, the addition of niacin can considerably stimulate milk performance.

ZUSAMMENFASSUNG

Aktuelle Prognosen weisen auf drastische und langfristig irreversible Klimaveränderungen in den nächsten Jahrzehnten hin. Steigende atmosphärische CO₂-Konzentrationen und Umgebungstemperaturen sowie veränderte Niederschlagsmuster mit erhöhter Sommertrockenheit stellen die bedeutsamsten Transformationen mit unmittelbarem Bezug zur Landwirtschaft dar.

Erhöhte atmosphärische CO₂-Gehalte stimulieren Photosynthese und Erträge von C₃-Pflanzen, diese Effekte gehen allerdings mit erheblichen Qualitätsminderungen und reduzierten Proteingehalten einher. Die Photosynthese von C₄-Pflanzen wie Mais könnte bereits bei aktuellen CO₂-Konzentrationen gesättigt sein. Allerdings fehlen die experimentellen Grundlagen zur Einschätzung der tatsächlichen Auswirkungen steigender atmosphärischer CO₂-Gehalte auf den Futterwert von Maissilage sowie möglicher Interaktionen mit vermehrt auftretender Sommertrockenheit und den Effekten höherer Umgebungstemperaturen während der Fütterung.

Ansteigende Umgebungstemperaturen lassen auf erhöhte Inzidenz und Intensität von Hitzestress einschließlich der bekannten negativen Folgen für Gesundheit und Leistung landwirtschaftlicher Nutztiere schließen. Bei Wiederkäuern werden höhere Konzentratanteile in der Ration und die Supplementierung von Niacin unter Nutzung seiner vasodilatativen Eigenschaften als mögliche Gegenmaßnahmen im Bereich der Fütterung diskutiert. Entsprechend wurden bei hitzestressen Milchkuhen durch die Gabe von Niacin Reduktionen der Körpertemperaturen beobachtet. Die Nutzung hoher Konzentratanteile unterliegt jedoch aufgrund von steigendem Pansenazidoserisiko physiologischen Restriktionen. Innovative Messgeräte (Boli) zur kontinuierlichen Messung von pH-Wert und Temperatur im Pansen könnten durch die Erfassung beider Parameter zu Diagnose und Verständnis von subakuter Pansenazidose beitragen.

Die Ziele der vorliegenden Arbeit umfassten die Evaluierung der Auswirkungen von erhöhten atmosphärischen CO₂-Konzentrationen und Trockenheit während des Anbaues von Mais auf den Futterwert der erstellten Silagen, die Kontamination mit ausgewählten Mykotoxinen und die Nährstoffverdaulichkeit und Metabolisierung von DON bei steigenden Umgebungstemperaturen während der Fütterung. Die Supplementierung von Niacin und erhöhte Konzentratanteile in der Ration sollten hinsichtlich ihrer Effekte auf Thermoregulation und Milchleistung von Milchkuhen bewertet werden. Dies erfolgte unter Anwendung innovativer Boli zur Messung von pH-Wert und Temperatur im Pansen.

In einem Maisfeld wurde durch CO₂-Expositionstechnik (FACE) in definierten Feldabschnitten eine CO₂-Konzentration von 550 ppm generiert. In einer Hälfte jeder Versuchsstelle wurde Niederschlag ausgeschlossen. Maisganzpflanzen wurden geerntet, einsiliert und ihre Nährstoff- und Mykotoxingehalte analysiert. In einem Bilanzversuch an Schafen wurden die Nährstoffverdaulichkeiten der Maissilagen bei unterschiedlicher Wärmeexposition der Tiere sowie Differenzialblutbild, thermoregulatorische Parameter und die Metabolisierung von DON in de-epoxy-DON untersucht.

Zusätzlich wurden vier am Pansen fistulierte Milchkühe mit Rationen verschiedener Konzentratanteile gefüttert. In dorsalen und ventralen Pansensack wurde je ein Bolus zur kontinuierlichen Messung von Pansen-pH und -temperatur eingebracht. Zur Durchführung eines Methodenvergleiches wurden im Vormagen in unmittelbarer Nähe zu den Boli an definierten postprandialen Zeitpunkten die Pansentemperaturen bestimmt und Panseninhalt zur manuellen pH-Messung entnommen.

Weiterhin wurden 20 erstlaktierende Milchkühe in vier Fütterungsgruppen mit einer täglichen Supplementierung von 0 oder 24 g Niacin sowie Konzentratanteilen von 30 % oder 60 % eingeteilt. Körpertemperaturen und Atemfrequenzen bei Hitzestress sowie Milchleistung, Pansen-pH und -temperatur wurden ermittelt. Die pH-Sensoren der genutzten Boli wurden auf die Entstehung zeitabhängiger Drift untersucht.

Erhöhte atmosphärische CO₂-Konzentration und Trockenheit hatten keinen Einfluss auf die Rohnährstoffgehalte der generierten Silagen und deren Nährstoffverdaulichkeiten, was auf einen unter diesen Bedingungen größtenteils unveränderten zukünftigen Futterwert von Maissilage schließen lässt. Wasserrestriktion war mit signifikant auf über 1500 µg/ kg Trockensubstanz steigenden DON Gehalten verbunden. Diese Konzentrationserhöhungen wurden allerdings durch die CO₂-Exposition annähernd auf Ausgangsniveau reduziert. Die geringsten Verdaulichkeiten von ADF, NDF und Rohfaser wurden bei den niedrigsten Umgebungstemperaturen beobachtet. Die simulierten zukünftigen Anbaubedingungen der verfütterten Maispflanzen hatten keinen Einfluss auf die untersuchten Blutparameter und Körpertemperaturen der Schafe, wohingegen Wärmeexposition signifikant erhöhte Körpertemperaturen und thermoregulatorische Aktivität induzierte. Die Metabolisierung von DON in de-epoxy-DON lag generell über 90 %, wurde nicht von Hitzestress beeinträchtigt und resultierte in niedrigen DON-Konzentrationen im Plasma von überwiegend unter 1 ng/ml.

Bland-Altman Vergleich und linearer Zusammenhang ($r=0.59$) zwischen Bolus und manueller pH-Messung wiesen auf eine mittlere Übereinstimmung beider pH-Messverfahren hin, wobei

auf Basis dieser Ergebnisse die mangelnde Kongruenz nicht präzise der mangelnden Genauigkeit einer Methode zugeordnet werden kann. Die pH-Bestimmung per Bolus war jedoch durch systematische Überschätzungen niedriger pH-Werte unter 6.0 sowie Überschätzungen hoher pH-Werte über 6.5 charakterisiert. Ein weiteres Indiz für die Notwendigkeit weiterer technischer Verbesserungen war das Auftreten zeitabhängiger positiver pH-Drift.

Steigende Konzentratanteile in der Ration induzierten signifikant reduzierte pH-Werte sowie erhöhte Temperaturen im Vormagen. Der Messort im Pansen hatte keinen Einfluss auf den pH-Wert, beeinträchtigte jedoch die Pansentemperatur. Konzentratreiche Fütterung kann zur Verringerung der negativen Effekte von Hitzestress auf Milchkühe beitragen, da hohe Kraftfutteranteile mit einer signifikanten Reduktion der Hauttemperaturen um 0.2 bis 0.3 °C einhergingen. Der Pansen-pH war bei Verfütterung von Rationen mit Konzentratanteilen von 60 % täglich für über 5 Stunden unter 5.6 reduziert, was auf ein steigendes Risiko der Entstehung von subakuter Pansenazidose hinweist. Konzentratanteile von 60 % stimulierten im Vergleich zu Rationen mit 30 % Kraftfutter bei reduzierten Milchfett- und Milchproteingehalten sowohl Milchleistung als auch Milchproteinmenge. Niacinsupplementierung hatte keinen Einfluss auf die Körpertemperaturen, war allerdings mit erhöhter Milchproduktion sowie steigender Milchprotein- und Laktosemenge verbunden.

Es kann geschlussfolgert werden, dass im Rahmen der durchgeführten Untersuchungen keine Hinweise auf zukünftige Veränderungen des Futterwertes von Maissilage durch Erhöhungen der atmosphärischen CO₂-Konzentration auf 550 ppm und Trockenheit während des Pflanzenwachstums festgestellt wurden. Mangelnde Wasserversorgung der Maispflanzen kann zu steigenden DON-Gehalten in den erstellten Silagen führen. Diese Kontamination kann jedoch durch erhöhte atmosphärische CO₂-Konzentrationen reduziert werden. Zukünftig vermehrt auftretende Wärmeexposition beeinträchtigt die beinahe vollständige Metabolisierung unterschiedlicher Mengen von DON im Pansen voraussichtlich nicht. Hohe Konzentratanteile in Milchkuhrationen können zur Milderung von Hitzestress beitragen und wirken sich positiv auf die Milchproduktion aus, unterliegen jedoch aufgrund von ruminalem pH-Depressionen physiologischen Restriktionen. Derartige pH-Reduktionen können durch innovative Boli unter Berücksichtigung etwaiger Störgrößen detektiert werden. Das Potential der Supplementierung von Niacin zur Verringerung von Hitzestress bei Milchkühen ist beschränkt, Niacinzulage kann jedoch zu deutlich stimulierter Milchleistung führen.

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich an Eides statt, die vorliegende Dissertation: “Methods for estimation and nutritional influencing of thermoregulation and heat stress in dairy cows and sheep as well as impacts of changing climatic conditions on the feed value of maize silage“ selbstständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel verfasst zu haben. Die Arbeit lag bisher in gleicher oder ähnlicher Form keiner Prüfungsbehörde vor.

Halle/Saale, den 24.08.2012

Malte Lohölter

DANKSAGUNG

Mein Dank gilt allen, die unmittelbar oder direkt zu dieser Arbeit beigetragen, mich nicht nur bei ihrer Erstellung beruflich und persönlich unterstützt haben und ohne deren Hilfe diese Untersuchungen nicht in dieser Form möglich gewesen wären:

Herrn Prof. Dr. Dr. Sven Dänicke für die Möglichkeit, die vorliegende Arbeit am Institut für Tierernährung in Braunschweig durchführen zu können sowie für die kompetente und stets freundliche Unterstützung und Begutachtung der Arbeit.

Ferner Herrn Dr. Ulrich Meyer für die wertvollen wissenschaftlichen Hilfestellungen, die engagierte Betreuung meiner Arbeit und die motivierende Anleitung.

Herrn Prof. Dr. Gerhard Flachowsky und Herrn Dr. Peter Lebzien für die Mitbetreuung der Arbeit und vielfältige fachliche Anregungen.

Den Mitarbeitern des Institutes für Tierernährung danke ich für die umfangreiche Unterstützung in allen relevanten Bereichen und die mir stets entgegengebrachte Hilfsbereitschaft.

Allen Doktoranden danke ich für die Hilfe bei der Versuchsdurchführung und die schönen gemeinsamen Aktivitäten.

Meiner Freundin Inga, meiner Familie und meinen Freunden danke ich für die wundervolle gemeinsame Zeit.