Institut für Tierwissenschaften

der Rheinischen Friedrich-Wilhelms-Universität zu Bonn

Silage maize variety and heat stress – effects on nutritive value of maize silage and on digestion events and performance of ruminants

Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

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Tag der mündlichen Prüfung: **3.11.2014**

Erscheinungsjahr: **2015**

für Martina

Silage maize variety and heat stress – effects on nutritive value of maize silage and on digestion events and performance of ruminants

The present thesis addresses two topics; brown-midrib (Bm) silage maize and heat stress in ruminants. The first part of this thesis focused on an experimental Bm silage maize hybrid with regard to dry matter (DM) intake (DMI), performance and digestibility in comparison to a commercial (Con) non-Bm hybrid. For the trials, 64 intact and 6 ruminally and duodenally cannulated German Holstein cows were used. They were fed rations based on silage of the Con or the Bm hybrid. The Bm maize silage-based diets had a lower DMI when fed as total mixed ration but no differences between Con and Bm were observed when silages were fed for ad libitum intake with restricted concentrate feeding. However, utilisation of energy was higher in animals fed Bm maize silage-based diets. Feeding Bm maize silage, furthermore, lowered milk fat content. Ruminal fermentation patterns, especially short chain fatty acid proportions, were not altered. Ruminal, as well as total tract apparent digestibility did not differ between Con and Bm, ruminal particle passage rate, however, was higher for the Bm diet. Microbial crude protein (MCP) synthesis and its efficiency were higher for Bm-fed animals. In part two of the thesis, the Bm and Con silages were tested in digestibility and nitrogen (N) balance trials with sheep. Trials were conducted at 15, 25 and 35°C ambient temperature. Ambient temperature did not affect nutrient digestibility but an interaction of temperature and silage maize variety was found. Results on N utilisation were inconsistent. In animals fed Con urinary N excretion was higher at 15°C compared to 25 and 35°C, whilst urinary N excretion was lower at 25°C compared to 15 and 35°C in animals fed Bm. Finally, the impact of summer temperatures in Lower Saxony, Germany on DMI and performance of mid-lactation dairy cows was evaluated. Feeding trials conducted at the experimental station of the Friedrich-Loeffler-Institute in Braunschweig, Germany between January 2010 and July 2012 were evaluated. Temperature-humidity indices (THI) were calculated for each day and a generalised linear mixed model was used to calculate the impact of THI on DMI, milk yield and milk composition. When THI increased above 60 DMI of the same day was decreased, whereat a decrease in milk yield was observed one day later. With a critical view on the literature on feeding strategies for heat-stressed dairy cows and in consideration of the present results, it can be concluded that Bm maize might reduce negative effects of heat stress. The higher efficiency of nutrient utilisation and the higher rate of ruminal passage of particles of Bm silage might help to maintain DMI during periods of heat stress. Increased efficiency of MCP synthesis may balance protein deficiency due to reduced DMI without the negative effects of increasing dietary crude protein concentration. Further research to characterise the interaction of heat stress and Bm feeding, however, is necessary, both under controlled and field conditions.

Silomaissorte und Hitzestress – Auswirkungen auf den Futterwert von Maissilage und Verdauungsvorgänge und Leistung von Wiederkäuern

Die vorliegende Arbeit befasst sich mit zwei Themen; Brown-Midrib (Bm) Silomais in der Wiederkäuerfütterung und Hitzestress bei Wiederkäuern. Im ersten Teil der Arbeit wurde eine Bm-Experimentalhybride im Hinblick auf Trockenmasseaufnahme (DMI), Leistung und Verdaulichkeit näher untersucht. Für die Versuche wurden 64 intakte Kühe sowie 6 am dorsalen Pansensack und am proximalen Duodenum fistulierte Kühe (Deutsche Holstein) mit einer Silage aus Bm-Mais oder einer Silage aus einer Kontrollvariante (Kon) gefüttert. Es zeigte sich, dass Bm-Mais nicht zu einer Steigerung der DMI, jedoch zu einer Verbesserung der Energieverwertung führte. Des Weiteren hat der Bm-Mais zu einer Reduzierung des Milchfettgehaltes geführt, wohingegen die Fermentation im Pansen, die scheinbare ruminale sowie die Gesamttrakt-Verdaulichkeit nicht beeinflusst wurden. Allerdings hatte die Silage aus Bm-Mais eine schnellere Partikelpassage durch den Pansen. Darüber hinaus waren die Menge an gebildetem mikrobiellem Rohprotein (CP) sowie die Effizienz der mikrobiellen CP-Synthese gesteigert, wenn Bm-Mais gefüttert wurde. Im zweiten Teil der Arbeit wurden die Bm- und Kon-Silagen im Verdaulichkeits- und Stickstoff (N) -Bilanzversuch an Hammeln getestet. Hierbei wurden mit jeder Silage Versuchsdurchgänge bei 15, 25 und 35 °C durchgeführt. Ein Einfluss der Umgebungstemperatur auf die Rohnährstoffverdaulichkeit konnte nicht nachgewiesen werden, allerdings wurden Interaktionen zwischen den Silagen und den Umgebungstemperaturen festgestellt. Die Ergebnisse der Untersuchung der N Verwertung waren uneinheitlich. Die N-Ausscheidung mit dem Harn war bei Tieren, die mit Kon-Silage gefüttert wurden, bei 15 °C höher als bei 25 und 35 °C, wohingegen die Ausscheidung von N mit dem Harn bei 25 °C niedriger war als bei 15 und 35 °C, wenn Bm-Silage gefüttert wurde. Schließlich wurden die Auswirkungen von Sommertemperaturen in Niedersachsen auf DMI und Milchleistung von Milchkühen im mittleren Laktationsdrittel untersucht. Zu diesem Zweck wurden Fütterungsversuche, die zwischen Januar 2010 und Juli 2012 auf der Versuchsstation des Friedrich-Loeffler-Institutes in Braunschweig durchgeführt wurden, ausgewertet. Ein Temperatur-Luftfeuchte-Index (THI) wurde für jeden Tag berechnet und mit einem generalisierten additiven gemischten Modell die Auswirkungen auf DMI, Milchleistung und Milchzusammensetzung untersucht. Die DMI verringerte sich wenn der THI über 60 stieg noch am selben Tag wohingegen eine verringerte Milchleistung mit einer Verzögerung von 24 h festgestellt wurde. Bei kritischer Betrachtung der Literatur zu Fütterungsstrategien für hitzegestresste Milchkühe und den Ergebnissen der vorliegenden Arbeit kann gefolgert werden, dass Bm-Mais das Potential hat, negative Auswirkungen von Hitzestress zum Teil aufzuwiegen. Die höhere Effizienz der Nährstoffverwertung und höhere ruminale Passageraten könnten einer Verringerung der DMI entgegenwirken. Die höhere Effizienz der mikrobiellen Proteinsynthese könnte darüber hinaus die geringere CP-Aufnahme durch verringerte DMI ausgleichen und gleichzeitig negative Effekte einer erhöhten CP-Konzentration vermeiden. Allerdings sind weitere Untersuchungen sowohl unter Labor- als auch unter Produktionsbedingungen nötig.

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Abbreviations

A	Undefined parameter of the ingesta flow model
a_i	Random effect of the individual animal ($_i$ = 1 to 8)
ADF	Acid detergent fibre
ADFom	Acid detergent fibre expressed exclusive of residual ash
ADL	Acid detergent lignin
aNDFom	Amylase pre-treated neutral detergent fibre expressed exclusive of residual ash
ARD	Apparent ruminal digestibility
B	Number of compartments of the ingesta flow model
BHB	Beta-Hydroxybutyrate
Bm	Brown-midrib
BW	Body weight
$BW^{0.75}$	Metabolic body size
C_3	Propionate
c_i	Random effect of the cow ($_i$ = 1 to 6)
CF	Crude fibre
CMRT	Compartment mean retention time
Con	Control
CP	Crude protein
CWC	Cell wall constituent
d_j	Fixed effect of the diet ($_j$ = 1 to 2)
d_0	Day of data collection
d_{-1}	One day prior to data collection
DCF	Digestible crude fibre
DEE	Digestible ether extract
DIM	Days in milk
DM	Dry matter
DMF	Dry matter flow
DMI	Dry matter intake
DMI_0	Dry matter intake at d_0
DMI_{-1}	Dry matter intake at d_1
DOM	Digestible organic matter
dt_{jl}	Interaction of diet and sampling time ($_j$ = 1 to 2, $_l$ = 1 to 7)
DWD	Deutscher Wetterdienst

E	Energy
e	Residual error
e	Base of the natural logarithm
ECP	Endogenous crude protein
EE	Ether extract
ESOM	Enzyme soluble organic matter
FCM	Fat-corrected milk
FLI	Friedrich-Loeffler-Institute
FOM	Fermented organic matter
FPR	Fat-to-protein ratio
G	Glucose
GP	Gas production
I	Insulin
IU	International unit
k	Fractional passage rate from Compartment 2
λ	Fractional passage rate from Compartment 1
L	Number of lactation
LAVES	Lower Saxony State Office for Consumer Protection and Food Safety
LSmeans	Least square means
µ	Overall mean
MCP	Microbial crude protein
MCPF	Microbial crude protein at the duodenum
ME	Metabolisable energy
N	Nitrogen
n	Number
NADP	Nicotinamide adenine dinucleotide phosphate
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fibre
NDFom	Neutral detergent fibre expressed exclusive of residual ash
NEFA	Non-esterified fatty acid
NEL	Net energy for lactation
NfE	Nitrogen-free extracts
NIR	Near-infrared reflectance spectroscopy
OM	Organic matter
ppm	Parts per million

q	Quotient of gross energy and metabolisable energy
R_0	Hours per day temperature was below 21°C at d_0
R_{-1}	Hours per day temperature was below 21°C at d_{-1}
RDP	Ruminally degraded protein
REML	Restricted maximum likelihood
RH	Relative humidity
RNB	Ruminal nitrogen balance
RUP	Ruminally undegraded protein
s_j	Fixed effect of silage maize variety ($_j$ = 1 to 2)
st_{jk}	Interaction of silage maize variety and ambient temperature ($_j$ = 1 to 2, $_k$ = 1 to 3)
SARA	Subacute ruminal acidosis
SCFA	Short chain fatty acids
SD	Standard deviation
SE	Standard error
SEM	Standard error of the means
SSE	Sum of squares error
T	Temperature
t_k	Fixed effect of ambient temperature ($_k$ = 1 to 3)
t_l	Fixed effect of sampling time ($_l$ = 1 to 7)
TD	Time delay between marker dose and first marker appearance
Tdb	Dry bulb temperature
Tdp	Dew point temperature
THI	Temperature-humidity index
THI_0	THI at d_0
THI_{-1}	THI at d_{-1}
TMR	Total mixed ration
TMRT	Pre-duodenal total mean retention time
TRT	Treatment
Twb	Wet bulb temperature
uCP	Utilisable crude protein
WI	Water intake
y_t	Marker concentration in duodenal chyme at time $_t$

Chapter 1 General introduction and review of literature

Introduction

The present thesis addresses two topics, brown-midrib (Bm) maize silage for ruminant feeding and heat stress in ruminants. These topics seem to be independent, but are closely linked with regard to climate change.

It is well known that climate largely affects lignification. Maize plants grown at increased temperature have an increased lignin content (Cone and Engels, 1990). Therefore, it can be concluded that climate change, which will lead to an increase in average temperature as well as intensity and frequency of heat waves (Meehl et al., 2007), will also increase lignification. Accumulation of lignified cell wall, however, decreases nutrient digestibility and, therefore, energy available to the animal. The Bm3 mutation of maize is known to reduce lignification and, therefore, has the potential to counteract negative effects of increased temperature on lignification.

It can, furthermore, be expected that due to increased intensity and frequency of heat waves heat stress in cattle will become a serious problem even in temperate climates. Silage prepared from Bm maize might be useful to develop feeding strategies for heat-stressed cattle. Brown-midrib maize silage-based diets lead to a higher nutrient digestibility (and, thus, content of metabolisable energy) as well as a higher intake (Table 2). Therefore, problems of feeding heat-stressed cattle (e.g. reduced dry matter intake (DMI) and a subsequent decrease in performance) might be (partly) compensated for by feeding maize silage prepared from Bm hybrids.

Review of literature

1. Brown-midrib mutations in maize

Brown-midrib mutations were already discovered in 1924 (Jorgenson, 1931) and six different mutants have been described, namely Bm1 (Eyster, 1926; Jorgenson, 1931), Bm2 (Burnham and Brink, 1932), Bm3 (Emerson et al., 1935), Bm4 (Burnham, 1947), Bm5 and Bm6 (Ali et al., 2010). A brief overview of the mutations is given in Table 1.

Brown-midrib mutants are characterised by a reddish-brown pigmentation of stem, roots, leafs, tassels and the cob. The pigmentation is covered by chlorophyll but can be seen in tissue with low chlorophyll content, e.g. the leaf midrib (Jorgenson, 1931). The pigmentation is a result of accumulation of phenolic derivatives because of disturbances in the lignin biosynthesis pathway (Vignols et al., 1995).

Table 1. Overview of characteristics of brown-midrib (Bm) mutations.

Mutation	Firstly described	Linkage group	Affected gene product
Bm1	Eyster (1926), Jorgenson (1931)	5 / short arm	Cinnamyl alcohol dehydrogenase
Bm2	Burnham and Brink (1932)	1 / long arm	Methylene tetrahydrofolate reductase
Bm3	Emerson et al. (1935)	4 / short arm	Caffeate O-methyl transferase
Bm4	Burnham (1947)	9 / long arm	-
Bm5	Ali et al. (2010)	5 / centromere	-
Bm6	Ali et al. (2010)	2 / short arm	-

The Bm3 mutation was characterised extensively, both, regarding its agronomic value (e.g. yield, stalk strength or susceptibility to diseases) and its impact on animal nutrition (e.g. DMI, digestibility, animal performance). Most studies particularly dealt with Bm3 maize (and, to a lesser extent with Bm1) because the properties of this mutant (cell wall content and composition, and agronomic value) were shown to be the most appropriate for breeding and feeding purposes (Barnes et al., 1971; Lechtenberg et al., 1972; Barriere and Argillier, 1993; Barriere et al., 1994). Especially the work of Barnes et al. (1971) and Lechtenberg et al. (1972) induced more intense research on the Bm3 mutants, likely because these authors observed a higher *in vitro* digestibility of Bm3 plants as compared to normal ones or to other brown-midrib genotypes.

1.1. Agronomic value of brown-midrib 3 mutants

The changes in lignin biosynthesis and its subsequent impact on lignin composition and concentration in Bm mutants have extensive effects on the agronomic value of maize. Several studies have shown that Bm mutants are inferior to their isogenic counterparts regarding yield, susceptibility to lodging, and diseases.

Brown-midrib mutants generally have a lower dry matter (DM) yield which can be attributed to reduced stover as well as grain yield and is reflected in reduced plant height and ear length (Miller et al., 1983; Lee and Brewbaker, 1984; Gentinetta et al., 1990). Recent results from the Wisconsin Corn Hybrid Performance Trials from 2008 to 2012 (Lauer et al., 2008; 2009; 2010; 2011; 2012) substantiate the aforementioned agronomic inferiority. The Bm hybrids yielded less DM and milk per hectare, yet they were superior when milk yield was expressed per ton of silage according to the method of Undersander et al. (1993) and Shaver and Lauer (2006).

An increased occurrence of stalk breakage and lodging (Gentinetta et al., 1990), a lower crushing strength (Zuber et al., 1977; Weller et al., 1985) and lower stem diameters (Lee and Brewbaker, 1984) have been reported for Bm mutants. However, Weller et al. (1985) and Gentinetta et al. (1990) also concluded that there is a genotype specific impact on susceptibility to lodging which is greater than the impact of the Bm mutation. A higher susceptibility to diseases was also discussed, as lignin which is reduced in Bm mutants serves as a physical barrier against, e.g., pathogen invasion (Buendgen et al., 1990).

1.2. Implications on digestion events, dry matter intake, and performance of dairy cows

Brown-midrib mutants were of interest for animal nutritionists because of their low lignin content and their altered lignin composition. Lignin contributes only to a low proportion to total DM of maize but largely affects digestibility of DM and fibre fractions (Van Soest, 1964; Sullivan, 1966; Allinson and Osbourn, 1970; Muller et al., 1972). The Bm mutation, additionally, leads to changes in lignin composition (i.e. proportion of phenolic monomers) which also affects digestibility (Taboada et al., 2010; Novo-Uzal et al., 2011). An increase in digestibility of the fibre fractions of Bm maize silage was reported for wethers and lambs (Muller et al., 1972; Block et al., 1982) and dairy cows (Sommerfeldt et al., 1979; Greenfield et al., 2001). Muller et al. (1972) and Block et al. (1982) concluded that the lower lignin content allowed for a faster digestion of cell wall constituents (CWC). In contrast, Sommerfeldt et al. (1979) found significant increases in CWC and cellulose digestibility at similar lignin contents of Bm and control silages and, therefore, assumed that alterations in lignin structure rather than lignin concentration may have influenced digestibility. Others found no effect of Bm maize silage on diet digestibility (Holt et al., 2010).

Furthermore, effects on ruminal ingesta kinetics and microbial crude protein (MCP) synthesis were observed. Decreased ruminal retention time and higher MCP synthesis of Bm based diets were shown by Oba and Allen (2000b) and increased *in situ* degradation of Bm maize silage was shown by Mustafa et al. (2005). From these results it may be expected that ruminal fermentation was also affected. However, the concentration of ruminal short chain fatty acids (SCFA), as well as the molar proportions of SCFA (i.e. acetic, propionic, butyric, valeric acids) did not change after feeding Bm maize silage (Oba and Allen, 2000a), although increased ruminal organic matter (OM) digestibility was observed. Contradictory, Qiu et al. (2003) and

Taylor and Allen (2005b, c) found changes in molar proportion of SCFA without changes in ruminal digestibility after feeding Bm miaze silage.

Several studies have shown that silage from Bm maize, fed either solely, supplemented with concentrate or with concentrate and other sources of roughage led to an increase in DMI. Others, in contrast, found no impact of feeding Bm mutants on DMI (Table 2). Literature data concerning the effect of feeding Bm maize on milk yield are also inconsistent. Increased DMI and milk yield, enhanced DMI without any effect on milk yield, and no effects on DMI and animal performance were observed (Table 2).

Table 2. Impact of brown-midrib (Bm) maize silage on dry matter (DM) intake (DMI) and performance of lactating dairy cows.

Reference	Effect on		DMI (kg/day)	Milk yield (kg/day)	Days in milk	Bm maize silage (% of diet DM)
	DMI	Milk yield				
Oba and Allen (1999)	↑	↑	~ 24.5	~ 40	89 ± 27	45
Ebling and Kung (2004)	↑	↑	~ 24.6	> 40	143 ± 32	42
Cherney et al. (2004)	↑	↑	~ 22.0	~ 40	84 ± 32	60
Oba and Allen (2000a)	↑	↑	~ 23.3	> 30	70 ± 7	35 - 55
Qiu et al. (2003)	↑	↔	~ 25.4	~ 35	162	31 - 39
Castro et al. (2010)	↑	↔	~ 25.4	~ 40	82 ± 19	34 - 40
Sommerfeldt et al. (1979)	↑	↔	~ 18.0	~ 25	~ 42	83
Taylor and Allen (2005a)	↔	↔	~ 24,8	> 40	72 ± 8	40
Tine et al. (2001)	↔	↔	~ 24.0	~ 34	155 ± 23	60
Greenfield et al. (2001)	↔	↔	~ 20.5	24 - 25	221 ± 20	60
Gehman et al. (2008)	↔	↔	~ 20.7	< 40	101 ± 34	58
Holt et al. (2010)	↔	↔	~ 26.8	< 40	26 - 39	25 - 31

These inconsistent results may be largely explained by two factors: Composition of the experimental diet (percentage of Bm maize silage and percentage and quality of the remainder diet ingredients) and requirements of the experimental animals. Holt et al. (2010) suggested that high quality of other forages in the diet (e.g. lucerne) and fine chopping might dilute potential effects of Bm. Greenfield et al. (2001) concluded that especially animals with a high energy demand increased DMI when fed silage prepared from Bm maize.

As shown in Table 2, especially cows with a high milk yield appear responsive to Bm maize silage because they had a higher DMI and/or yielded more milk. Animals yielding less milk or in later lactation stages, respectively, did not respond to Bm maize silage in the same way.

Dairy cows in early lactation are not able to cover their energy demand from DMI due to limited DMI capacity. Brown-midrib maize silage may allow them to increases DMI due to reduced ruminal retention time and higher digestibility. Increased DMI as well as increased digestibility may, therefore, improve nutrient supply of the animal and alleviate it from energy deficiency early post partum. In that way, Bm maize may allow to reduce mobilization of body tissue and the risk of ketosis. Additionally, increased forage intake offers the possibility to increase concentrate intake at the same time without decreasing the forage to concentrate ratio. Thus, energy intake can be increased without exposing the animals to the risk of acidosis, particularly subacute ruminal acidosis (SARA).

2. Heat stress in ruminants

Climate change is very likely to increase incidence and severity of heat waves (Meehl et al., 2007) and might, therefore, become an increasing problem for dairy production in Germany. For the US dairy industry, annual losses due to heat stress of about $900 million were estimated (St-Pierre et al., 2003).

According to Yousef (1985) and Bligh and Johnson (1973) it can be defined that an animal is heat-stressed once ambient temperature exceeds the upper critical temperature. That is “the ambient temperature above which thermoregulatory evaporative heat loss processes of a resting thermoregulating animal are recruited.” (Bligh and Johnson, 1973; Figure 1).

The most important environmental factors affecting animals are temperature, relative humidity, radiant heat, precipitation, atmospheric pressure, ultraviolet light, wind velocity and dust (Khalifa, 2003). Temperature and relative humidity (RH), however, are the most important, especially regarding closed housing systems.

The impact of climate on animals depends on duration and severity of climatic factors and might, therefore, induce variable results (Khalifa, 2003). Morbidity, production and reproduction are mainly affected by chronic heat stress, whereas acute heat stress affects mortality (Khalifa, 2003). Furthermore, the upper critical temperature is not a constant but varies depending on age, performance and physiological stage of an animal (Bianca, 1965; Yousef, 1985).

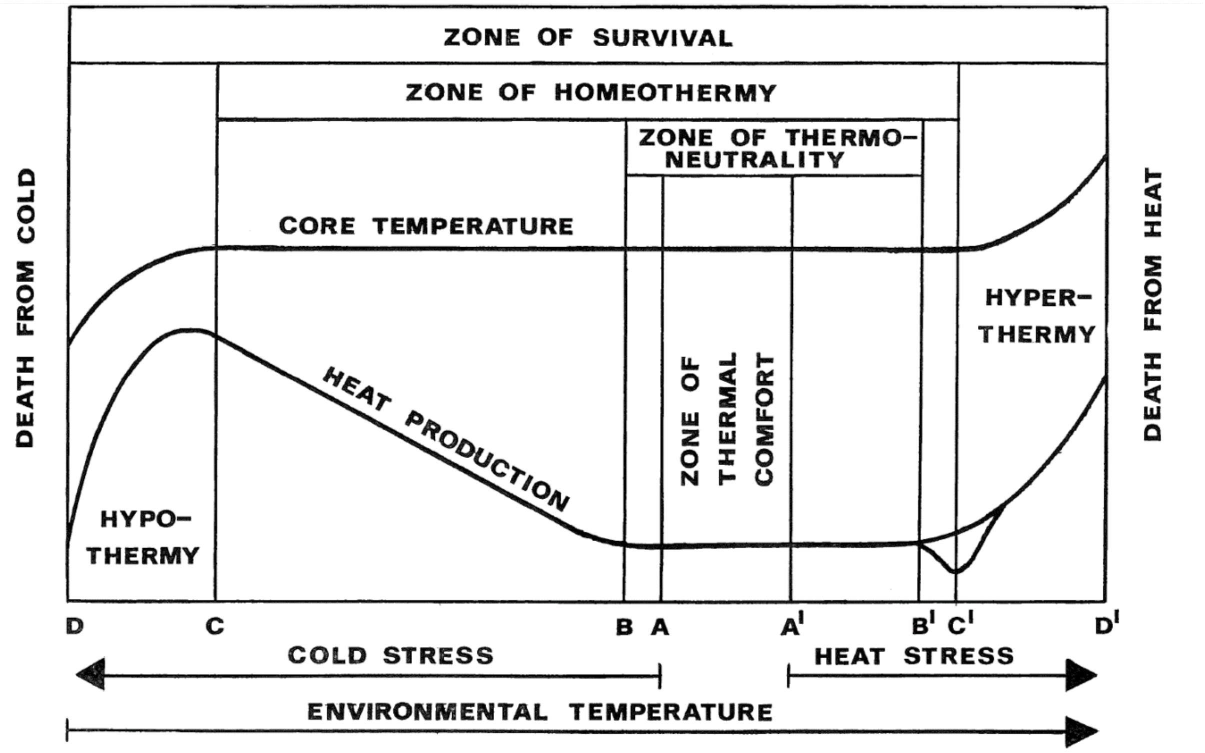

Figure 1. The thermoneutral zone (adapted from Bianca, 1968). Notes: ***A*** to ***A'***, comfort zone, blood vessels are neither dilated nor constricted, evaporation is minimal; ***B***, lower critical temperature, the animal begins to produce heat to maintain body temperature if ambient temperature decreases further; ***B'***, upper critical temperature, animal dissipates excess heat to maintain body temperature; ***C***, animal is not able to balance heat loss, body temperature falls; ***C'***, animal is not able to maintain homeothermy, body temperature rises; **D**, lower lethal temperature, animal dies from cold; **D'**, upper lethal temperature, animal dies from heat.

Table 3. Classification of climates according to their main effects on animals (adapted from Khalifa, 2003).

Climate	Region	Köppen classification	Main effect
Hot			
Hot wet climate	Tropical and subtropical	A and C	Heat stress in summer, rain, wind, storm
Hot dry climate	Low latitude, arid (desert)	B	Heat stress in summer, salinity, Dehydration, starvation Cold stress in winter
Cold	High latitude, semiarid	A	Cold stress in winter
	Savannah	B	Cold stress in winter
	Arid and semiarid	E	Cold stress, rain
Altitude	Polar	D	Cold stress low pressure
	Moist continental		
	Mid-latitude		

Notes: A, Tropical climates; B, Dry climates; C, Temperate wet climates; D, Boreal climates; E, Polar climates.

2.1. Estimating critical climatic conditions for temperate climates

Originally developed to describe the impact of ambient temperature and relative humidity on man and the necessity of air conditioning, the discomfort index was developed (Thom, 1959). Since then, several different equations were developed to calculate the so called temperature-humidity index (THI) and describe the impact of climatic conditions on animals. Subsequently, several THI thresholds were derived. An overview over THI equations and thresholds is given in Tables 4 and 5.

Generally, THI 70 or 72 were accepted as heat stress threshold for dairy cows (Hahn, 1985; Johnson, 1985; Dupreez et al., 1990; Chase, 2006; Table 5)). Igono et al. (1992) defined THI 64, 72 and 76 as minimum, mean and maximum threshold. Igono et al. (1992) also suggested considering hours per day with a temperature below 21°C because such "cool periods" at night relieve animals from heat stress. Shishido et al. (1983) showed that a constant ambient temperature of 29°C was more detrimental than a diurnal temperature variation which had the same average temperature.

Adequacy of THI equations, however, depends on the scope of application and different THI equations may lead to different thresholds within the same application range (Brügemann et al., 2012). Hahn et al. (2003) discussed different heat stress indices and concluded that the THI might not be the best thermal index that can be developed. The THI disregards radiation, airflow and cold conditions, but it takes into account temperature and relative humidity which represent a large amount of the impact of heat, especially for animals in sheltered environments. Therefore, Hahn et al. (2003) concluded that the THI might be a useful index except for the winter season and is used as a de facto standard especially for cattle.

Most of the THI equations and the thresholds were developed in hot regions. Literature on the impact of mild heat stress is scarce. A recent study showed that THI equations and THI thresholds to describe heat stress in hot climates cannot be transferred directly to temperate regions (Brügemann et al., 2012). For temperate regions in Lower Saxony, Germany they suggested a THI threshold of 60 or 70 for the equations of Bohmanova et al. (2005) and Ravagnolo and Misztal (2000), respectively (Table 4), which denoted a substantial decline in test-day milk yield.

Table 4. Equations to calculate the temperature-humidity index (THI).

Equations using dry bulb temperature and relative humidity	
(1) Mader et al. (2006) (2) Hahn (1999)	$= (0.81 \cdot Tdb°C) + [(RH/100) \cdot (Tdb°C - 14.4)] + 46.4$
(3) NRC (1971)	$= (1.8 \cdot Tdb°C + 32) - [(0.55 - 0.0055 \cdot RH) \cdot (1.8 \cdot Tdb°C - 26)]$
(4a) Ravagnolo and Misztal (2000)^1, (4b) Bohmanova et al. (2005)^2	$= (1.8 \cdot Tdb°C + 32) - [(0.55 - 0.0055 \cdot RH) \cdot (1.8 \cdot Tdb°C - 26)]$
Equations using dry bulb temperature and wet bulb temperature	
(5) Thom (1959)^3	$= [0.4 \cdot (Tdb°F + Twb°F)] + 15$
(6) NRC (1971)	$= (Tdb°C + Twb°C) \cdot 0.72 + 40.6$
Equations using dry bulb temperature and dew point temperature	
(7) NRC (1971)	$= (0.55 \cdot Tdb°F + 0.2 \cdot Tdp°F) + 17.5$
(8) Hahn (1999)	$= Tdb°C + 0.36 \cdot Tdp°C + 41.2$

Notes: Tdb, Dry bulb temperature; RH, Relative humidity [%]; Twb, Wet bulb temperature; Tdp, Dew point temperature; ^{1}Daily maximum of Tdb and daily minimum of RH were used; ^{2}Daily mean of hourly Tdb and RH were used; ^{3}Originally named discomfort index.

Table 5. Thresholds of the temperature-humidity index (THI) to characterise heat stress in dairy cows.

THI	Stress level	Comments
		Thresholds according to Chase (2006)
< 72	None	-
72 – 79	Mild	Dairy cows will adjust by seeking shade, increasing respiration rate and dilation of blood vessels. The effect on milk production will be minimal
80 – 89	Moderate	Both saliva production and respiration rate will increase. Feed intake may be depressed and water consumption will increase. There will be an increase in body temperature. Milk production and reproduction will be decreased.
90 – 98	Severe	Cows will become very uncomfortable due to high body temperature, rapid respiration (panting) and excessive saliva production. Milk production and reproduction will be markedly decreased.
> 98	Danger	Potential cow deaths can occur.
		Thresholds according to Hahn (1985) and Dupreez et al. (1990)
≤ 70	Normal	-
71 – 78	Alert	-
79 – 83	Danger	-
≥ 83	Emergency	-

Adapted from and Hahn (1985), Dupreez et al. (1990), Chase (2006).

2.2. Implications on dry mater intake, digestion events, and performance of sheep and dairy cows

Depending on its severity, heat stress may largely affect DMI, digestion events, and performance of dairy cows. However, it is worthwhile to mention that DMI, digestion events and performance interact.

Dairy cows respond to heat stress by decreasing DMI and increasing water intake. Decreased DMI due to increased ambient temperature was observed by Wayman et al. (1962), West (1999) and Bouraoui et al. (2002). Similarly, Olbrich et al. (1972) and Bernabucci et al. (1999) observed reduced DMI in heat-stressed heifers and Maloiy et al. (2008) in several domesticated and wild ruminants in East Africa.

In dairy cows, reduced DMI can be attributed to either a decrease in total DMI or a decrease in DMI from forage, if animals are able to separate the ration or if concentrate and forage are fed separately (Bouraoui et al., 2002). Heat stress might decrease total DMI as animals try to decrease heat production emerging from feed intake. Decreased forage intake might be a further strategy of the animals to reduce heat production (Bouraoui et al., 2002). Forage intake was associated with heat production from ruminal fermentation of fibre (Czerkawski, 1980; Coppock, 1985; Webster et al., 1975) and energy expenditure for ingestion (Osuji et al., 1975; Susenbeth et al., 1998; Susenbeth et al., 2004). It has, however, to be taken into account that reduced heat emerging from fermentation is rather related to the proportion of OM of a certain feedstuff that is fermented than to its fermentation characteristics (Webster et al., 1975). Decreased forage to concentrate ratio and, thus, reduced ruminal acetate to propionate proportion, furthermore, decreases heat increment of feeding because metabolic use of propionate releases less energy than metabolic use of acetate. Reduced DMI may also be explained by the increase in water intake, as well as by a reduced ruminal motility leading to an increase in ruminal fill, which in turn reduces DMI capacity. Reduced ruminal motility and, therefore, a reduced rate of ingesta passage may be a result of reduced pH and higher ruminal concentration of lactic acid during heat stress (Mishra et al., 1970; Gregory, 1987).

Several studies reported increased digestibility of fibre fractions or DM in steers, wethers and dairy heifers in response to heat stress (Miaron and Christopherson, 1992; Weniger and Stein, 1992; Bernabucci et al., 1999). Reduced rate of ingesta passage and increased ingesta mean retention time may cause the increase in digestibility independently of DMI (Weniger and Stein, 1992). Others, however, did not find changes in digestibility when animals were exposed to heat stress (Mathers et al., 1989; Lourenco et al., 2010) or observed reduced digestibility (Bhattacharya and Hussain, 1974). According to Christopherson (1985), in such cases heat stress might have been not severe enough to affect digestibility. Additionally, as changes in digestibility under heat stress conditions were related to reduced ruminal motility, it has to be considered that reduced ruminal motility might not only enhance digestibility. Enhanced digestibility due to reduced ruminal motility can be explained by reduced ingesta passage and thus, increased time for fermentation. Indeed, reduced ruminal motility might also hamper ruminal digestion. Firstly, reduced mixing of ruminal content could reduce inoculation of feed particles with microorganisms. Secondly, contact of fermentation products with the ruminal mucosa and thus absorption might be reduced.

Decreased performance and productivity of dairy cows due to heat stress was observed by Wayman et al. (1962), Moody et al. (1967), Bouraoui et al. (2002) and Gantner et al. (2011). It was assumed that decreased milk yield was a consequence of decreased DMI (Wayman et al., 1962). In quantitative terms, however, reduced DMI only explained 35 - 50% of milk yield reduction in heat-stressed animals (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard and Rhoads, 2012). In a thermoneutral environment lactating dairy cows are able to adapt to energy deficiency to some degree to support their dominant physiological state (i.e. milk synthesis; Baumgard and Rhoads, 2013). If DMI is reduced, less insulin is produced, insulin sensitivity decreases and somatotropin production increases. Adipose tissue can be mobilised and used for milk synthesis. Lipogenesis, furthermore, is reduced and glucose availability for milk synthesis is increased (Baumgard and Rhoads, 2013; Figure 2). In heat-stressed cows in contrast, reduced DMI does not reduce but increase basal and stimulated insulin levels. Therefore, adipose tissue mobilisation is hampered and glucose is directed to lipogenesis (Baumgard and Rhoads, 2013). Muscle catabolism, furthermore, is increased which may be due to use of glucogenic amino acids for gluconeogenesis (Baumgard and Rhoads, 2013; Figure 2). Thus, heat-stressed animals fail to spare glucose to maintain milk production (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard and Rhoads, 2012; Baumgard and Rhoads, 2013; Figure 2). Moreover, it was assumed that panting may increases maintenance requirement about 7 to 25% (NRC, 1981), which may also contribute to decreased performance. Heat stress, furthermore, reduces blood flow to the gastrointestinal tract (Bell et al., 1983). Therefore, nutrient transport from the gastro intestinal tract may be lowered and nutrient utilisation be decreased. The former might especially be true for cows early post partum that rely on tissue mobilisation to cover their energy demand. Animals in advanced stages of lactation might be less affected. However, it can be assumed that acute heat stress might decrease DMI to an extent that even cows in advanced stages of lactation enter negative energy balance. Indeed such a situation might not

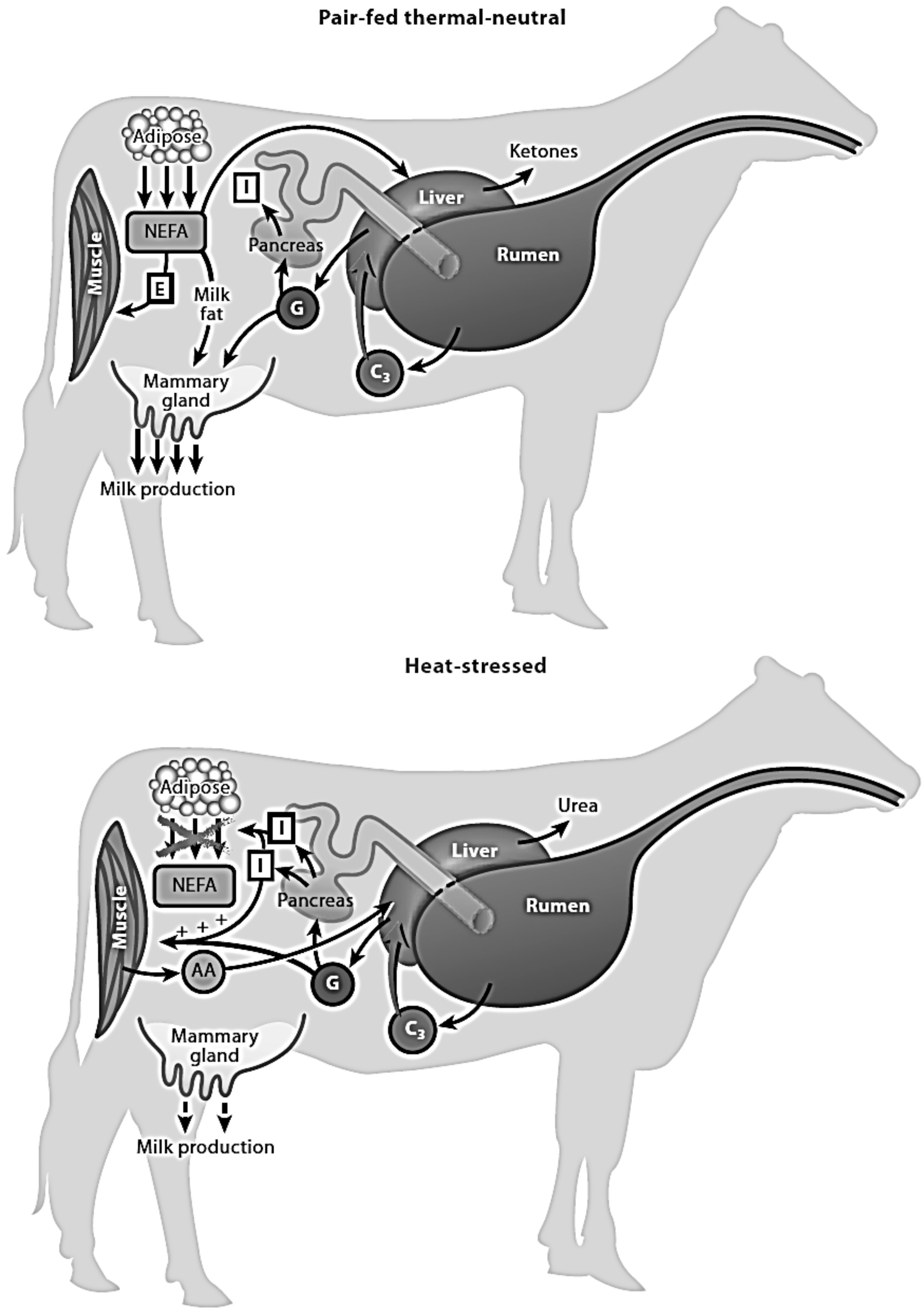

Figure 2. Differences in the insulin metabolism of thermoneutral and heat-stressed dairy cows when fed at the same intake level (adapted from Baumgard and Rhoads, 2013). Notes: I, Insulin; E, Energy; G, Glucose; C_3, Propionate; NEFA, Non-esterified fatty acids.

be induced by mild heat stress but it becomes obvious that research will have to pay attention to graduation of heat stress.

Though positive effects of heat stress on nutrient digestibility were observed, in its entirety heat stress is negative. Yet, it remains difficult to define particular mechanisms as they are running simultaneously. Hence, it is unclear in how far positive and negative effects cancel each other. Knowledge about mild heat stress, furthermore, is scarce which requires attention as heat stress and it effects do not change on a linear scale.

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Chapter 2 Scope of the thesis

The present thesis is a cumulative thesis based on a series of trials conducted at the experimental station of the Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany. The trials address fibre quality of maize silage and heat stress in ruminants. In chapters three and four, effects of a brown-midrib (Bm) silage maize variety compared to a control silage maize variety on dry matter (DM) intake (DMI), performance, and digestion events is evaluated. In Chapter five and six, the effects of heat stress on nutrient digestibility in sheep and on performance and DMI of dairy cows is evaluated. It was hypothesised that silage prepared from a Bm maize hybrid would increase DMI, DM digestibility and milk yield in dairy cows and, therefore, improve productivity. It was assumed that increased degradation rate of fibre fractions and decreased ruminal mean retention time would contribute to enhanced performance and also improve efficiency of microbial crude protein synthesis. So, the first objective of the present thesis was to investigate the feeding value of a Bm silage maize variety and its impact on digestion events and performance. The second objective was to evaluate the impact of heat stress on sheep and dairy cows. It was hypothesised that acute heat stress would increase nutrient digestibility in sheep, and comparably mild heat stress, like summer temperatures in Lower Saxony, Germany, reduces DMI, milk yield, and milk fat and protein yield of dairy cows. The third objective was to discuss possible adaptation and mitigation strategies to heat stress for the temperate climate in Lower Saxony. Feeding strategies to offset negative effects of heat stress on dry matter intake and performance were addressed. Especially the use of Bm maize silage as an option to feed heat-stressed cows was focused.

Chapters three to six were published in peer reviewed journals.

Chapter 3

Effects of a brown-midrib corn hybrid on nutrient digestibility in wethers and on dry matter intake, performance, rumen and blood variables in dairy cows

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Journal of Animal Physiology and Animal Nutrition, 2014
Vol. 98. Issue 2, 300–309, DOI: 10.1111/jpn.12080

Summary

The aim of the present trials was to determine the effect of an experimental brown-midrib (Bm) corn hybrid in relation to a commercial corn hybrid (Con) on digestibility in wethers and on dry matter (DM) intake (DMI), milk yield and milk composition in dairy cows. Digestibility of crude fibre (CF), neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) were higher for Bm (CF Con: 57.8%; Bm: 67.2%; NDFom Con: 56.8%; Bm: 64.8%; ADFom Con: 52.0%; Bm: 63.9%), but concentration of net energy for lactation did not differ (Con: 6.4 MJ/kg DM; Bm: 6.3 MJ/kg DM). A total of 64 lactating German Holstein cows were assigned to one of the two dietary treatments Con or Bm according to milk yield, lactation number, days in milk and body weight. In Trial 1, cows were fed a total mixed ration consisting of 50% corn silage (Con or Bm) and 50% concentrate on DM basis. In Trial 2, the same animals were fed the respective silage for *ad libitum* intake and 5.3 kg of concentrate DM per animal per day. In Trial 1, DMI and milk fat content were decreased significantly for the Bm treatment (DMI Con: 22.5 kg/day; Bm: 21.5 kg/day; milk fat Con: 3.8%; Bm: 3.3%). In Trial 2, milk yield and fat-corrected milk (FCM) were increased significantly, whereas milk fat% was decreased significantly (milk yield Con: 25.8 kg/day; Bm: 29.4 kg/day; FCM Con: 27.2 kg/day; Bm: 29.6 kg/day; fat Con: 4.4%; Bm: 4.0%). Diets did not influence ruminal pH or temperature. Diets, furthermore, did not influence rumination in either trial. Additional research on digestibility and rumen fermentation should, however, be carried out using dairy cows at respective intake levels as trials with wethers cannot be transferred to high-yielding *ad libitum* fed cows.

Keywords: brown-midrib, dairy cow, fibre, digestibility, milk yield, milk composition

Introduction

Brown-midrib (Bm) mutations in *Zea mays* L. have been known since 1924 (Ali et al., 2010). Brown-midrib mutants are characterised by a reddish brown pigmentation of the leaf midrib (Barriere and Argillier, 1993) and were described to have a reduced dry matter (DM) yield (Lee and Brewbaker, 1984). Bm mutations, furthermore, were shown to improve fibre digestibility (Sommerfeldt et al., 1979; Greenfield et al., 2001), dry matter intake (DMI) (Oba and Allen, 1999; Cherney et al., 2004) and performance (Oba and Allen, 1999, 2000a) in ruminants.

It was assumed that improved fibre digestibility was based on a reduced lignin content (Block et al., 1982) and/or changes in the structure of lignin (Sommerfeldt et al., 1979), while the increase in DMI could be a result of the improved fibre digestibility (Oba and Allen, 1999). The increase in performance may be based on the increase in DMI or digestibility or both (Oba and Allen, 1999; Ebling and Kung, 2004). Mechanisms of the effects have not yet been completely elucidated, especially as fibre content, fibre digestibility and DMI may interact.

Besides increased DMI and increased performance, an improvement in fibre digestibility may lead to a shift in ruminal SCFA (short chain fatty acid) proportions (Qiu et al., 2003; Gehman et al., 2008). Changes could be expected in ruminal pH due to an increased SCFA production. Dry matter intake and forage to concentrate ratio, furthermore, affect heat increment of feeding. Therefore, changes in ruminal temperature which may be an important aspect with regard to heat stress might occur. Indeed, effects of Bm on ruminal fermentation (Gehman et al., 2008; Holt et al., 2010) are not consistent.

Therefore, the aim of the present trials was to investigate the effects of an experimental Bm hybrid on DMI and milking performance of dairy cows in relation to a common corn hybrid (Con) with special regard to fibre digestibility. As the roughage composition of experimental diets varied widely in the literature, the present trials were conducted using corn silage as the sole roughage. As the impact of Bm on ruminal fermentation was inconsistent, continuous measurements of ruminal pH and temperature were evaluated.

It was hypothesised that Bm would increase DMI due to higher fibre digestibility and thus increase performance. It was, furthermore, hypothesised that feeding Bm would change rumination activity and ruminal fermentation which will cause changes in ruminal pH and temperature.

Materials and methods

Corn silages, animals, treatments and experimental design

The Con and Bm corn were grown at the experimental station of the Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany. Cutting height was approximately 18 to 20 cm, theoretical chopping length was 5.5 mm, harvest date was 11 October 2010 (Con) and 12 October 2010 (Bm), DM at harvesting was 34.4% (Con) and 33.4% (Bm), corn was harvested in the dough stage. Dry matter yield per ha was 20.8 t (Con) and 17.8 t (Bm). The Bm hybrid was an experimental hybrid (‘Saaten-Union GmbH’, Isernhagen, Germany) the Con hybrid was ‘Ronaldinio’ (‘KWS-Saat AG’, Einbeck, Germany).

Digestibility trial with wethers

For calculation of energy content of the two silages, a digestibility trial with four wethers was conducted according to the recommendations of GfE (1991). Wethers received 1 kg of silage

DM per day; to adapt crude protein content, 20 g of urea was added per animal per day; thus, crude protein content of the diet was 134 g/kg DM. Animals were adapted to the silage for 13 days followed by 8 days of total collection of faeces.

Feeding trials with dairy cows (Trials 1 and 2)

Two trials were conducted at the experimental station of the FLI in Braunschweig, Germany according to the regulations concerning protection of experimental animals of the European Union and were approved by the 'Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit' (File number 33.9- 42502-04-10/0304).

A total of 64 lactating dairy cows (German Holstein) were assigned to one of the two dietary treatments (32 per treatment) according to daily milk yield (Con 34.7 ± 6.7 kg, Bm 35.2 ± 5.7 kg), lactation number (Con 1.6 ± 0.8, Bm 1.6 ± 0.7), days in milk (DIM) (Con 91.6 ± 18.4, Bm 92.3 ± 16.0) and body weight (BW) (Con 597 ± 75.2 kg, Bm 573 ± 58.9 kg) at the beginning of Trial 1. Respective values prior to Trial 2 were: Milk yield; Con 28.9 ± 4.6 kg/day, Bm 29.6 ± 4.4 kg/day, number of lactation; Con 1.6 ± 0.8, Bm 1.6 ± 0.7, DIM; Con 174.6 ± 18.4, Bm 175.3 ± 16.0, BW; Con 554 ± 66.7 kg, Bm 535 ± 57.8 kg. Number of lactation was considered for statistical evaluation, 33 animals were in their first lactation and 31 were in their second or third lactation. During Trial 1, animals received a total mixed ration (TMR) consisting of corn silage of Con or Bm and concentrate [50% each on DM basis] for *ad libitum* intake. During Trial 2, animals had *ad libitum* access to Con or Bm silage, the concentrate was restricted to 5.3 kg DM per animal per day and fed via an automatic feeding system. Each trial lasted 56 days. Between the trials, both groups were fed Con TMR for 28 days.

Ingredient composition of the concentrates is given in Table 1. Chemical composition of the silages and concentrates is given in Table 2. Diets were created to meet the nutrient and energy requirements of dairy cows according to GfE (2001).

Animals were kept in a cubicle housing system with two group pens, one for each dietary treatment. Each group pen was equipped with two concentrate feeding stations (Insentec, B.V., Marknesse, The Netherlands), 30 automatic self-feeding stations and two automatic drinking troughs (both Insentec, B.V., Marknesse, The Netherlands). Animals were equipped with ear tags to be identified individually at the concentrate feeder (Trial 2), the automatic feeding and drinking stations, in the milking parlour, and at the balance. Due to health problems during Trial 1, data of two animals from treatment Con and of one animal from treatment Bm were excluded from statistical analysis. Therefore, 61 of 64 animals completed Trial 1. All animals that completed Trial 1 took part in Trial 2.

Table 1 Components of the concentrates of Trial 1 and Trial 2.

	Concentrate1	Concentrate2
Wheat (%)	36.5	36.5
Solvent-extracted soybean meal (%)	25.0	25.0
Solvent-extracted rapeseed meal (%)	13.0	13.0
Dried sugar beet pulp (%)	22.3	18.3
Calcium carbonate (%)	1.0	2.5
Sodium chloride (%)	0.2	0.2
Mineral and vitamin premix* (%)	2.0	2.0
Urea (%)	-	2.5
MJ NEL / kg DM†	7.9	7.6

* per kg mineral feed: 170 g Ca; 50 g P; 120 g Na; 45 g Mg; 800,000 IU vitamin A; 100,000 IU vitamin D3; 4,000 mg vitamin E; 4,000 mg Mn; 6,000 mg Zn; 1,300 mg Cu; 120 mg I; 35 mg Co; 40 mg Se; NEL, net energy for lactation; DM, dry matter; † based on table values (Universität Hohenheim – Dokumentationsstelle 1997).

Table 2 Nutrient content of the corn silages (LSmeans of n = 4 pool samples for each silage, two pool samples from Trial 1 and two pool samples from Trial 2 with standard errors) and concentrates (arithmetic means of n = 2 pool samples from each trial with standard errors).

	Con silage	Bm silage	Concentrate1	Concentrate2
DM (g/kg)	319 ± 8.6	335 ± 8.6	877 ± 2.8	882 ± 0.5
Ash (g/kg DM)	43 ± 1.2	45 ± 1.2	80 ± 1.3	88 ± 0.6
CP (g/kg DM)	78 ± 0.9	78 ± 0.9	239 ± 2.9	312 ± 1.3*
EE (g/kg DM)	31 ± 0.8	29 ± 0.8	28 ± 1.7	27 ± 1.5
CF (g/kg DM)	223 ± 6.6	218 ± 6.6	85 ± 3.3	75 ± 2.5
NDFom (g/kg DM)	481 ± 9.8	488 ± 9.8	242 ± 5.5	225 ± 2.8
ADFom (g/kg DM)	258 ± 4.9	244 ± 4.9	111 ± 2.7	105 ± 3.3
ADL (g/kg DM)	34 ± 2.0	17 ± 2.0	-	-
Starch (g/kg DM)	272 ± 7.6	297 ± 7.6	-	-

Con, control; Bm, brown-midrib; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDFom, neutral detergent fibre expressed without residual ash; ADFom, acid detergent fibre expressed without residual ash; ADL, acid detergent lignin; *including 12 g urea nitrogen.

Data and sample collection

Feed intake of each cow was recorded continuously. Representative samples of TMR (Trial 1) were collected daily and pooled at 4-week intervals for chemical analysis. Silages were collected twice a week, and DM was determined to adapt TMR composition according to DM changes of the silages (Trial 1). Concentrate samples were collected once a week and pooled at 4-week intervals for chemical analysis.

Milk yield was recorded at each milking (05:30 and 15:00). Samples of milk were taken on 2 days per week at two consecutive milking times. Animals were weighed after each milking.

Blood samples were collected at the beginning and the end of each trial from a *vena jugularis externa* using serum and plasma sampling tubes (Sarstedt 26.323 10 ml with serum clotting activator and Sarstedt 26.369 10 ml with 16 IU Heparin/ml blood; Sarstedt, Nümbrecht, Germany).

Ten animals of each group were equipped with a wireless rumen probe for continuous pH and temperature measurement (KB1000; Kahne Animal Health, Auckland, New Zealand) which was inserted via an intubation cannula. Probes were set to measure pH and temperature every 20 min; data were saved on an internal memory and transmitted to a portable computer once a week. Further technical details and validation of the rumen probes were described by Lohölter et al. (2013).

Daily mean, minimum and maximum values were calculated to evaluate pH measurements. According to Oba and Allen (2000a) and Taylor and Allen (2005b), the hours per day for which ruminal pH was below 6.0, 5.8 and 5.5 were calculated.

In both trials, rumination activity of all animals was determined using a 24-h observation technique as described by Maekawa et al. (2002). Due to feasibility, a time interval of 10 instead of 5 min was chosen and no behavioural patterns except rumination were recorded, whereby chewing activity without feed intake was considered to be rumination. It was assumed that rumination activity lasted the entire 10 min between two observations, and, therefore, total rumination duration was calculated from the number of intervals animals were observed to ruminate. Observations started after the morning milking at about 08:00, were disrupted during evening milking from 15:00 to 17:00 and ended with the beginning of the morning milking the next day at 5:00. Hence, animals were observed for 19 h and results were extrapolated to 24 h.

Analyses

Feedstuffs were dried at 60°C for 72 h and ground to pass through a 1-mm screen using a Retsch mill (SM 1; Retsch, Haan, Germany) and analysed for DM, ash, crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre expressed without residual ash (NDFom), acid detergent fibre expressed without residual ash (ADFom) and acid detergent lignin (ADL) according to the methods of VDLUFA (2007) including amylase pre-treatment for NDFom analysis. Starch was analysed using a polarimeter (Kernchen Gyromat; Kernchen GmbH, Seelze, Germany). Milk samples were analysed for fat and protein using an infrared milk analyser (Milkoscan FT 6000; Foss Electric, Hillerød, Denmark). Blood samples were analysed for plasma concentration of beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) using a photometrical blood analyser (Eurolyser CCA 180 VET; Greiner Diagnostic, Bahlingen, Germany).

Calculations

Metabolisable energy (ME) and net energy for lactation (NEL) of the corn silages were calculated based on the digested nutrients gained from trials with wethers (GfE, 1991). Energy content of the concentrate was calculated from table values (Universität Hohenheim – Dokumentationsstelle, 1997).

Fat-corrected milk (4%) (FCM) was estimated according to Gaines (1928).

Energy balance was calculated as daily energy intake minus requirement for maintenance (GfE, 2001) minus requirement for milk production (Tyrrell and Reid, 1965).

Statistical analysis

Statistical analysis was carried out using SAS (Software package 9.2; SAS Institute; Cary, NC, USA). For statistical analyses, the MIXED procedure of SAS was used. Variances were evaluated with the restricted maximum likelihood method; the method of Kenward-Rogers was used to estimate degrees of freedom. Post-hoc test was carried out using the ‘Tukey-Kramer’ test.

For energy content and nutrient digestibility, diet was considered to be the fixed factor. For performance and feed intake, diet and interaction of diet and number of lactation were considered to be fixed factors. For rumination activity, diet was considered to be the fixed factor. For analysis of blood parameters, day of sampling was included into the model.

For ruminal pH and temperature, daily mean values of pH, temperature and duration per day below pH 6.0, 5.8 and 5.5 were calculated and diet was considered to be the fixed factor. The results are presented as least square means (LSmeans) with standard errors. Differences were accepted to be significant for $p < 0.05$.

Results

Chemical analysis, digestibility and energy content of the silages

Nutrient content of the two corn silages are shown in Table 2. Digestibility of organic matter (OM) did not differ between the two silages; therefore, no difference in energy content was found (Table 3). Digestibility values of CF, NDFom and ADFom were increased when Bm silage was fed and digestibility of CP was reduced as shown in Table 3. *In situ* NDF degradation was, furthermore, higher for Bm (degradation rate: Con 3.6%/h; Bm 4.9%/h, effective degradability: Con 26.4%; Bm 35.3%; Gorniak et al., 2013).

Dry matter intake, milk yield and composition

Average BW did not differ significantly between treatments within trials, but there was a tendency for a higher BW for Con in both trials. Animals, however, lost weight during the course of Trial 1 and recovered during Trial 2, as shown in Table 4.

Table 3 Digestibility values from trials with wethers and energy content of the silages (LSmeans with standard errors).

	Con	Bm
	Digestibility %	
OM	72.3 ± 1.42	72.1 ± 1.42
CP	62.5^a ± 1.32	45.5^b ± 1.32
EE	73.1 ± 1.22	73.1 ± 1.22
CF	57.8^b ± 3.21	67.2^a ± 3.21
NDFom	56.8^b ± 2.49	64.8^a ± 2.49
ADFom	52.0^b ± 2.51	63.9^a ± 2.51
NfE	78.2 ± 1.04	77.4 ± 1.03
	Energy Content MJ/kg DM	
ME	10.6 ± 0.19	10.5 ± 0.19
NEL	6.4 ± 0.14	6.3 ± 0.14

Con, control; Bm, brown-midrib; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDFom, neutral detergent fibre expressed without residual ash; ADFom, acid detergent fibre expressed without residual ash; NfE, nitrogen-free extracts; DM, dry matter; ME, metabolisable energy; NEL, net energy for lactation; values with different superscripts differ significantly ($p < 0.05$).

In Trial 1, animals fed the Bm silage had a lower DMI and a lower milk fat content, but milk yield and FCM did not differ between groups (Table 4). Dry matter intake as percentage of BW, however, did not differ between treatments. As milk yield did not differ, decreased milk fat content of Bm-fed animals led to decreased milk fat yield per day. Milk protein content and protein yield per day were not influenced by treatment, but milk urea was lower in animals fed the Bm silage. Fat-to-protein ratio (FPR), furthermore, was decreased in animals fed the Bm silage because of the lower milk fat content.

In Trial 2, results were different as DMI and DMI as percentage of BW were the same for both treatment groups, but milk yield and FCM were higher for Bm-fed animals. Because of the increased milk yield, the decrease in fat content did not alter the daily yield of milk fat. Milk protein content was the same for both diets, but due to the increased milk yield, protein yield per day was higher in Bm-fed animals. Similar to Trial 1, milk urea content and FPR were lower in animals fed the Bm diet.

Table 4 Dry matter intake, milk yield and milk composition from Trials 1 and 2 (LSmeans with standard errors).

	Dietary treatment		*p*-values		
	Con	Bm	diet	number of lactation*	Diet x number of lactation
		Trial 1			
BW (kg)	593 ± 9.7	568 ± 9.6	0.071	<0.001	0.626
BW change (kg)	- 42 ± 3.2	- 41 ± 3.2	0.833	0.671	0.936
DMI TMR (kg/d)	22.5 ± 0.30	21.5 ± 0.30	0.014	<0.001	0.914
DMI (% BW)	3.82 ± 0.064	3.79 ± 0.063	0.743	0.012	0.458
DMI (% $BW^{0.75}$)	18.79 ± 0.27	18.47 ± 0.27	0.402	<0.001	0.497
NDFom intake (kg/d)	8.16 ± 0.11	7.78 ± 0.11	0.014	<0.001	0.913
milk yield (kg/d)	33.3 ± 0.83	34.8 ± 0.82	0.216	<0.001	0.873
FCM (kg/d)	32.3 ± 0.74	31.0 ± 0.73	0.234	<0.001	0.873
Milk fat (%)	3.8 ± 0.12	3.3 ± 0.12	0.004	0.110	0.670
Milk fat (kg/d)	1.26 ± 0.04	1.14 ± 0.04	0.036	<0.001	0.910
Milk protein (%)	3.4 ± 0.04	3.4 ± 0.04	0.492	0.247	0.600
Milk protein (kg/d)	1.13 ± 0.02	1.16 ± 0.02	0.223	<0.001	0.598
FPR	1.1 ± 0.03	1.0 ± 0.03	< 0.001	0.156	0.439
Milk Urea (ppm)	245 ± 5.9	197 ± 5.9	< 0.001	0.315	0.585
		Trial 2			
BW (kg)	566 ± 9.5	549 ± 9.3	0.192	<0.001	0.487
BW change (kg)	+ 17 ± 2.4	+ 26 ± 2.3	0.010	0.111	0.878
DMI total (kg/d)	19.8 ± 0.24	19.8 ± 0.24	0.979	<0.001	0.345
DMI (% BW)	3.5 ± 0.065	3.6 ± 0.064	0.255	0.281	0.937
DMI (% $BW^{0.75}$)	17.1 ± 0.268	17.5 ± 0.265	0.305	0.988	0.917
NDFom intake (kg/d)	8.18 ± 0.12	8.16 ± 0.11	0.902	<0.001	0.340
milk yield (kg/d)	25.8 ± 0.58	29.4 ± 0.57	< 0.001	0.187	0.534
FCM (kg/d)	27.1 ± 0.58	29.5 ± 0.57	0.005	0.013	0.464
Milk fat (%)	4.4 ± 0.10	4.0 ± 0.10	0.009	0.206	0.867
Milk fat (kg/d)	1.12 ± 0.03	1.17 ± 0.03	0.237	0.010	0.481
Milk protein (%)	3.3 ± 0.04	3.3 ± 0.04	0.694	0.312	0.314
Milk protein (kg/d)	0.85 ± 0.02	0.98 ± 0.015	<0.001	0.017	0.162
FPR	1.3 ± 0.02	1.2 ± 0.02	<0.001	0.356	0.689
Milk Urea (ppm)	286 ± 5.5	211 ± 5.5	<0.001	0.038	0.467

Con, control; Bm, brown-midrib; BW, body weight; DMI, dry matter intake; TMR, total mixed ration; $BW^{0.75}$, metabolic body size; NDFom, neutral detergent fibre expressed without residual ash; FCM, fat-corrected milk; FPR, fat protein ratio; *primiparous vs. multiparous cows.

Blood parameters and energy balance

As energy content of the silages did not differ, the lower DMI of the animals receiving the Bm diet in Trial 1 reduced energy intake (Con 160.2 ± 2.10 MJ NEL/day; Bm 152.7 ± 2.07 MJ NEL/day). Energy requirement for maintenance (Con 35.2 ± 0.43 MJ NEL/day; Bm 34.1 ± 0.43 MJ NEL/day), requirements for milk production (Con 103.1 ± 2.23 MJ NEL/day; Bm 100.8 ± 2.20 MJ NEL/day) and energy balance (Con 22.0 ± 1.83 MJ NEL/day; Bm 17.9 ± 1.80 MJ NEL/day) were, however, unaffected.

In Trial 2, energy intake (Con 132.1 ± 1.53 MJ NEL/day; Bm 132.2 ± 1.51 MJ NEL/day) and energy requirement for maintenance (Con 34.0 ± 0.43 MJ NEL/day; Bm 33.2 ± 0.42 MJ NEL/day) did not differ between the two groups, but according to the increased requirement for milk production (Con 84.9 ± 1.70 MJ NEL/day; Bm 92.9 ± 1.68 MJ NEL/ day), the positive energy balance was reduced in the Bm treatment (Con 13.2 ± 1.14 MJ NEL/day; Bm 6.2 ± 1.13 MJ NEL/day).

In Trial 1, plasma BHB concentration was higher for Bm, but in Trial 2, plasma BHB concentration was higher for Con. Plasma NEFA concentration was not affected (Table 5).

Table 5 Plasma BHB and Plasma NEFA concentrations in Trial 1 and 2 (LSmeans with standard errors).

	Con		Bm		*p*-value	
	Day 1*	Day 56	Day 1	Day 56	Diet	Diet x day of sampling
Trial 1						
BHB (mmol/L)	0.72 ± 0.095	0.87 ± 0.095	1.16 ± 0.095	0.94 ± 0.093	0.008	0.061
NEFA (mmol/L)	0.38 ± 0.031	0.24 ± 0.031	0.45 ± 0.031	0.22 ± 0.030	0.528	0.185
Trial 2						
BHB (mmol/L)	0.94 ± 0.070	1.13 ± 0.070	0.95 ± 0.069	0.82 ± 0.069	0.037	0.026
NEFA (mmol/L)	0.21 ± 0.012	0.19 ± 0.012	0.22 ± 0.012	0.21 ± 0.012	0.305	0.578

Con, control; Bm, brown-midrib; BHB, beta-hydroxybutyrate; NEFA, non-esterified fatty acids; *day 1, beginning; day 56 end of the respective trial.

Ruminal pH, ruminal temperature and rumination activity

Because of technical problems, only data from weeks 1 to 3 of Trial 1 could be considered for calculations of ruminal pH and ruminal temperature. For pH values, no differences were found between the diets. Temperature measurements were not affected by diet either (Table 6).

Rumination activity averaged 526 ± 14.9 min per day for Con treatment and 520 ± 14.7 min per day for Bm treatment in Trial 1. In Trial 2, rumination activity averaged 561 ± 12.2 min per day for Con treatment and 542 ± 12.2 min per day for Bm treatment. In both trials, diet did not influence rumination (Table 6).

Table 6 Ruminal fermentation characteristics (pH value and temperature) in Trial 1 and rumination in Trials 1 and 2 (LSmeans with standard errors).

	Diet		*p*-values
	Con	Bm	Diet
Minimum pH	5.2 ± 0.04	5.2 ± 0.04	0.760
Maximum pH	6.8 ± 0.06	6.8 ± 0.06	0.769
Mean pH	6.0 ± 0.06	6.0 ± 0.05	0.908
pH < 6.0 duration (h/d)	11.4 ± 1.17	11.3 ± 1.04	0.952
pH < 5.8 duration (h/d)	7.4 ± 1.14	7.6 ± 1.02	0.932
pH < 5.5 duration (h/d)	2.8 ± 0.78	3.1 ± 0.69	0.765
Minimum temperature °C	36.0 ± 0.31	35.4 ± 0.28	0.174
Maximum temperature °C	40.7 ± 0.10	40.6 ± 0.09	0.355
Mean temperature °C	39.4 ± 0.08	39.2 ± 0.07	0.058
	Rumination (min/d)		
Trial 1	526 ± 15.2	520 ± 14.9	0.795
Trial 2	561 ± 12.4	542 ± 12.4	0.277

Con, control; Bm, brown-midrib.

Discussion

Chemical analysis, digestibility and energy content of the silages

Consistent with the present results, reduced contents of lignin in Bm mutants in relation to isogenic variations and to common corn silage hybrids have been described frequently (Muller et al., 1972; Block et al., 1982; Greenfield et al., 2001; Qiu et al., 2003; Kung et al., 2008). Corresponding to the results above, however, the literature is inconsistent concerning differences between Bm hybrids and common hybrids regarding NDFom and ADFom contents (Muller et al., 1972; Block et al., 1982; Greenfield et al., 2001; Qiu et al., 2003; Kung et al., 2008).

Although a higher CF digestibility was found for the Bm silage, OM digestibility and energy content were not increased at the same time because of the lower digestibility of CP and the relative low proportional contribution of CF digestibility to ME (Table 3). It remains, however, unclear why CP digestibility was lower in Bm. An increase in digestibility of the fibre fractions of Bm corn silage was reported for wethers and lambs, (Muller et al., 1972; Block et al., 1982) and dairy cows (Sommerfeldt et al., 1979; Greenfield et al., 2001). Muller et al. (1972) and Block et al. (1982) concluded that the lower lignin content allowed for a faster digestion of cell wall constituents (CWC). Sommerfeldt et al. (1979) in contrast observed significant increases in CWC and cellulose digestibility at similar lignin contents of their Bm and control silages. Therefore, they assumed that alterations in lignin structure rather than lignin content may have influenced digestibility. Beyond alterations in lignin content, modifications in lignin structure were described (Mechin et al., 2000; Marita et al., 2003) and seem to be important for changes in digestibility of the fibre fractions in Bm mutants. In contrast to the present findings, Holt et al. (2010) did not find changes of fibre digestibility when feeding a Bm silage-based diet. They suspected contradictory effects of components of the total ration, which could have diluted potential effects of the Bm silage.

Dry matter intake

With regard to the higher digestibility and *in situ* degradation of NDFom of the Bm silage, an increase in DMI could be expected (Oba and Allen, 1999). The lower DMI of Bm silage in Trial 1 may be explained by differences in BW. Differences between groups were not significant, but BW tended to be higher for Con-fed animals; therefore, DMI should be referred to BW. Dry matter intake per kg of BW and DMI per kg of $BW^{0.75}$ did not differ between Con and Bm in Trials 1 and 2, which has been shown for wethers (Block et al., 1982) and heifers (Ballard et al., 2001). Greenfield et al. (2001), Taylor and Allen (2005c) and Holt et al. (2010), furthermore, showed that Bm did not affect total DMI in dairy cows. Several authors, however, showed that feeding Bm silage positively affects DMI (Rook et al., 1977; Oba and Allen, 1999; Cherney et al., 2004; Ebling and Kung, 2004). According to Greenfield et al. (2001), it may be concluded that there is a trend for animals with a high energy demand to increase DMI when fed Bm. Oba and Allen (1999), Cherney et al. (2004) and Ebling and Kung (2004), for example who found an increased DMI while feeding Bm, used early to midlactating cows (80–140 DIM) with a high milk yied (>40 kg/day). Authors who found no effects on DMI in contrast used cows late in lactation (220–240 DIM) with a lower milk yield (24–33 kg/day) (Ballard et al., 2001 and Greenfield et al., 2001). Taylor and Allen (2005c) and Holt et al. (2010), who used high-yielding early lactation cows, however, did not show an increase in DMI.

Milk yield and composition

Effects of Bm silages on milk yield were described in the literature, but were not consistent and sometimes contradictory to effects on DMI (Rook et al., 1977; Castro et al., 2010). According to Rook et al. (1977), Sommerfeldt et al. (1979), Greenfield et al. (2001), Qiu et al. (2003), Castro et al. (2010) and Holt et al. (2010), no effects on milk yield were found in Trial 1. According to Cherney et al. (2004), Ebling and Kung (2004) and Kung et al. (2008), milk yield and/or FCM was increased in the Bm-fed animals in Trial 2.

Impact of Bm silages on milk yield may be an effect of increased *in vitro* fibre digestibility as suggested by Ballard et al. (2001) or increased DMI (Oba and Allen, 1999, 2000a,c), leading to an increase in energy available for milk production. As total DMI was lower at the same milk yield in Trial 1 and DMI was the same at a higher milk yield in Trial 2, it seems to be most likely that the present results are based on the higher fibre digestibility of the Bm silage. An increase in fibre digestibility should increase energy supply from fibre which should explain the impact on milk yield, as milk yield is mainly related to energy intake. Contradictory to this assumption, the higher total tract fibre digestibility and *in situ* fibre degradation did, however, not increase energy content of the Bm silage when fed to wethers at maintenance level (Table 3).

In both trials, milk fat content was lower in the Bm treatment, as described by Oba and Allen (2000a), Taylor and Allen (2005a) and Holt et al. (2010). Several other studies (Sommerfeldt et al., 1979; Oba and Allen, 1999; Castro et al., 2010) showed that there was no impact of Bm hybrids on milk fat content. Milk fat yield, however, was changed only in the trials of Holt et al. (2010), who found a decrease, and Oba and Allen (1999) who found an increase in milk fat yield. In Trial 1, milk fat yield was decreased while feeding the Bm diet, whereas in Trial 2, milk fat yield did not differ between diets.

The higher ADFom content of diets in Trial 2 may explain the higher milk fat content in relation to Trial 1. The slight difference in ADFom content between the Con and the Bm diet may, however, not explain the large difference in milk fat content within trials. As fibre content and fibre digestibility may affect ruminal SCFA proportions, further trials should be carried out with regard to ruminal fibre degradation and its impact on milk composition.

Milk urea content, furthermore, was decreased when Bm silage was fed. A decrease in milk urea content was described by Taylor and Allen (2005a) and Kung et al. (2008). According to Kung et al. (2008), milk protein content did not differ between diets in either trial, and milk protein yield was increased in Trial 2. The decrease in milk urea content might indicate reduced protein availability to the animal, which is supported by the decreased total tract CP digestibility from digestibility trials with wethers. Validity of CP digestibility, however, is limited in ruminants; therefore, further research on the impact of Bm on nitrogen utilization is necessary. Furthermore, amount of fermentable carbohydrates and rate of degradation of carbohydrates from the silages will have to be taken into account when evaluating nitrogen utilization. Changes in extent and rate of fibre breakdown might impact synchronicity of energy and nitrogen availability to rumen microorganisms and, thus, might impact efficiency and/or yield of microbial protein synthesis. With regard to the FPR, one could assume that animals in the

Bm treatment were suffering from subacute ruminal acidosis. Yet, for Weeks 1–3 of Trial 1, it could be shown that animals had mean ruminal pH values of 6.0, indicating that animals did not suffer from acidosis (Beauchemin and Yang, 2005). Furthermore, pH value did not differ between diets showing that there was presumably no dietary impact.

Blood parameters and energy balance

In general, it has to be considered that only one blood sample was taken per sampling day and as animals were fed *ad libitum* time since the last feed intake is unknown. According to Nielsen et al. (2003), however, feeding a TMR *ad libitum* may smooth diurnal variations in plasma BHB concentrations, and, thus, the impact of feed intake can be neglected.

Loss of BW was similar for both treatments, showing that there was an appreciable use of adipose tissue for energy supply in Trial 1 and animals regained BW in Trial 2. Thus, the positive energy balances based on digestibility data from trials with wethers may be not appropriate to draw conclusions on absolute energy supply to dairy cows. According to Enjalbert et al. (2001) and Hachenberg et al. (2007), the BHB values are, however, well within the normal range and show that animals did not suffer from subclinical or clinical ketosis despite the considerable loss of BW in Trial 1. In Trial 2, animals regained BW, but weight gain was higher for Bm-fed animals and Bm-fed animals had lower plasma BHB concentrations. Plasma concentrations of NEFA were also within the normal range (Enjalbert et al., 2001; Hachenberg et al., 2007), showing that animals were in good health status with regard to energy supply. Milk fat content, furthermore, was not increased to an extent that could be expected for animals suffering from ketosis. It has, however, to be mentioned that thresholds given in literature are adapted to animals early in lactation and cannot be transferred to mid-lactation cows in general.

Ruminal pH, ruminal temperature and rumination activity

Oba and Allen (2000a) and Taylor and Allen (2005b) found a reduced mean ruminal pH when feeding Bm. According to the results from Trial 1, Holt et al. (2010) did not find changes in ruminal pH.

The present results, furthermore, showed that also minimum and maximum pH as well as duration per day below pH 6.0, 5.8 and 5.5 did not differ between diets. Taylor and Allen (2005b) who found decreased mean and minimum pH and a tendency for increased time per day below pH 6.0, concluded that higher ruminal SCFA concentration contributed to pH changes. Oba and Allen (2000a), who found similar changes in ruminal pH, in contrast, did not observe changes in ruminal SCFA concentration.

The present results, thus, may indicate that increased total tract fibre digestibility did not influence ruminal fermentation despite a remarkable increase in *in situ* fibre degradation (Gorniak et al., 2013).

According to Oba and Allen (2000b), rumination activity did not differ between the diets in the present trials. It might be concluded that supply with dietary fibre was similar for both treatments despite a reduced total NDFom intake for Bm in Trial 1. Taylor and Allen (2005a), in contrast, noticed significantly reduced daily rumination duration in their Bm treatment; they concluded that increased fibre degradability and particle fragility made Bm silage less effective in stimulating rumination.

With regard to Webster et al. (1975) and Reynolds et al. (1991), it could be assumed that the higher DMI and energy intake of animals fed the Con diet accounted for the trend of the increased mean ruminal temperature. Heat production, however, does not arise from ruminal fermentation on its own; physical work, chemical energy for digestion and intracellular synthesis of adenosine triphosphate, for example, also contribute to the heat increment and have to be considered.

Conclusion

In relation to the Con hybrid, the experimental Bm hybrid had a higher total tract digestibility of CF, NDFom and ADFom in wethers but did not improve DMI in dairy cows. Total tract digestibility of CP was lower for Bm. Positive effects on milk yield and FCM yield, however, were shown, and the Bm silage decreased milk fat content. Brown-midrib corn silage did not impact ruminal pH values and rumination activity. The present trials showed that the Bm hybrid might have some advantages in relation to common hybrids. Further research, however, is necessary to rank actual Bm hybrids in relation to other standard non-Bm hybrids.

Acknowledgements

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 the trials and analyses is gratefully acknowledged. The authors further acknowledge the financial support of these trials by the 'Niedersächsisches Ministerium für Wissenschaft und Kultur' within the research network 'KLIFF' (climate impact and adaptation research) and 'Saaten-Union GmbH' Isernhagen, Germany.

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Chapter 4

Digestibility, ruminal fermentation, ingesta kinetics and nitrogen utilisation in dairy cows fed diets based on silage of a brown-midrib or a standard maize hybrid

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Archives of Animal Nutrition, 2014

Vol. 68, No. 2, 143-158, http://dx.doi.org/10.1080/1745039X.2014.897531

Digestibility, ruminal fermentation, ingesta kinetics and nitrogen utilisation in dairy cows fed diets based on silage of a brown-midrib or a standard maize hybrid

The aim of the present experiment was to investigate an experimental brown-midrib (Bm) maize hybrid in comparison with a control (Con) non-Bm maize hybrid on ruminal and total tract digestibility, ruminal fermentation, ruminal ingesta kinetics, nitrogen (N) utilisation and microbial efficiency. A total of six ruminally and duodenally cannulated German Holstein cows were used. Animals were fed diets of either 11.5 kg dry matter (DM) of a Con or a Bm maize silage plus 4.1 kg DM of concentrate. Ruminal and total tract digestibility of organic matter, neutral detergent fibre and acid detergent fibre did not differ between hybrids. Short-chain fatty acid concentrations and pH in the rumen were not affected, but ruminal mean retention time was lower for Diet Bm (Con: 45.4 ± 2.39 h; Bm: 40.6 ± 2.39 h; least squares means ± standard error). Cows fed Diet Bm had greater efficiency of N utilisation (Con: 30.1 ± 1.37%; Bm: 33.1 ± 1.37%) and increased flow of microbial crude protein at the duodenum (MCPF) (Con: 7.0 ± 0.37 g/MJ metabolisable energy (ME); Bm: 8.1 ± 0.37 g/MJ ME). Thus, MCPF and utilisable crude protein at the duodenum (uCP) were greater for Diet Bm (MCPF – Con: 1117 ± 52.1 g/d; Bm: 1306 ± 52.1 g/d; uCP – Con: 1594 ± 57.9 g/d; Bm: 1807 ± 57.9 g/d) and ruminal N balance was lower for Diet Bm (Con: 98.7 ± 8.92 g/d; Bm: 65.6 ± 8.92 g/d). The present results show that the Bm maize hybrid might be advantageous for dairy cow nutrition with regard to N utilisation and MCPF. However, further research is necessary to draw more precise conclusions on the potential of Bm maize hybrids in general.

Keywords: dairy cows; hybrid varieties; maize silage; microbial protein; nitrogen metabolism; rumen fermentation

1. Introduction

Brown-midrib (Bm) mutations in *Zea mays* L. were already discovered in 1924 (Ali et al. 2010) and have been investigated intensively in the last decades. The Bm mutations were characterised by a lower lignin content and an altered lignin structure and were reported to enhance performance in ruminants compared to non-Bm maize hybrids (Oba and Allen 1999, 2000a). It was assumed that improved performance was due to increases in dry matter intake (DMI) and/or digestibility (Oba and Allen 1999; Ebling and Kung 2004). As literature on effects of Bm maize on DMI, digestibility, ruminal fermentation and ruminal ingesta kinetics is inconsistent (Oba and Allen 2000b; Greenfield et al. 2001) and response variables may interact, mechanisms of effects cannot be characterised in detail. However, in Germany, no variety is available commercially at the time, which might be due to agronomic inferiority.

Therefore, the aim of the present experiment was to compare an experimental Bm maize hybrid with a control (Con) non-Bm maize hybrid as related to ruminal and total tract digestibility, nitrogen (N) utilisation and ruminal ingesta kinetics in lactating dairy cows fed maize silage-based diets. As proportion of Bm maize of total roughage DM varied widely in previous studies (Oba and Allen 2000a; Ballard et al. 2001; Greenfield et al. 2001; Kung et al. 2008), maize silage was used as the sole roughage in this study.

In situ experiments on ruminal degradation of the same silages (Gorniak et al. 2013) revealed that the Bm maize had a higher effective degradability. Therefore, it was hypothesised that the Bm maize hybrid would enhance ruminal fermentation and thus affect molar proportions of short-chain fatty acid (SCFA) and ruminal pH. It was, furthermore, hypothesised that ruminal rate of passage and the flow of microbial crude protein (CP) at the duodenum (MCPF) would be increased. Yet, the present study will not present a general overview but rather give a first insight into the properties of a Bm maize hybrid for dairy cow nutrition in Germany.

2. Materials and methods

2.1. Experimental design, animals and maize hybrids

The Con and Bm maize hybrids were grown at the experimental station of the Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany. Cutting height was approximately 18–20 cm, chopping length was 5.5 mm and kernels were crushed at harvesting. Harvest dates were 11 October 2010 (Con) and 12 October 2010 (Bm), dry matter (DM) at harvesting was 34.4% (Con) and 33.4% (Bm) and maize plants were harvested in the dough stage. DM yield per hectare was 20.8 t (Con) and 17.8 t (Bm). The Bm maize was an experimental hybrid "SUM 2368" (Saaten-Union GmbH, Isernhagen, Germany) and the Con maize hybrid was "Ronaldinio" (KWS-Saat AG, Einbeck, Germany), and it was chosen as a representative for medium maturing silage maize varieties regarding time of flowering, plant height, cold sensitivity, susceptibility to lodging, tillering, maturation, starch content and digestibility (Anonymous 2013). Silages were preserved in big bales and sealed with stretch foil.

The experiment was carried out at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Germany. A total of six lactating German Holstein cows were used. The animals were equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter 10 cm; Bar Diamond Inc. Parma, ID, USA) and T-shaped cannulas at the proximal duodenum close to the pylorus (inner diameter 2 cm;

MEDVET, Laatzen, Germany). The animals were housed in a tethered stall with neck straps and individual troughs with free access to water. Cows were milked at 05:30 and 15:30 h daily.

A two-treatment crossover design was used. Thus, the experiment consisted of two experimental periods with both treatments represented in both experimental periods. At the beginning of the experiment, body weight of the animals was 668 kg ± 41.1 kg, milk yield was 24.4 ± 5.4 kg/d and the animals were 88.5 ± 3.7 days in milk (means ± standard deviation). One cow was nine years and the others were six years of age. One cow was in her third lactation, four were in their fourth and one was in her fifth lactation. Cows were assigned to one of the two treatments according to milk yield. Treatments were: (1) Diet Con consisting per day on DM basis of 11.5 kg maize silage from the common hybrid “Ronaldinio” plus 4.1 kg concentrate and (2) Diet Bm consisting of 11.5 kg maize silage of the experimental Bm maize hybrid “SUM 2368” plus 4.1 kg concentrate. To ensure the intended maize silage to concentrate ratio, the DM content of maize silage was determined twice a week. Maize silage and concentrate were given in two equal meals at 5:30 h and 15:00 h. The pelleted concentrate was hand-mixed with the silage in the trough. The concentrate contained on DM basis 36.5% wheat grain, 25.0% solvent-extracted soybean meal, 13.0% solvent-extracted rapeseed meal, 18.3% dried sugar beet pulp, 2.5% calcium carbonate, 2.5% urea, 0.2% sodium chloride and 2.0% mineral and vitamin premix. The mineral and vitamin premix contained per kilogram 170 g Ca, 50 g P, 120 g Na, 45 g Mg, 800,000 IU vitamin A, 100,000 IU vitamin D3, 4000 mg vitamin E, 4000 mg Mn, 6000 mg Zn, 1300 mg Cu, 120 mg I, 35 mg Co and 40 mg Se.

2.2. Sample collection

Each experimental period lasted five weeks, beginning with two weeks of adaptation to the diet followed by three weeks of sample collection. During collection weeks one and two, samples of maize silage, concentrate and feed refusals were collected daily and pooled weekly for chemical analyses.

In the first sampling week, total collection of urine and faeces was carried out using urine devices that were adhered around the vulva using a contact adhesive. The urine devices were joined to a plastic container via a pressure-resistant plastic tube, thus allowing for quantitative collection of urine. Sulphuric acid (20% v/v, 250 ml per animal and d) was used as receiver to prevent ammonia losses from urine containers. Faeces were collected in stainless steel vats beneath the slates of the stalls. Urine and faeces were weighed daily; a subsample was taken and stored at −20°C for further analyses. Milk samples were collected on two days per week on two consecutive milking times. A portion of 50 ml was conserved with bronopol and stored at 8°C and a portion of 100 ml was stored at −20°C until further analyses.

In the second sampling week, duodenal chyme was collected every 2 h for five consecutive days. Each sample consisted of four 100-ml subsamples, and the pH of each subsample was measured immediately after withdrawal using a pH meter (pH525) equipped with a glass electrode (SenTix 21; both WTW, Weilheim, Germany). The sample with the lowest pH was added to the daily collective sample for each cow and stored at −20°C until further analyses (Rohr et al. 1979).

Chromium oxide (Cr_2O_3) was used as a marker to estimate duodenal ingesta flow. The marker consisted of 19.8% Cr_2O_3, 1.0% Al_2SO_4 and 79.2% wheat flour. Beginning 10 days before duodenal sampling, 100 g of marker was inserted into the rumen in two portions of 50 g every 12 h. One day before and during the entire sampling week, the marker was given in four portions of 25 g every 6 h.

During the third sampling week, ruminal fermentation characteristics and particle turnover were determined. To determine ruminal fermentation characteristics, ruminal fluid was collected from the ventral sac of the rumen via the rumen cannula using a manually operated vacuum pump. Approximately 100 ml of fluid was taken immediately before the morning feeding at 5:30 and 30, 60, 90, 150, 240 and 480 min afterwards.

To determine ruminal particle turnover, a single dose of 200 g of chromium-mordanted fibre was administered into the rumen via rumen cannula. Chromium-mordanted fibre was prepared according to Udén et al. (1980). Duodenal chyme was collected at 2, 4, 6, 8, 10, 12, 14, 16, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 66, 78, 90, 102, 114, 126 and 138 h after marker administration. Approximately 300 ml of duodenal chyme was collected per sampling time and stored at −20°C until samples were prepared for chromium analysis.

2.3. Analyses

Feedstuffs and feed refusals were dried at 60°C for 72 h in a forced-air oven and faeces were freeze-dried. Samples were ground to pass through a 1 mm screen using a Retsch mill (SM 1; Retsch, Haan, Germany) and analysed according to the methods of Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (2007, method numbers are given) for DM (3.1), ash (8.1), CP (feedstuffs: Dumas method, 4.1.2; faeces: Kjeldahl method, 4.1.1), starch (7.2.1), neutral detergent fibre (NDFom; 6.5.1), acid detergent fibre (ADFom; 6.5.2), and acid detergent lignin (ADL; 6.4.1). NDF and ADF were expressed without residual ash and are therefore referred to as NDFom and ADFom.

Milk samples were analysed for protein, fat and urea using an infrared milk analyser (Milkoscan FT 6000; Foss Analytical, Hillerød, Denmark). Milk N was calculated as milk protein divided by 6.38. Nitrogen concentration of duodenal chyme and urine were determined using the Kjeldahl method (4.1.1). For all matrices, CP content was calculated from N analysis ($CP = N \cdot 6.25$), except for milk. Ammonia concentration in duodenal chyme and ruminal fluid was determined by steam distillation into a boric acid solution and subsequent titration with hydrochloric acid (Anonymous 1998). Total N and ammonia content of duodenal chyme was analysed in freshly thawed material; all other analyses were carried out using freeze-dried and ground material. The DM, ash, CP and ammonia and chromium contents of duodenal chyme were determined from the daily pooled samples (sample week two). For Cr_2O_3 analyses in duodenal chyme samples were prepared according to Williams et al. (1962) and chromium content was quantified using an optical emission spectrometer with inductively coupled plasma (ICPOES Quantima; GBC Scientific Equipment Pty. Ltd., Melbourne, Victoria, Australia) (sample week two and three). For analyses of NDFom and ADFom, chyme samples were pooled per animal on a weekly basis according to duodenal DM flow (DMF) calculated from chromium contents (sample week two).

Microbial CP content was estimated by near-infrared reflectance spectroscopy of freeze-dried duodenal chyme according to Lebzien and Paul (1997) using a NIR Analyser (Foss NIRSystems Model 5000; FOSS Analytical, Hillerød, Denmark) (sample week two). The NIR system estimates the proportion of microbial N of non-ammonia nitrogen (NAN). From the NAN content in duodenal chyme and the proportion of microbial N of NAN, the amount of microbial N can be calculated.

Directly after collection of ruminal fluid, the pH was determined using a pH meter (pH525) equipped with a glass electrode (SenTix 21; both WTW). Afterwards the ammonia-N concentration was analysed as reported above (Anonymous 1998) and SCFA concentrations were analysed as described by Geissler et al. (1976) using a gas chromatograph (5890 II) equipped with an automatic injector (7673 II) and an integrator (3396 II; all Hewlett-Packard, Avondale, PA, USA). Furthermore, ruminal pH was recorded every 10 min for 48 h for each cow in the first sampling week of each period. For this purpose, rumen probes equipped with a glass electrode (KB1000; Kahne Animal Health, Auckland, New Zealand) were inserted into the animals per fistulam. From these recordings, the time of pH below 6.0, 5.8 and 5.5 [min/d] was calculated.

2.4. Calculations and statistics

2.4.1. Digestibility and nitrogen utilisation

Metabolisable energy (ME) and net energy for lactation (NEL) of the diets were calculated according to Gesellschaft für Ernährungsphysiologie (2001) (nutrients in g/kg):

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

$$\text{NEL [MJ/kg]} = 0.6 \cdot [1 + 0.004 \cdot (q - 0.57)] \cdot \text{ME [MJ/kg]} \quad (2)$$

where DEE is the digestible ether extract, DCF is the digestible crude fibre, DOM is the digestible organic matter (OM) and q is the quotient of ME and gross energy.

Fat-corrected milk (4% fat) (FCM) was calculated according to Gaines (1928).

$$\text{FCM [kg/d]} = (\text{milk fat content [\%]} \cdot 0.15 + 0.4) \cdot \text{milk yield [kg/d]} \quad (3)$$

Nitrogen balance was calculated as N intake minus faecal N excretion minus urinary N excretion minus N excretion with milk. Efficiency of N utilisation was expressed as percentage of N intake excreted with milk.

DMF was calculated as follows:

$$\text{DMF [kg/d]} = \text{Cr-application [mg/d]}/ (\text{duodenal Cr concentration [mg/g DM]} / 1000) \quad (4)$$

2.4.2. Duodenal ingesta flow

Daily duodenal OM and nutrient flows were then calculated by multiplication of their concentration in duodenal chyme with DMF.

Utilisable CP at the duodenum (uCP) was estimated according to Lebzien and Voigt (1999):

$$\text{uCP [g/d]} = \text{CP flow at the duodenum [g/d]} - NH_3\text{-N [g/d]} \cdot 6.25 - \text{endogenous CP [g/d]} \quad (5)$$

MCPF was calculated from NAN content of duodenal chyme and microbial N proportion of NAN as follows:

$$\text{MCPF [g/kg DM]} = [(\text{N-content of duodenal chyme [g/kg DM]} \\ - \text{NH}_3\text{-N of duodenal chyme [g/kg DM]}) \cdot \\ \text{microbial N proportion of NAN}] \cdot 6.25 \quad (6)$$

Endogenous CP was estimated from DMF according to Brandt et al. (1980):

$$\text{Endogenous CP [g/d]} = 3.6 \cdot \text{DMF [kg]} \cdot 6.25 \quad (7)$$

Ruminal N balance (RNB) and ruminally degraded CP (RDP) as well as ruminally undegraded feed CP (RUP) and ruminally fermented OM (FOM) were estimated using the following equations:

$$\text{RNB [g/d]} = (\text{CP intake [g/d]} - \text{uCP [g/d]}) / 6.25 \quad (8)$$

$$\text{RUP [g/d]} = 6.25 \cdot (\text{NAN flow at the duodenum [g/d]} \\ - \text{microbial N flow at the duodenum [g/d]}) \\ - \text{Endogenous CP flow at the duodenum [g/d]} \quad (9)$$

$$\text{RDP [g/d]} = \text{CP intake [g/d]} - \text{RUP [g/d]} \quad (10)$$

$$\text{FOM [kg/d]} = \text{OM intake [kg/d]} - (\text{Duodenal OM flow [kg/d]} \\ - \text{Microbial OM [kg/d]}) \quad (11)$$

The microbial OM was calculated according to Schafft (1983):

$$\text{Microbial OM [kg/d]} = 11.8 \cdot \text{Microbial N [g/d]} \quad (12)$$

2.4.2. Ruminal ingesta kinetics

Duodenal marker flow curves were fitted to a series of two-compartment models with no age dependency of the rate parameters in Compartment 1 and Compartment 2 (G1G1) or increasing order of gamma age dependency in Compartment 1 and no age dependency in Compartment 2 [GnG1, where n is the order of gamma age dependency (2–4)] using Proc NLIN (SAS Version 9.2., SAS Institute Inc., Cary, NC, USA) and Marquardt method for iteration (Marquardt 1963) as described by Pond et al. (1988). As ingesta flow was considered to be a multi-compartmental process, the regression equation consisted of an exponential and a double-exponential term, as follows:

$$y_t = Ae^{-kt} \exp [-Be^{-\lambda t}] \quad (13)$$

where yt is the marker concentration in duodenal chyme at time t, A is an undefined parameter, B is the number of compartments, λ is the fractional passage rate from the first compartment and k is the fractional passage rate from Compartment 2.

Model fit was estimated on the basis of average sum of squares error (SSE). The G3G1 model was chosen, as average SSE was lower compared to G4G1 indicating better fit. The G1G1 and G2G1 models did not converge for all animals and were thus not used. Mean retention time in the Compartment 1 (CMRT1) was calculated as n/λ.

Mean retention time in Compartment 2 (CMRT2) was calculated as 1/k. Pre-duodenal total mean retention time (TMRT) was calculated as CMRT1 + CMRT2 + TD, where TD denotes the time delay between marker dose and first marker appearance in duodenal chyme.

2.4.3. Statistical analyses

For statistical analyses, the SAS software package was used (SAS Version 9.2., SAS Institute Inc.) applying the MIXED procedure. Diet, experimental period and diet by period interaction were considered to be the fixed factors. For ruminal pH, ruminal ammonia and ruminal SCFA concentrations, sampling times were added to the model. As the same cows were used in both experimental periods for different treatments, the RANDOM statement was used to take individual cow effect into account. The following models were used:

$$y = \mu + c_i + d_j + p_k + dp_{jk} + e_{ijk} \quad (14)$$

(all variables except ruminal pH and concentration of ammonia and SCFA)

$$y = \mu + c_i + d_j + p_k + t_l + dp_{jk} + dt_{jl} + e_{ijkl} \quad (15)$$

(ruminal pH and concentration of ammonia and SCFA), where μ is the overall mean, c is the random effect of the cow (i = 1–6), d is the fixed effect of the diet (k = 1–2), p is the fixed effect of the period (j = 1–2), t is the fixed effect of sampling time (l = 1–7), dp is the interaction of diet and period, dt is the interaction of diet and sampling time and e_{ijk}/e_{ijkl} is the residual error.

Evaluation of variances was done using the restricted maximum likelihood (REML) method, and the Kenward–Rogers method was applied to estimate degrees of freedom. Tukey–Kramers test was used for post-hoc analyses. Unless otherwise stated, results are presented as least squares means (LSmeans) with the standard error of the means (SEM). Differences between LSmeans were accepted to be significant if F-test calculated a p-value < 0.05.

3. Results

3.1. Chemical composition

The mean values of the chemical composition of silages, concentrate and diets are given in Table 1. Silages and diets did not differ, except for a numerically higher content of starch and a numerically lower content of ADL in the Bm treatment. Although maize kernels were cracked at harvesting, 5% of the kernels of both Con and Bm maize silage were intact.

3.2. Milk yield and composition

Milk and FCM yields and milk fat yield and milk composition did not differ between diets. Protein yield was higher and milk urea concentration was lower for Diet Bm. Milk yield was not different for treatments Con and Bm (milk yield: Con 22.3 kg/d, Bm 24.1 ± 1.67 kg/d; FCM: Con 18.7 kg/d, Bm 20.6 ± 1.49 kg/d). Milk composition and yield of milk ingredients did also not differ, except for protein yield, which was higher for Diet Bm (milk protein: Con 3.0%, Bm 3.0 ± 0.13%; fat: Con 2.9%, Bm 3.1 ± 0.19%; protein: Con 665 g/d, Bm 732 ± 30.6 g/d; fat: Con 652 g/d, Bm 730 ± 64.5 g/d). Milk urea content was higher for Diet Con (Con 147.5 mg/L, Bm 93.4 ± 18.26 mg/L). Values are LSmeans with SEM.

3.3. Ruminal and total tract digestibility

Apparent total tract digestibility of the diets did not differ between Diets Con and Bm (Table 2). Energy content was the same for both diets (Table 1), and apparent ruminal digestibility of OM, NDFom, and ADFom was not influenced by diet (Table 2).

Table 1. Nutrient content of silages, concentrate and total diets and energy content of the total diets.

	Silages[†] (n = 6)[*]			Diets[♦] (n = 6)	
	Control maize	Brown-midrib maize	Concentrate[◊] (n = 6)	Control (Con)	Brown-midrib (Bm)
Nutrients [g/kg DM[‡]]					
Organic matter	954 ± 2.2	951 ± 2.5	915 ± 2.8	944 ± 1.5	942 ± 1.5
Crude protein	84 ± 3.3	82 ± 3.8	309 ± 9.9	143 ± 3.2	141 ± 4.6
Ether extract	33 ± 2.7	28 ± 3.2	26 ± 2.1	31 ± 2.0	27 ± 2.5
Starch	257 ± 6.2	283 ± 11.2	304 ± 7.6	269 ± 5.9	289 ± 8.4
Crude fibre	239 ± 10.1	228 ± 7.1	84 ± 2.0	198 ± 7.5	190 ± 5.4
Neutral detergent fibre	497 ± 15.5	472 ± 28.5	242 ± 24.0	431 ± 16.2	412 ± 25.2
Acid detergent fibre	272 ± 9.1	249 ± 11.4	123 ± 8.1	233 ± 7.8	216 ± 10.1
Acid detergent lignin	35 ± 2.5	15 ± 1.2	27 ± 3.8	33 ± 2.6	18 ± 1.8
ME[§] [MJ/kg DM]	$10.5^b \pm 0.16$	$10.9^a \pm 0.13$	12.9 ± 0.09	10.2 ± 0.14	10.2 ± 0.14
NEL[¶] [MJ/kg DM]	$6.3^b \pm 0.12$	$6.6^a \pm 0.10$	8.1 ± 0.06	6.1 ± 0.08	6.1 ± 0.08

Notes: [†]Analysed nutrient contents (arithmetic means ± standard deviation [SD]), energy contents were estimated by enzyme soluble organic matter (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten 2007, Method No. 6.6.1) (LSmeans ± standard errors [SE]); [*]Number of feed samples analysed; [◊]Analysed nutrient contents (arithmetic means ± SD), energy contents were estimated by the Hohenheim gas test (Menke and Steingass 1988) (LSmeans ± SE); [♦]Nutrient content was calculated from analysed nutrients of silages and concentrate (arithmetic means ± SD), energy content of the total diet was estimated *in vivo*, see Sections 2.2. and 2.4. (LSmeans ± SE); [‡]DM, Dry matter; [§]ME, Metabolisable energy; [¶]NEL, Net energy lactation; [ab]Means with different superscripts are significantly different ($p < 0.05$).

Table 2. Effects of silage maize variety on ruminal and total tract digestibility (LSmeans with SEM[†]).

	Experimental diets			*p*-values[‡]		
	Con	Bm	SEM	Hybrid	Period	Hybrid × period
Organic matter						
Intake [kg/d]	14.7	14.8	0.14	0.122	0.004	0.809
Apparently digested in the rumen [%]	49.1	46.2	1.83	0.234	0.045	0.790
Truly digested in the rumen [%]	63.4	62.9	1.33	0.718	0.056	0.990
Apparently digested in the total tract [%]	69.5	70.4	1.05	0.349	0.395	0.190
NDFom[¶]						
Intake [kg/d]	6.6	6.5	0.73	0.177	0.001	0.126
Apparently digested in the rumen [%]	47.9	50.4	2.90	0.435	0.036	0.918
Apparently digested in the total tract [%]	55.4	57.6	1.86	0.085	0.235	0.099
ADFom[◊]						
Intake [kg/d]	3.6	3.5	0.04	0.003	0.001	0.649
Apparently digested in the rumen [%]	45.1	47.8	3.03	0.383	0.036	0.666
Apparently digested in the total tract [%]	49.4	54.3	2.47	0.092	0.979	0.145

Notes: [†]SEM, Standard error of the means; [‡]Effects of maize hybrid, experimental period and maize hybrid × period interaction; [¶]NDFom, Neutral detergent fibre expressed without residual ash; [◊]ADFom, Acid detergent fibre expressed without residual ash.

3.4. Ruminal fermentation and ingesta passage

Ruminal measurements revealed no differences in fermentation characteristics between the two diets, as there were no differences in mean, minimum and maximum pH. Diets did not affect the time when the pH was below 6.0, 5.8 and 5.5 (Table 3). Ruminal ammonia concentration and SCFA concentrations did not differ between Diets Con and Bm except for molar proportions of isovaleric acid which was higher for Diet Con (Table 3).

Rate of passage from the Compartment 1 was the same for both diets. The rate of passage from the Compartment 2 was faster and TMRT was lower after feeding Diet Bm (Table 4).

3.5. Nitrogen utilisation and MCPF

Faecal N excretion was higher in cows fed Diet Bm, whereas urinary N excretion tended to be lower in these animals ($p = 0.066$; Table 5). Furthermore, animals that received Diet Bm excreted more N via milk; thus, the efficiency of N utilisation was greater.

Feeding Diet Bm resulted in a higher total N and MCPF. When expressed per MJ ME or per g RDP, MCPF was also greater when cows received the Bm diet. Flow of RUP and RUP as percentage of CP intake were not affected. RNB [g/d and g/MJ ME] was lower and uCP was higher after feeding Diet Bm (Table 5).

Table 3. Effects of silage maize variety on ruminal fermentation (LSmeans with SEM[†]).

	Experimental diets			*p*-values[‡]		
	Con	Bm	SEM	Hybrid	Period	Hybrid × period
Mean pH	6.45	6.41	0.047	0.486	0.587	0.776
Maximum pH	7.09	7.14	0.086	0.395	0.209	0.464
Minimum pH	5.72	5.66	0.064	0.341	0.099	0.189
pH < 6.0 duration [min/d]	112	206	54.1	0.281	0.488	0.313
pH < 5.8 duration [min/d]	35	89	29.7	0.195	0.296	0.253
pH < 5.5 duration [min/d]	8	17	7.8	0.060	1.000	0.344
NH_3 [mmol/L]	13.8	13.8	0.42	0.977	< 0.001	< 0.001
SCFA[§] total [mmol/L]	89.4	84.5	6.49	0.626	0.003	0.003
Acetic acid [mol%]	65.7	63.8	1.01	0.252	< 0.001	< 0.001
Propionic acid [mol%]	19.8	20.4	0.57	0.470	0.003	< 0.001
Butyric acid [mol%]	13.1	14.3	0.84	0.351	0.942	< 0.001
Valeric acid [mol%]	0.4	1.1	0.19	0.070	< 0.001	< 0.001
Isobutyric acid [mol%][¶]	-	-		-	-	-
Isovaleric acid [mol%]	1.0	0.4	0.16	0.047	< 0.001	< 0.001
Acetic acid : propionic acid	3.4	3.2	0.13	0.465	< 0.001	< 0.001

Notes: [†]SEM, Standard error of the means; [‡]Effects of maize hybrid, experimental period and maize hybrid × period interaction; [§]SCFA, Short-chain fatty acids; [¶]Concentrations were outside analytical accuracy.

Table 4. Effects of silage maize variety on ruminal ingesta kinetics (LSmeans with SEM[†]).

	Experimental diets			*p*-values[‡]		
	Con	Bm	SEM	Hybrid	Period	Hybrid × period
λ^* [h^{-1}]	0.179	0.172	0.0102	0.491	0.338	0.221
$k^\#$ [h^{-1}]	0.039	0.048	0.0038	0.037	0.402	0.275
TD[◊] [h]	1.3	1.4	0.40	0.664	0.041	0.687
CMRT1[§] [h]	17.1	17.6	0.90	0.554	0.365	0.202
CMRT2[$] [h]	27.0	21.6	2.49	0.097	0.904	0.314
TMRT[+] [h]	45.4	40.6	2.39	0.041	0.182	0.616

Notes: [†]SEM, Standard error of the means; [‡]Effects of maize hybrid, experimental period and maize hybrid × period interaction; [*]λ, Passage rate from Compartment 1; [#]k, Passage rate from Compartment 2; [◊]TD, Time delay to first marker appearance in duodenal chyme; [§]CMRT1, Mean retention time in Compartment 1; [$]CMRT2, Mean retention time in Compartment 2; [+]TMRT, Pre-duodenal total mean retention time.

4. Discussion

4.1. Silages

Reduced lignin and increased starch contents were described for Bm maize hybrids compared to isogenic hybrids as well as other non-Bm maize hybrids (Muller et al. 1972; Mustafa et al. 2005). In fact, no general conclusions for the examined Bm maize hybrid can be drawn, as only one hybrid was available for comparison. The present results, however, show that a more detailed examination of nutrient composition of the Bm maize hybrid is necessary to characterise differences to commercial non-Bm maize hybrids that are already available.

4.2. Milk yield and composition

Milk and FCM yields were not affected in the present experiment, which is in line with the findings of Rook et al. (1977), Sommerfeldt et al. (1979) and Greenfield et al. (2001). In a recent experiment with the same silages that were offered for ad libitum intake, Bm maize increased milk yield of cows compared to the Con group (Gorniak et al. 2013). Increased milk yield in response to Bm maize was also shown by Oba and Allen (2000a), Ballard et al. (2001) and Kung et al. (2008). The main reason was that Bm maize might lead to an increase in energy available for milk production due to increased DMI (Oba and Allen 1999), increased fibre digestibility (Ballard et al. 2001), more consistent energy supply from the rumen (Oba and Allen 2000a) or greater postruminal starch digestion (Oba and Allen 2000b). For the present experiment, however, DMI, energy intake and ruminal fibre digestion were the same for both diets, and therefore, changes in milk yield were not expected.

The higher milk protein yield in response to Bm maize agrees with Oba and Allen (1999, 2000a) and Gorniak et al. (2013). Lower milk urea nitrogen or milk urea contents were described by Kung et al. (2008) and Gorniak et al. (2013). Increases in milk protein yield can largely be attributed to increased energy intake from non-fibre carbohydrates (Oba and Allen 1999). As energy intake was the same for both diets and rations did not differ in ruminal fermentation characteristics (Table 3), it seems likely that the increase in protein yield can be attributed to a greater N use efficiency in cows fed Diet Bm. This conclusion, furthermore, agrees well with the lower milk urea concentration in cows fed Diet Bm. The absolute level of milk protein content is surprisingly low but cannot be explained finally.

Table 5. Effects of silage maize variety on nitrogen utilisation and nitrogen balance (LSmeans with SEM[†]).

	Experimental diets			*p*-Values[‡]		
	Con	Bm	SEM	Hybrid	Period	Hybrid × period
N intake [g/d]	353.8	354.7	0.98	0.501	< 0.001	0.246
Faecal N [g/d]	91.5	102.5	2.84	0.024	0.753	0.697
Urinary N [g/d]	123.0	102.1	7.38 / 6.42[§]	0.066	0.474	0.623
Milk N [g/d]	104.2	114.7	4.79	0.021	0.035	0.320
Efficiency of N utilisation [%]	30.1	33.1	1.37	0.022	0.005	0.279
N-balance [g/d]	36.2	35.5	6.92 / 5.73[§]	0.941	0.004	0.103
N-flow [g/d]	300.1	332.0	10.64	0.054	0.019	0.249
NAN[◊]-flow [g/d]	287.3	323.6	10.20	0.037	0.020	0.225
MCPF[*] [g/d]	1117.2	1305.8	52.10	0.018	0.023	0.527
MCPF [g/kg FOM[$]]	120.6	141.6	7.67	0.066	0.048	0.565
MCPF[g/kg ME[#]]	7.0	8.1	0.37	0.029	0.077	0.882
MCPF [g/g RDP[¶]]	0.64	0.76	0.031	0.025	0.088	0.301
RUP[♦]-flow [g/d]	477.2	501.2	23.03	0.483	0.049	0.043
RUP-flow [% of feed CP[||]]	25.4	26.4	1.20	0.567	0.296	0.084
RDP [g/d]	1733.7	1715.7	24.45	0.575	0.074	0.166
RNB[&] [g/d]	98.7	65.6	8.92	0.028	0.170	0.236
RNB [g/MJ ME]	0.62	0.41	0.053	0.023	0.105	0.197
uCP[+]-flow [g/d]	1594.4	1807.0	57.86	0.036	0.022	0.201

Notes: [†]SEM, Standard error of the means; [‡]Effects of maize hybrid, experimental period and interaction of maize hybrid × period; [§]Due to one missing value in treatment control, standard error instead of SEM is presented; [◊]NAN, Non-ammonia nitrogen; [*]MCPF, Flow of microbial crude protein at the duodenum; [$]FOM, Ruminally fermented organic matter; [#]ME, Metabolisable energy; [¶]RDP, Rumen degradable protein; [♦]RUP, Rumen undegradable protein; [||]CP, Crude protein, corrected for urea nitrogen; [&]RNB, Ruminal nitrogen balance; [+]uCP, Utilisable crude protein at the duodenum.

4.3. Ruminal and total tract digestion and ruminal ingesta kinetics

4.3.1. OM digestibility

Silages did not differ in apparent total tract digestibility of OM. Similar results were reported by Oba and Allen (1999) and Qiu et al. (2003). Furthermore, silages did not differ in true ruminal digestibility of OM which is in accordance with Qiu et al. (2003) and Taylor and Allen (2005b). Greenfield et al. (2001) reported a trend for a greater apparent ruminal OM digestibility for Bm maize, and Oba and Allen (2000b) reported a lower true ruminal OM digestibility for Bm maize, indicating that differences in ruminal digestion were compensated in the lower gut, as in both studies total tract digestibility did not differ between silages.

4.3.2. Fibre digestibility and rate of passage

Results of fibre digestibility were in line with OM digestibility; maize silage neither affected ruminal nor total tract digestibility. This agrees with Oba and Allen (2000b) and Qiu et al.

(2003). In contrast, in other studies, it was found that Bm maize silage had greater total tract and/or ruminal digestibility of NDF or ADF than silages from conventional hybrids (Greenfield et al. 2001; Ebling and Kung 2004; Gorniak et al. 2013). The Bm maize may have a higher digestibility because of its lower lignin contents (Muller et al. 1972), altered lignin structure (Sommerfeldt et al. 1979) or both. Holt et al. (2010) suggested that characteristics of the total diet may compensate for positive effects of Bm maize. Wethers fed the same silages without concentrate showed higher NDFom and ADFom digestibilities for Bm maize than for Con maize (Gorniak, Meyer, and Dänicke 2013). On the contrary, in the present experiment, only a trend was observed for an increased digestibility of NDFom ($p = 0.085$) and ADFom ($p = 0.092$); thus the concentrate proportion in the experimental diet might have diluted the Bm maize effects.

Rate of passage and rate of *in situ* degradation of NDFom might, furthermore, help to explain the present results on NDFom digestibility. Passage rate from the rumen of Diet Bm was higher than passage rate of Diet Con, which agrees with Oba and Allen (2000b). The enhanced rate of passage might be explained by the higher degradation rate of NDFom found *in situ* (Gorniak et al. 2013). This also indicates that Bm maize particles were more susceptible to microbial fermentation which might have increased particle breakdown and thus affected physical properties of ingesta particles (e.g. buoyancy) in a manner that particles had a greater probability of leaving the rumen. Increased rate of passage due to increased degradation would explain missing effects on ruminal digestibility of NDFom. Thus, it appears that, in comparison to Con maize, Bm maize was not digested to a greater extent in the same time, but less time was necessary to reach the same extent of digestion which might increase DMI in animals fed ad libitum.

4.4. Fermentation characteristics

Ruminal fermentation obviously was unaffected by Bm maize silage as neither total SCFA concentration, molar proportions of SCFA (except for isovaleric acid), nor pH or ammonia concentrations differed between Diets Con and Bm.

Literature on the effects of Bm maize on ruminal fermentation is inconsistent. Holt et al. (2010) observed no effect on ruminal SCFA concentrations and ruminal pH. Oba and Allen (2000a) showed that ruminal SCFA concentrations were not affected, although ruminal OM digestibility of Bm maize was reduced. Qiu et al. (2003) and Taylor and Allen (2005a, 2005b) reported changes in molar proportions of SCFA and total SCFA concentrations without any changes in extent of ruminal digestion. Greenfield et al. (2001) showed an increase in ruminal fibre digestion and a decrease in ruminal starch digestion, leading to lower molar proportions of isobutyrat, valerate and isovalerate.

Extent and rate of ruminal digestion will, however, not necessarily affect ruminal SCFA concentration or molar SCFA proportion as ruminal mucosa might adapt to changes in SCFA production and thus adjust absorption (Dijkstra et al. 1993). Therefore, in further experiments, analyses of ruminal SCFA production rates and blood concentrations of glucose, insulin and SCFA would be of interest, especially with regard to metabolic use of end products of fermentation (i.e. direction of nutrients and energy towards milk production or towards tissue deposition).

4.5. MCPF and N utilisation

The Diet Bm resulted in higher faecal N excretion; higher flow of NAN, MCPF and uCP and a lower RNB. These results might be due to the higher efficiency of microbial protein synthesis. The lower milk urea concentration, the tendency for a lower N excretion with urine and the lower RNB substantiate that ruminal microorganisms used the available N more efficiently. Similar results were found by Oba and Allen (2000b), and it can be concluded that the higher MCPF can be attributed to the higher rate of passage of fibre fractions in Bm maize-fed cows (Table 4). Higher ruminal passage rates are associated with a decrease in microbial turnover (National Research Council 1985; Wallace and McPherson 1987; Wells and Russell 1996) because of reduced predation of bacteria by protozoa and reduced microbial lysis and a decrease in microbial maintenance requirement. Furthermore, the increased passage rate of fibre fractions (Table 4) and the higher *in situ* degradation of DM, NDFom, and ADFom of Bm maize silage (Gorniak et al. 2013) denote improved energy availability to rumen microorganisms also leading to an increase in MCPF.

The absolute value of MCPF was rather low. However, animals had a comparably low DMI and milk yield. Nevertheless, as 5% of the maize kernels were intact, energy available to ruminal microorganisms might have been reduced. Therefore, the low MCPF might be in part a result of limited energy available in the rumen. Indeed, the situation was the same for both silages, and therefore validity of the results is not limited.

The similar amount of RDP and the higher MCPF (i.e. the higher uCP flow) in cows fed Bm maize shows that the higher faecal N excretion of Bm maize silage was either related to lower postruminal CP disappearance, higher postruminal secretion of endogenous CP or hindgut fermentation (National Research Council 1985). Despite the higher faecal N output in cows fed Bm maize, CP was used more efficiently (see above). As N intake did not differ between Diets Con and Bm, the higher efficiency of N utilisation can be attributed to the increase in milk protein yield. Together with the increase in milk N, the trend (p = 0.065) for a decrease in urinary N led to similar results for both treatments regarding the N balance. The unexpectedly high positive N balances cannot be fully explained, but it has to be taken into account that N balances in adult ruminants are often greater than expected (Spanghero and Kowalski 1997). In contrast to the present findings, it was already reported that Bm maize silage did not affect N utilisation in ruminants (Qiu et al. 2003; Taylor and Allen 2005a). However, it has to be considered that the Con variety represents only one possible counterpart to the Bm maize hybrid. Therefore, the present results have to be validated in further experiments, compared to isogenic variations and other commercial hybrids.

5. Conclusions

The experimental Bm maize hybrid did not affect total tract and ruminal digestibility of OM and fibre fractions; moreover, ruminal SCFA concentrations and ruminal pH were not altered. Nonetheless, Bm maize affected ruminal ingesta kinetics and MCPF as well as efficiency of N utilisation. The increase in MCPF and its efficiency as well as the decreased ruminal retention time indicate that Bm maize might have some advantages compared to standard maize hybrids for dairy production. Further research on Bm maize, however, is needed to describe the mode of action of Bm maize hybrids in more detail and to evaluate the potential of Bm maize hybrids for dairy production compared to standard hybrids, e.g. increased DMI due to reduced ruminal retention time. Especially a more detailed investigation of Bm maize hybrids, their isogenic counterparts and a wider range of commercial hybrids will be necessary to rank the tested Bm maize hybrid and to validate the first results shown.

Acknowledgements

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 the experiment and analyses is gratefully acknowledged. The authors, furthermore, thank Merle Alex, Julius-Kühn-Institut, Braunschweig, Germany, for assistance with NIR analysis.

Funding

The authors acknowledge financial support provided by the "Niedersächsisches Ministerium für Wissenschaft und Kultur" within the research network "KLIFF" (climate impact and adaptation research) and by Saaten-Union GmbH, Isernhagen, Germany.

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Chapter 5

Effect of ambient temperature on nutrient digestibility and nitrogen balance in sheep fed brown-midrib maize silage

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Archives of Animal Nutrition, 2014
Vol. 68, No. 4, 336-344, http://dx.doi.org/10.1080/1745039X.2014.931017

Effect of ambient temperature on nutrient digestibility and nitrogen balance in sheep fed brown-midrib maize silage

The aim of the experiment was to determine the impact of heat stress on nutrient digestibility and nitrogen balance in sheep fed silages differing in fibre quality. The digestibility trial was conducted at three different ambient temperatures (15°C, 25°C and 35°C for 24 h/d). The tested brown-midrib maize (Bm) silage had a higher nutrient digestibility, except for ether extract (EE) and a higher metabolisable energy (ME) content than the control maize (Con) silage. Nitrogen (N) excretion with faeces was higher but N excretion with urine was lower for sheep fed Bm silage, subsequently N balance did not differ between the two silages. Temperature had no effect on nutrient digestibility, except for crude protein (CP), but N excretion with urine was lower at elevated temperatures. A diet by temperature interaction was found for dry matter (DM) and organic matter (OM) digestibility. When the ambient temperature increased from 15°C to 25°C, the DM and OM digestibility increased in animals fed Con silage, but decreased in animals fed Bm silage. Concomitantly, ME estimated from digestible nutrients was higher for Bm than for Con at 15°C, but no differences were found at 25°C and 35°C. Effects of diet by temperature interaction, furthermore, were observed for EE and CP digestibility. Therefore, forage quality has to be considered when feeding heat-stressed animals.

Keywords: digestibility; heat stress; hybrid varieties; maize silage; nitrogen balance; sheep

1. Introduction

The effects of heat stress on nutrient digestibility were extensively characterised during the last years. Results in literature are, however, inconsistent. Due to elevated ambient temperature, increased (Miaron and Christopherson 1992; Weniger and Stein 1992; Lohölter et al. 2012) as well as decreased nutrient digestibility (Bhattacharya and Hussain 1974; Llamas-Lamas and Combs 1990; Bernabucci et al. 2009) or no effects on digestibility (Mathers et al. 1989; Lourenço et al. 2010) were described. In general, dry matter intake (DMI) was not affected by increased temperature, except in the studies of Bhattacharya and Hussain (1974) and Llamas-Lamas and Combs (1990) who reported a decrease in DMI due to heat stress. Many authors compared only two temperature levels (e.g., McDowell et al. 1969; Bhattacharya and Hussain 1974; Bhattacharya and Uwayjan 1975; Mathers et al. 1989; Bernabucci et al. 1999, 2009). However, responses to temperature changes may be non-linear, which might be a result of interactions between temperature and relative humidity (Weniger and Stein 1992). It was also shown that forage to concentrate ratio might interact with ambient temperature (Bhattacharya and Hussain 1974; Bhattacharya and Uwayjan 1975). In contrast, studies concerning the impact of fibre quality on DMI and digestive events are scarce. Indeed, fibre quality might be important because of its impact on heat emerging from fermentation (Czerkawski 1980) and energy requirement for ingestion (Susenbeth et al. 2004).

The aim of the present experiment was to compare the impact of different ambient temperatures on digestibility and nitrogen (N) balance in sheep. It was assumed that animals might adapt to elevated ambient temperature (Bernabucci et al. 1999, 2009). Sheep were, therefore, adapted to ambient temperature to avoid effects of adaptation during the experiment. Furthermore, two maize silages were chosen differing in *in situ* fibre degradability and metabolisable energy (ME) content to overcome effects of different forage to concentrate ratios while investigating the impact of fibre quality and energy content.

2. Materials and methods

The experiment was conducted in temperature-controlled rooms at the Friedrich-Loeffler-Institute in Braunschweig, Germany according to the directive 2010/63/EU of the European Parliament and the Council of the European Union (Anonymous 2010) concerning the protection of experimental animals. The trial was approved by the State Office for Consumer Protection and Food Safety (LAVES) in Oldenburg, Lower Saxony, Germany (file number 33.9-42502-04-11/0595). Eight adult castrated male German Blackheaded Mutton sheep, weighing (mean ± standard deviation) 101 ± 5.8 kg were randomly split into two groups of four animals and the two groups were randomly assigned to the treatments. Experimental diets were a control maize (Con) silage or a brown-midrib maize (Bm) silage. Each diet was fed at ambient temperatures of 15°C, 25° C or 35°C. Harvest dates of the maize hybrids were 11 October 2010 for Con and 12 October 2010 for Bm, and the dry matter (DM) contents of maize at harvesting were 34.4% for Con and 33.4% for Bm. Maize plants were harvested in the dough stage. The DM yield per ha was 20.8 and 17.8 t for Con and Bm, respectively. Cutting height was approximately 18 to 20 cm, chopping length was 5.5 mm and kernels were crushed at harvesting. The Bm hybrid was an experimental hybrid “SUM 2368” (Saaten-Union GmbH, Isernhagen, Germany) the Con hybrid was “Ronaldinio” (KWS-Saat AG, Einbeck, Germany). Silages were preserved in big bales with stretch foil. Ambient temperature and relative humidity were recorded using Tinytag Plus 2 Dataloggers (Gemini Dataloggers, Chichester, UK). From ambient temperature and relative humidity a temperature–humidity index (THI) was calculated according to Hahn (1999):

$$\mathrm{THI} = 0.81 \cdot T + (RH/100) \cdot (T - 14.4) + 46.6$$

where T is the mean hourly temperature [°C] and RH is the mean hourly relative humidity [%].

Animals were adapted to ambient temperature and diet for 13 d in individual boxes and then moved into metabolism crates for total collection of faeces and urine for 8 d. Animals received daily 1 kg silage (on DM-basis) and 20 g urea, fed in two equal portions at 6:30 and 13:30 h. Animals had ad libitum access to water. During the collection period, respiration rate and rectal temperature were determined on four random days of each collection period. Respiration rate was determined by counting flank movements for 30 s. Afterwards, rectal temperature was measured using a standard clinical thermometer.

Total amount of faeces and urine were weighed daily; subsamples were taken and stored at −20°C for further analysis. Feed samples were taken daily, pooled per treatment and stored at −20°C for further analysis. Feedstuffs were dried at 60°C for 72 h in a forced-air oven and faeces were freeze-dried. Samples were ground to pass through a 1-mm screen using a Retsch mill (SM 1; Retsch, Haan, Germany) and analysed according to the methods of VDLUFA (2007, method numbers in brackets) for DM (3.1), ash (8.1), ether extract (EE; 5.1.1), crude protein ($CP = N \cdot 6.25$; Dumas method, 4.1.2), neutral detergent fibre (NDF; 6.5.1), acid detergent fibre (ADF; 6.5.2) and acid detergent lignin (ADL; 6.5.3). NDF and ADF were expressed without residual ash and amylase pretreatment was done for NDF, therefore, they are referred to as aNDFom (neutral detergent fibre assayed with amylase and expressed exclusive of residual ash) and ADFom (acid detergent fibre expressed exclusive of residual ash). Energy content of the silages was estimated from enzyme soluble organic matter (ESOM; 6.6.1) and regression equations of GfE (2008), as follows:

$$\text{ME[MJ/kg DM]} = 7.15 + 0.00580 \cdot \text{ESOM} - 0.00283 \cdot \text{NDFom} + 0.03522 \cdot \text{EE}$$

Energy content of the silages was also estimated from digestible nutrients according to GfE (1991). Urine was analysed for N (Kjeldahl method; 4.1.1).

Statistical analyses were done using the MIXED procedure of SAS (SAS Version 9.2., SAS Institute Inc., Cary, NC, USA). Silage maize variety, ambient temperature and silage maize variety by ambient temperature interaction were considered to be fixed factors. The RANDOM statement was used to estimate the individual animal effect. The statistical model was as follows:

$$y = \mu + a_i + s_j + t_k + st_{jk} + e_{ijk}$$

where μ is the overall mean, a is the individual animal (i = 1 to 8), s is the silage maize variety (j = 1 to 2); t is the ambient temperature (k = 1 to 3) and e is the residual error.

3. Results and discussion

The nutrient and energy contents of tested silages, and ambient temperature and THI during the experimental periods are presented in Table 1. Rising ambient temperature increased respiration rate and rectal temperature (Table 2), thus it can be assumed that sheep experienced heat stress. Diet affected all estimated variables, except digestibility of EE. For the Bm silage superior digestibilities of DM, OM, aNDFom and ADFom and a higher energy content was estimated *in vivo*. In contrast, the digestibility of CP was lower in animals fed Bm silage (Table 2). Moreover, after feeding the Bm silage the N excretion with faeces was higher and the N excretion with urine was lower. Furthermore, this diet affected the apparently absorbed N (AAN) but no effects on the ratio of urinary N to AAN were observed.

Table 1. Chemical composition and energy content of the maize silages, measured temperatures and temperature-humidity indices (THI) during the experimental periods.[*]

	Con[¶]	Bm[◊]
Dry matter (DM) [g/kg]	282 ± 5.1	335 ± 37
Ash [g/kg DM]	49 ± 2.1	48 ± 0.7
Crude protein [g/kg DM]	87 ± 1.8	83 ± 1.8
Ether extract [g/kg DM]	34 ± 1.6	28 ± 0.5
Starch [g/kg DM]	258 ± 3.8	292 ± 21.0
Neutral detergent fibre[†] [g/kg DM]	472 ± 10.4	458 ± 10.0
Acid detergent fibre[‡] [g/kg DM]	263 ± 5.7	248 ± 7.5
Acid detergent lignin [g/kg DM]	32 ± 1.8	16 ± 0.7
Metabolisable energy[§] [MJ/kg DM][§]	10.5 ± 0.17	10.9 ± 0.13
Temperature [°C]		
Target ambient temperature		
15°C	15.9 ± 0.02	16.4 ± 0.30
25°C	25.5 ± 0.02	25.1 ± 1.56
35°C	33.5 ± 2.42	34.3 ± 1.61
THI		
Target ambient temperature		
15°C	59.8 ± 0.08	60.9 ±0.63
25°C	70.2 ±0.17	70.7 ±2.90
35°C	77.0 ± 5.74	80.0 ± 1.95

Notes: [*]Means with standard deviation, n = 3 analyses per silage, n = 8 for mean daily temperature and n = 8 for mean daily THI; [¶]Con, Control maize silage; [¥]Bm, Brown-midrib maize silage; [†]Amylase pre-treated, without residual ash; [‡]Without residual ash; [§]Estimated from enzyme soluble organic matter (VDLUFA 2007, Method No. 6.6.1) and regression equation (GfE 2008).

Ambient temperature did not affect digestibility of DM, OM, aNDFom and ADFom, but interactions between varieties and temperatures were observed for DM and OM digestibility. The increase in ambient temperature increased the digestibility of DM and OM in animals fed Con silage, whilst the DM and OM digestibilities were decreased in animals fed Bm silage (Figure 1). Urinary excretion of N was affected by higher ambient temperature, as was AAN. Furthermore, diet by temperature interaction was found for N intake, N balance and AAN (Table 2).

In accord with the present results, Bhattacharya and Uwayjan (1975) and Lourenço et al. (2010) showed that ambient temperature did not affect nutrient digestibility in sheep. Mathers et al. (1989) reported the same for cattle. Other authors, in contrast, found higher nutrient digestibilities in sheep (Weniger and Stein 1992; Lohölter et al. 2012) and cattle (McDowell et al. 1969; Miaron and Christopherson 1992; Bernabucci et al. 1999) when temperature rose. It was, however, also observed that elevated ambient temperature lowered nutrient digestibility in sheep and dairy cows (Bhattacharya and Hussain 1974; Llamas-Lamas and Combs 1990; Goetsch and Johnson 1999; Bernabucci et al. 2009).

Reduced ruminal outflow rate of fluid (Miaron and Christopherson 1992) and particles (Weniger and Stein 1992) positively affected nutrient digestibility in animals exposed to increased ambient temperature. In contrast, Goetsch and Johnson (1999) concluded that the retention time of ruminal ingesta would decrease due to increased water intake, and thus, lower digestibility. Fibre digestibility might be enhanced by the increase in ruminal pH due to increased ambient temperature, as observed in high-concentrate diets by Weniger and Stein (1992). The increase in digestibility might also be a result of reduced DMI and a shift of forage to concentrate ratio in favour of concentrate (McDowell et al. 1969). In the present experiment, the target DMI was 1 kg/d. However, due to variations of DM content of the silages, DMI varied and was slightly below 1 kg/d for the treatment Con at ambient temperatures of 15°C, 25°C, 35°C and for treatment Bm at 25°C and 35° C, whereas animals receiving Bm maize at 15°C had a DMI of about 1.1 kg/d. However, the higher DMI in the treatment Bm at 15°C might be irrelevant for digestibility measurements, as the difference in DMI was likely not sufficient to affect nutrient digestibility and the nutritional level was below maintenance (Gabel et al. 2003). Authors who did not find effects of temperature on digestibility (Bhattacharya and Uwayjan 1975; Mathers et al. 1989) concluded that the tested temperatures (25°C and 33°C) were not adverse for sheep and cattle. Higher temperature may also redirect blood flow for cooling purposes from the digestive tract to peripheral tissues, which may subsequently reduce nutrient absorption (Christopherson 1985; Lu 1989).

Table 2. Impact of ambient temperature on digestibility and nitrogen utilisation in sheep (LSmeans).

	Experimental Diets										
	Con[¶]				Bm[◊]				*p*-Values		
	15°C	25°C	35°C	SEM[#]	15°C	25°C	35°C	SEM	Diet	Temp[♦]	Diet × Temp[*]
Dry matter (DM) intake [g/d]	943	977	944	1.9	1132	938	947	1.8	0.033	0.032	0.026
Digestibility [%]											
DM	60.3	64.1	64.3	0.89	72.5	68.0	69.3	0.84	<0.001	0.879	0.023
Organic matter	62.2	66.0	66.9	0.87	74.1	70.0	71.6	0.83	<0.001	0.686	0.025
Crude protein	53.5	55.0	56.3	0.88	52.5	43.3	47.9	0.83	<0.001	0.041	0.005
Neutral detergent fibre[†]	43.9	48.7	49.3	1.67	64.0	59.8	62.8	1.54	<0.001	0.712	0.325
Acid detergent fibre[‡]	43.1	46.9	49.1	1.49	63.6	59.8	61.9	1.41	<0.001	0.635	0.232
ME[§] [MJ/kg DM]	9.2	9.7	9.8	0.12	10.7	10.1	10.4	0.11	<0.001	0.754	0.016
N intake [g/d]	22.5	22.5	22.5	0.03	24.0	21.5	22.1	0.03	0.248	<0.001	<0.001
Faecal N [g/d]	6.2	6.0	5.8	0.12	7.0	7.0	6.7	0.12	<0.001	0.295	0.924
Urinary N [g/d]	16.1	13.1	13.5	0.50	13.5	11.8	13.0	0.47	0.021	0.012	0.504
N balance [g/d]	0.2	3.4	3.3	0.47	3.5	2.7	2.4	0.45	0.355	0.172	0.031
Apparently digested N [%]	72.5	73.4	74.2	0.53	70.7	67.5	69.6	0.50	<0.001	0.268	0.087
Apparently absorbed N [g/d]	16.3	16.5	16.7	0.12	17.0	14.5	15.4	0.11	<0.001	<0.001	<0.001
Urinary N / AAN [g/g]	0.99	0.79	0.81	0.029	0.80	0.82	0.85	0.028	0.193	0.100	0.060
Respiration rate [bpm[$]]	29	90	109	5.1	32	87	113	4.8	0.820	<0.001	0.894
Rectal temperature [°C]	38.6	39.0	39.3	0.07	38.7	39.0	39.3	0.07	0.867	<0.001	0.923

Notes: [¶]Con, Control maize silage; [#]SEM, Standard error of the means; [◊]Bm, Brown-midrib maize silage; [♦]Temp, Ambient temperature (15°C, 25°C or 35°C); [*]Diet by temperature interaction; [†]Amylase pre-treated, without residual ash; [‡]Without residual ash; [§]ME, Metabolisable energy, calculated from digestible nutrients according to GfE (1991); [$]bpm, Breaths per minute.

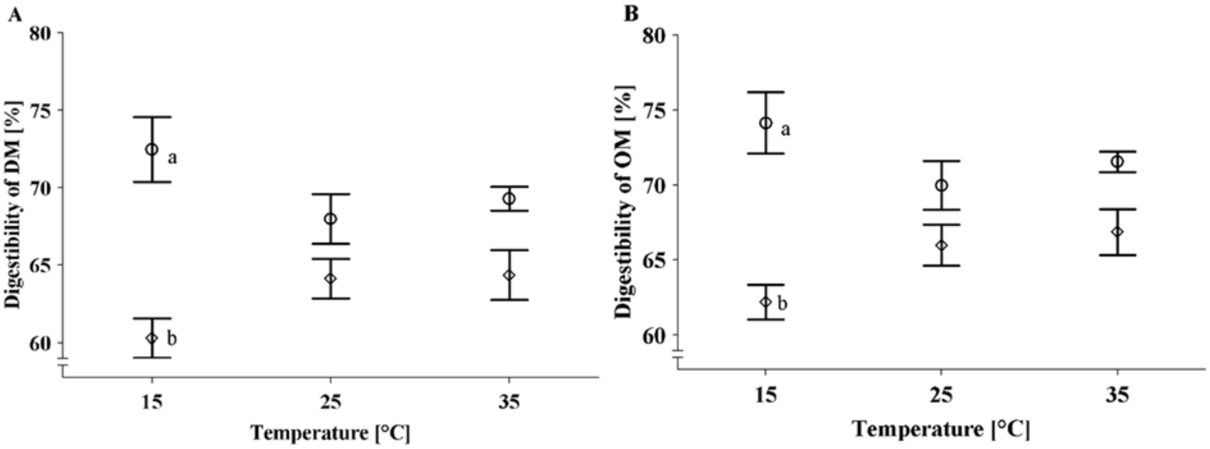

Figure 1. Digestibility of dry matter (DM, panel A) and organic matter (OM, panel B) as affected by silage maize variety × temperature interaction.
Notes: Con, Control maize silage (diamonds); Bm, Brown-midrib maize silage (circle); Values indicated with different letters differ significantly ($p < 0.05$).

In agreement with the present findings, Fike et al. (2005) found no effect of ambient temperature on faecal N excretion and a lower urinary N excretion in heat-stressed animals. In contrast, Dixon et al. (1999) reported higher urinary and lower faecal N excretions at high temperature. Al-Mamun et al. (2008) observed that urinary N excretion was not affected by ambient temperature, faecal N excretion was reduced and N absorption was increased at high temperature. On the contrary, Bunting et al. (1992) reported no effects of ambient temperature on urinary and faecal N excretion and absorbed N. In animals fed Bm silage, the differences in N excretion between lower and higher ambient temperatures were likely a result of the higher N intake due to higher DMI. Especially the high AAN at 15°C reflects the higher N intake, as faecal N excretion and apparent N digestibility were unaffected by temperature. Ratio of urinary N to AAN varied widely between treatments; however, due to the high variance no differences were observed. From the inconsistent results no general conclusion on the impact of ambient temperature on N utilisation can be drawn, but with regard to energy requirement for maintenance and energy cost of N excretion, N utilisation under heat-stress conditions should be investigated in further trials.

According to Goetsch and Johnson (1999), who observed an increase in water intake by forage-fed ewes, water intake increased with increasing temperature in the present experiment (15°C, 10.4 ± 3.44 l/d; 25°C, 19.2 ± 4.25 l/d; 35°C 32.1 ± 3.67 l/d, $p < 0.001$). Therefore, it can be concluded that ruminal fluid outflow and subsequently efficiency of microbial protein synthesis would also increase. Hence, ruminal NH_3 formation and absorption and, subsequently, urea excretion via urine would decrease. However, Weniger and Stein (1992) reported that ruminal NH_3 concentration was not affected by elevated temperatures in animals fed roughage-based diets. Yet, ruminal NH_3 concentration might not be the best indicator to estimate ruminal NH_3 production. Retention of ingesta, furthermore, might be more important in higher performing animals than in animals fed at maintenance level as in the present trial.

While the present experiment did not reveal a general temperature effect on digestibility, the factors temperature and diet interacted, which was also observed by Bhattacharya and Hussain (1974) for DM, CP and EE, and Bhattacharya and Uwayjan (1975) for crude fibre. DM and OM digestibility of treatments Con and Bm differed at 15°C ambient temperature, but due to an increase of the digestibility of the Con diet and a decreased digestibility of the Bm diet no differences were observed at 25°C and 35°C (Figure 1). When the Con silage was fed, positive effects of high ambient temperature (e.g., increased ingesta retention time) might have enhanced digestibility, whereas, negative effects (e.g., reduced cellulolytic activity) did not offset positive effects. In contrast, the Bm silage had a high OM digestibility, thus, an increased retention time would not further increase digestibility. Negative effects as redirecting of blood flow to the peripheral tissue (Christopherson 1985; Lu 1989) or reduced cellulolytic activity (Bernabucci et al. 2009) would, therefore, be adverse. However, such relations might be of importance in high-performing animals and should not be overestimated in sheep fed at maintenance level. Indeed, it has to be regarded that the present experiment was designed to draw general conclusions on the impact of heat stress. Further trials are necessary to investigate the impact of heat stress under production conditions.

4. Conclusion

Ambient temperature affected digestibility but led to inconsistent results. From the interaction of dietary treatments and ambient temperatures it can be concluded that forage quality has to be considered when animals are fed under heat-stress conditions. Thus, feeding strategies using different types of forage may exist to mitigate negative effects of increased temperature on animal production. Utilisation of N was affected by dietary treatment and ambient temperature; results, however, were inconsistent. Yet, excretion of N via urine (i.e. urea formation) is an energy intensive process, therefore, N utilisation should be considered in further investigations.

Acknowledgements

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 the trials and analyses is gratefully acknowledged.

Funding

The authors acknowledge financial support provided by the “Niedersächsisches Ministerium für Wissenschaft und Kultur” within the research network “KLIFF” (climate impact and adaptation research) and by Saaten-Union GmbH, Isernhagen, Germany.

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Chapter 6

Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate

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Archives of Animal Nutrition, 2014
Vol. 68, No. 5, 358-369, http://dx.doi.org/10.1080/1745039X.2014.950451

Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate

The aim of the present study was to evaluate the impact of summer temperatures in a temperate climate on mid-lactation Holstein dairy cows. Therefore, a data set was examined comprising five trials with dairy cows conducted at the experimental station of the Friedrich-Loeffler-Institute in Braunschweig, Germany. The temperature–humidity index (THI) was calculated using temperature and humidity data from the barns recorded between January 2010 and July 2012. By using a generalised additive mixed model, the impact of increasing THI on dry matter intake, milk yield and milk composition was evaluated. Dry matter intake and milk yield decreased when THI rose above 60, whilst water intake increased in a linear manner beyond THI 30. Furthermore, milk protein and milk fat content decreased continuously with increasing THI. The present results revealed that heat stress exists in Lower Saxony, Germany. However, further research is necessary to describe the mode of action of heat stress. Especially, mild heat stress has to be investigated in more detail and appropriate heat stress thresholds for temperate climates have to be developed.

Keywords: dairy cattle; feed intake; heat stress; milk yield; relative humidity; temperate climate

1. Introduction

The impact of heat stress on ruminants has been extensively studied (Wayman et al. 1962; Olbrich et al. 1972; West et al. 1999; Bouraoui et al. 2002). Temperature and temperature–humidity index (THI) thresholds were established and relationships between heat stress and production were characterised (Thom 1959; Bianca 1962; Yousef 1985; Mader et al. 2006). The focus of most studies, however, was on very hot regions, for example, Israel (Berman et al. 1985), Tunisia (Bouraoui et al. 2002) and Arizona, USA (Bohmanova et al. 2007). Due to climate change, extreme climatic conditions such as heat waves will occur more often in temperate regions (Meehl et al. 2007) and cattle will be exposed more often to conditions compromising their ability to lose heat and to maintain all physiological functions. Literature on the impact of heat stress in temperate regions is scarce and thresholds developed for tropical or subtropical regions may not fit to temperate conditions. For example, Brügemann et al. (2012) have shown that THI of 60–70 were thresholds denoting substantial declines in milk and protein yield in Lower Saxony, Germany, whereas thresholds of 68–78 (Bouraoui et al. 2002) and 68–83 (Bohmanova et al. 2007) were described for Tunisia and Arizona, USA. Recent studies from the USA, furthermore, showed that a THI of 68 might represent a threshold for heat stress in high-yielding dairy cows (Zimbelman et al. 2009). As most of the studies on the impact of heat stress on dry matter intake (DMI) and milk yield were conducted in hot regions, the aim of the present study was to evaluate the impact of mild heat stress on mid-lactation dairy cows in a temperate climate in Lower Saxony, Germany. Data of mid-lactation cows (100–200 d in milk) were used as mid-lactation cows might be even more susceptible than cows in early lactation (Maust et al. 1972; Broucek et al. 2007).

2. Materials and methods

2.1. Database description

Data from five feeding trials with dairy cows conducted between September 2009 and August 2012 at the experimental station of the Friedrich-Loeffler-Institute (FLI) inBraunschweig, Germany, were evaluated. Data included the daily registration of DMI, water intake and milk yield and weekly twice milk composition (protein and fat content, aliquots from consecutive evening and morning milking) was recorded. During the trials, cows were housed in naturally ventilated free-stall barns. Animals were fed once daily at 10:00 h. The barns were equipped with automatic self-feeding stations, automatic drinking troughs and automatic concentrate feeders (all Insentec, B.V., Marknesse, The Netherlands). Animals were equipped with ear tags to be identified at the feeding and drinking stations. Details on animals and feeding are given in Tables 1 and 2. Energy content of the rations was estimated from digestibility trials with wethers (forages Trials 1 and 2) according to GfE (1991) or from table values (forages Trials 3, 4 and 5; concentrates Trials 1–5; Universität Hohenheim – Dokumentationsstelle 1997). Data from cows between 100 and 200 d in milk were used for statistical evaluation; therefore, data were considered between 6 January and 9 June 2010, 22 January and 22 July 2011 and 1 February and 17 July 2012. Data of 449 d from 138 cows were used for statistical evaluation.

Table 1. Body weight, number of lactation, days in milk and milk yield of the cows used for statistical evaluation (mean ± standard deviation).

Trial	Body weight [kg]	Number of lactation	Days in milk	Milk yield [kg/d]
1	632 ± 67.2	2.0 ± 1.28	150 ± 28.9	29.0 ± 7.29
2	558 ± 69.5	1.6 ± 0.73	151 ± 28.9	31.0 ± 5.48
3	630 ± 92.6	2.4 ± 0.58	186 ± 10.1	32.0 ± 3.01
4	596 ± 65.4	2.0 ± 1.13	108 ± 5.7	30.8 ± 5.65
5	641 ± 73.4	2.7 ± 1.34	171 ± 21.3	30.5 ± 6.15

An overview over the data base is given in Table 2. Commonly, in Germany the warmest months of the year are June, July and August, but hot days may also occur in April, May and September. December, January and February are in general the coldest months and March, April, October and November are in between.

Temperature and relative humidity (RH) in the barns were recorded using Tinytag Plus 2 Dataloggers (Gemini Dataloggers, Chichester, UK). Each barn was equipped with four data loggers to take temperature and humidity fluctuations within each barn into account. Data loggers were set to record temperature and RH every 10 min. After elimination of biased data, hourly means of temperature and RH and subsequent daily mean, minimum and maximum temperature and RH were calculated. Values of RH >99% and <30% were considered to be measurement errors because these values were generally shown only by one of the four data loggers in each barn at the same time.

Table 2. Summary of data used for statistical evaluation.

Trial	Duration	Period used for statistical calculations (number of days)	Number of animals/ treatments	Feeding	Forage to concentrate ratio$^{\#}$	Ratio MS† to GS‡
1	29 September 2009 – 8 August 2010	6 January 2010 – 9 June 2010 (155 d)	63 / 4	TMR§	40 : 60 70 : 30	All diets 60 : 40
2	22 January 2011 – 15 June 2011	(1) 22 January 2011 – 19 April 2011 (88 d) (2) 20 April 2011 – 15 June 2011 (57 d)	61 / 4	(1) TMR (2) MS *ad libitum* concentrate restrictively	(1) 50 : 50 (2) 73 : 27	All diets MS only
3	18 June 2011 – 22 July 2011	18 June 2011 – 22 July 2011 (35 d)	60 / 2	TMR	74 : 36	50 : 50
4	26 October 2011 – 24 February 2011	1 February 2012 – 24 February 2012 (24 d)	30 / 3	TMR	50 : 50	GS only
5	19 April 2012 – 30 July 2012	(1) 19 April 2012 – 12 July 2012 (85 d) (2) 13 July 2012 – 17 July 2012 (5 d)	65 / 4	(1) TMR (2) TMR	(1) 80 : 20 or 40 : 60 (2) 60 : 40	(1) GS or MS (2) 50 : 50

Notes: $^{\#}$On dry matter basis; †MS, Maize silage; ‡GS, Gras silage; §TMR, Total mixed ration.

2.2. Calculation of heat stress indicators

The equation described by Hahn (1999) was used to calculate the THI as a heat stress indicator.

$$\mathrm{THI} = 0.81 \cdot T + (RH/100) \cdot (T - 14.4) + 46.6 \quad (1)$$

where T is the mean hourly ambient temperature [°C] and RH is the mean hourly relative humidity [%]. Values were calculated for each of the four data loggers and averaged to one barn value. Furthermore, from the temperature recorded every 10 min, the time was calculated when the temperature was below 21°C [h/d]. For this purpose it was assumed that the temperature was continuous for the entire 10 min. Therefore, the 10-min-intervals per day when the temperature was below 21°C were summed up to calculate the time when temperature was below 21°C. This threshold was chosen because Igono et al. (1992) have shown that 21°C denotes a critical threshold for cooling from heat stress.

For statistical evaluation, average THI of the day of data collection (d0), as well as average THI of one day prior to data collection (d−1) were considered, as Collier et al. (1981) and West et al. (2003) have shown that a delay of responses to heat stress may occur.

Meteorological data outside the experimental barn were provided by the Agrometeorological Research Centre of the Deutscher Wetterdienst (DWD) in Braunschweig, Germany. From meteorological data, THI outside the barn were calculated. Performance and climatic data were merged using PROC SQL in SAS (Software package 9.2, SAS Institute; Cary, NC, USA).

2.3. Statistical analyses

Statistical analyses were carried out using the gamm (generalised additive mixed model) procedure of R (Version 3.1.0, The R-Foundation; Vienna, Austria). Three different models were constructed as follows:

Model equation for DMI and energy (net energy lactation [NEL]) intake:

$$y = \alpha + f(\mathrm{THI}_0) + f(\mathrm{DIM}) + \mathrm{TRT} + \mathrm{L} + \text{milk yield}$$
$$+ \mathrm{BW}^{0.75} + \mathrm{WI} + \mathrm{R}_0 + \mathrm{cow} + \varepsilon \quad (2)$$

Model equation for water intake:

$$y = \alpha + f(THI_0) + f(DMI_0) + f(DIM) + TRT + L + \text{milk yield} + R_0 + cow + \varepsilon \quad (3)$$

Model equation for milk yield and composition and milk yield per kg of DMI:

$$y = \alpha + f(THI_{-1}) + f(DIM) + f(DMI_{-1}) + TRT + L + WI + R_{-1} + cow + \varepsilon \quad (4)$$

where α is the intercept and the effects of the model were as follows:

THI_0 is the THI at d_0, THI_{-1} is the THI at d_{-1}, TRT is the treatment within the respective trial, L is the number of lactation, R_0 is the number of hours per day temperature was below 21°C at d_0, R_{-1} is the number of hours per day temperature was below 21°C at d_{-1}, DIM is the number of days in milk, WI is the water intake, $BW^{0.75}$ is the metabolic body size, DMI_0 is DMI at d_0, DMI_{-1} is DMI at d_{-1}, milk yield is the daily milk yield, cow is the individual animal, ε is the residual error.

For DMI and water intake THI_0 was considered and for milk yield $THI_{-1}$was considered, as it can be expected that effects of increased THI on milk yield are delayed for 24–48 h (Collier et al. 1981; West et al. 2003). Treatments within the trials, DIM, number of lactation and metabolic body size (the latter for DMI only) were also considered to correct for these effects. In addition, it was assumed that individual animal effects affected the results. Therefore, a random intercept and a random slope were added to the model to take individual animal effects and changes in milk yield over the course of lactation into account. Heat stress thresholds were determined by visual inspection of the plots derived from the respective models (Figure 1).

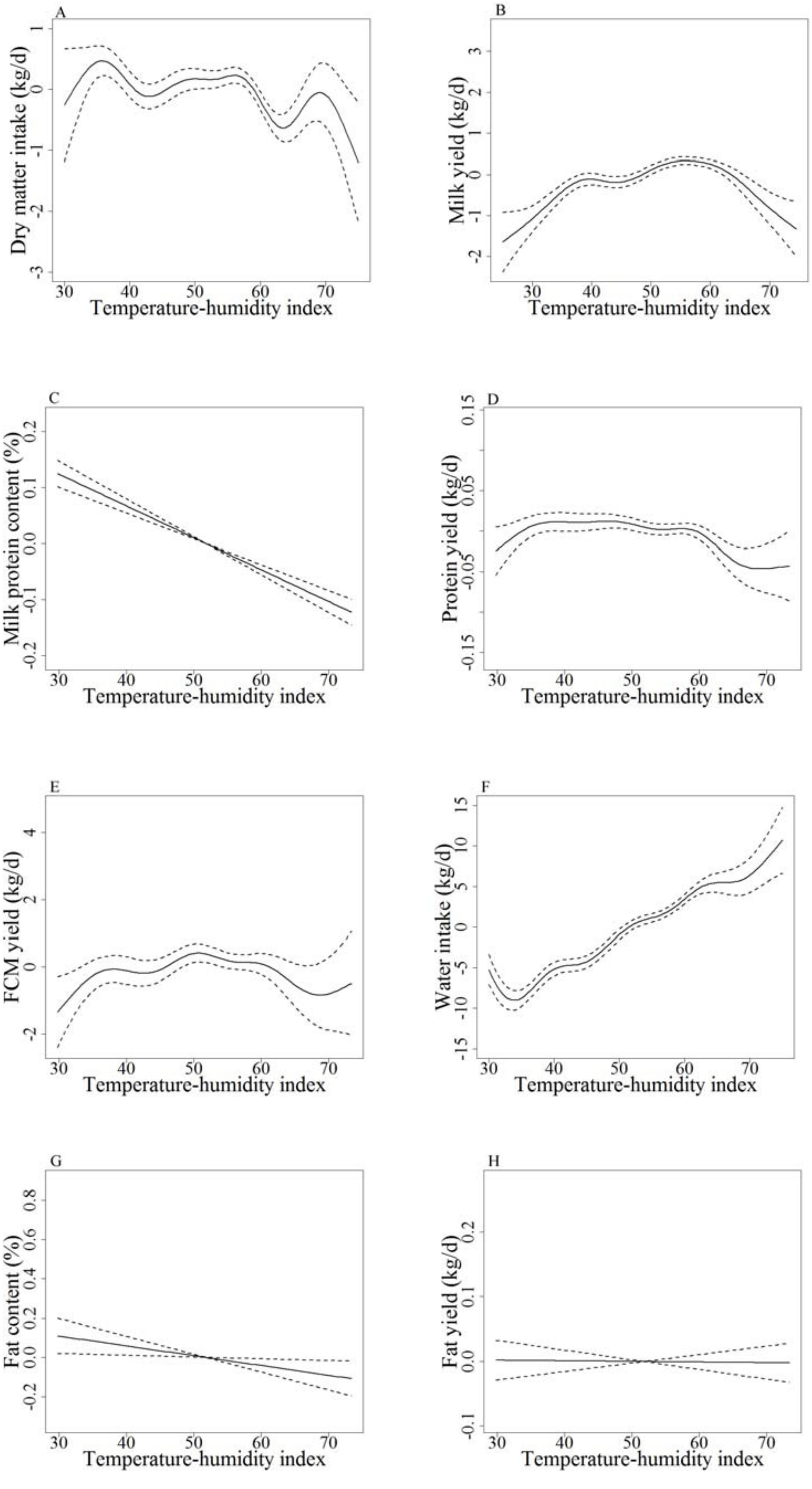

Figure 1. Impact of daily mean temperature-humidity index on dry matter and water intake (Panels A and F), milk and nutrients yields (Panels B, D, E and H) and milk composition (Panels C and G).

3. Results

3.1. Average THI inside and outside the barn

Figure 2 shows daily average THI inside the barn for 2010, 2011 and 2012 and Figure 3 shows the monthly maximum of THI inside and outside the barn. The highest THI were found from April to September with average THI around 60 and 80. In March and October, THI exceeded 60 on some days. During the whole year, the THI outside the barn was lower than inside the barn.

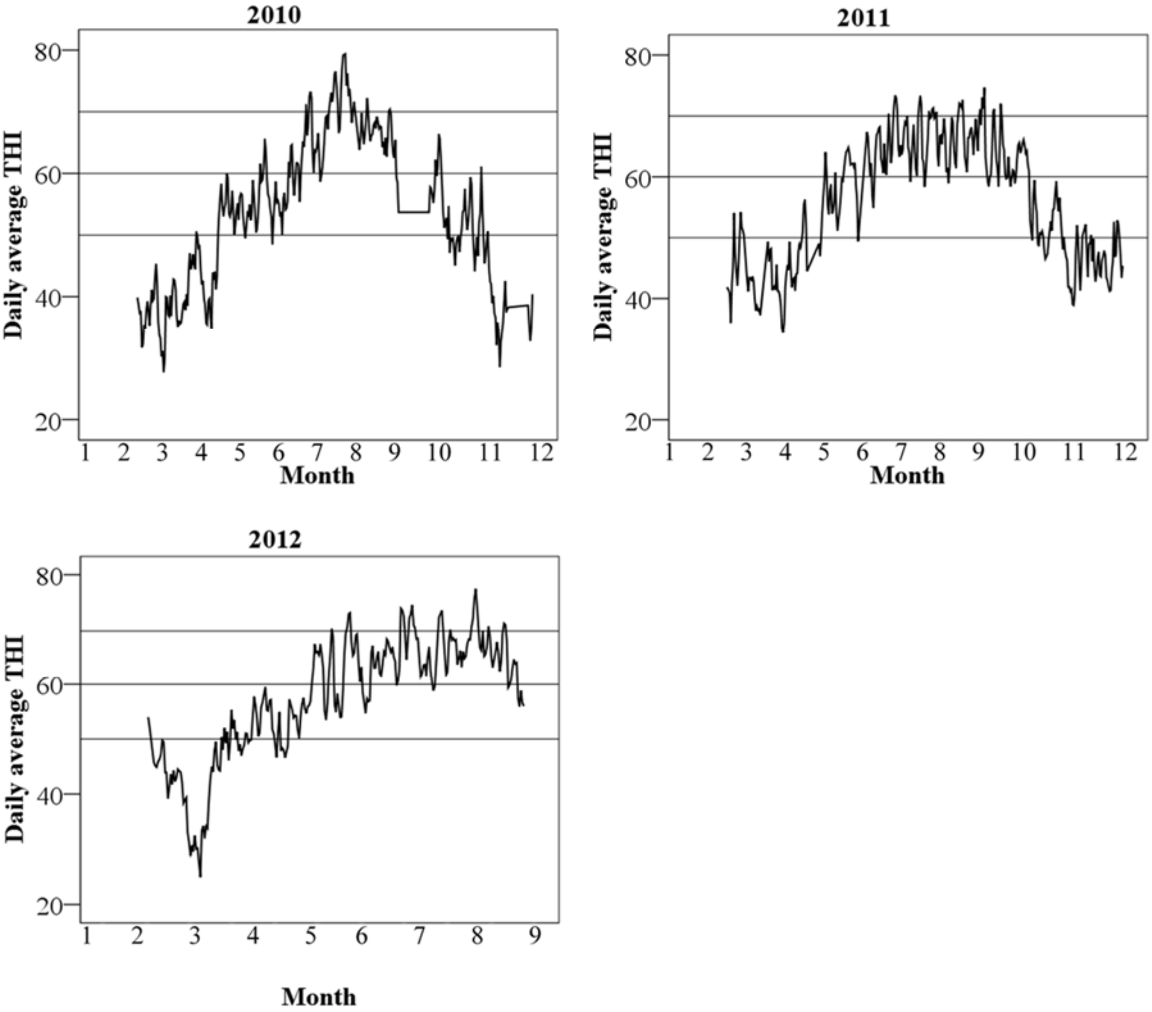

Figure 2. Trend of daily average temperature-humidity index (THI) inside the barn 2010, 2011 and 2012.

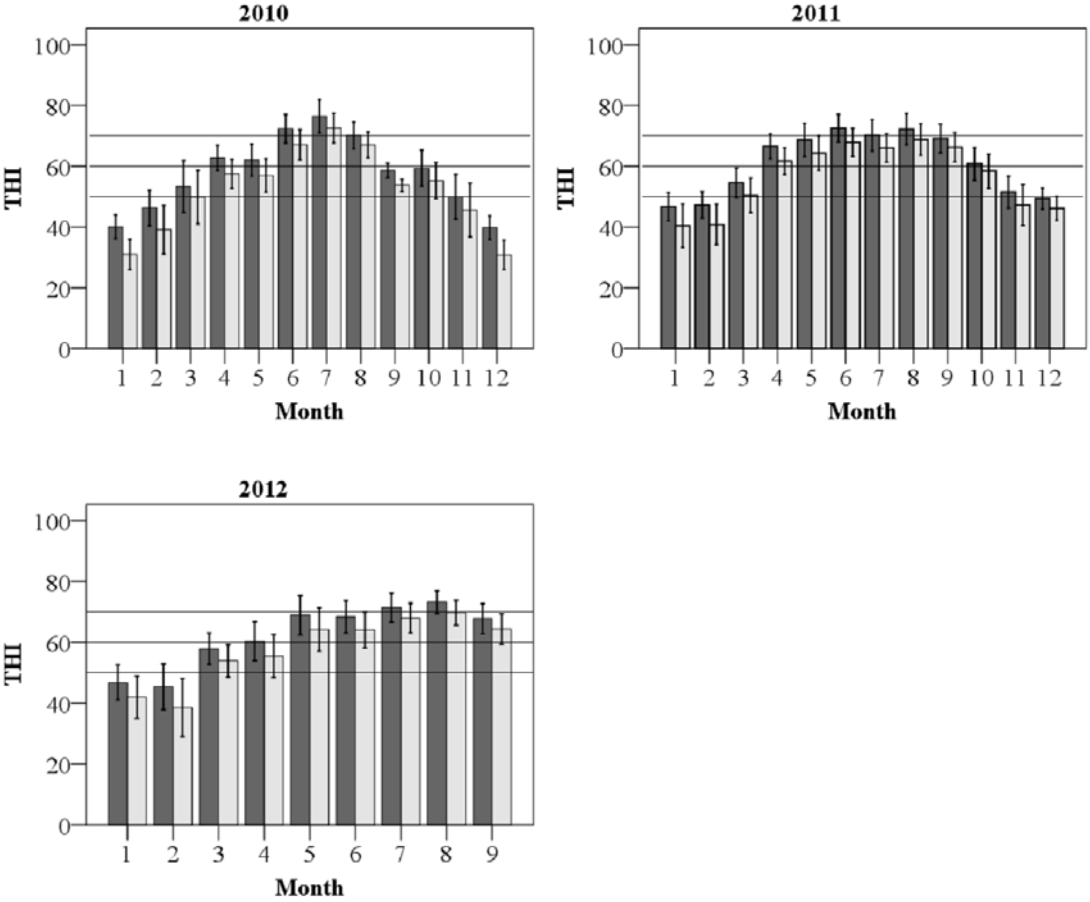

Figure 3. Monthly average of daily maximum temperature-humidity index (THI) inside (dark bars) and outside the barn (light bars) in 2010, 2011 and 2012 (mean with standard error).

3.2. Dry matter intake and water intake

Dry matter intake was affected by THI and the time per day when temperature was below 21°C at the day of data collection (Table 3). DMI increased up to THI 35, reached a plateau and dropped, when THI exceeded 60. At THI 70, DMI had a slight peak (Figure 1A). Results were similar for energy intake, yet the decrease beyond THI 60 was less pronounced.

Water intake was affected by THI and time per day when temperature was below 21°C at the day of data collection. Water intake decreased up to THI 35 and increased beyond THI 35 in a linear manner (Table 3 and Figure 1F).

Table 3. Impact of the THI and time when temperature was below 21°C [h/d] on dry matter intake, energy intake, and water intake.

	THI at day of data collection (d_0) (p-value)	Time [h] below 21°C at day of data collection (d_0) (p-value)	r^2
Dry matter intake [kg/d]	< 0.001	0.005	0.451
Energy intake [MJ NEL[†] /d]	< 0.001	0.025	0.507
Water intake [kg/d]	< 0.001	< 0.001	0.579

Note: [†]NEL, Net-energy for lactation.

Table 4. Impact of THI and time when temperature was below 21°C [h/d] on milk yield and composition, nutrient utilisation and FCM[†] yield.

	THI one day prior to data collection (d_{-1}) (p-value)	Time [h] below 21°C one day prior to data collection (d_{-1}) (p-value)	r^2
Milk yield [kg/d]	<0.001	0.001	0.368
Milk yield [kg/kg DMI[‡]]	<0.001	0.029	0.020
Protein content [%]	<0.001	0.092	0.177
Protein yield [kg/d]	<0.001	0.247	0.544
Fat content [%]	0.015	0.880	0.316
Fat yield [kg/d]	0.894	0.106	0.326
FCM yield [kg/d]	0.045	0.604	0.360

Notes: [†]FCM, Fat-corrected milk (4% fat) calculated according to Gaines (1928); [‡]DMI, dry matter intake.

3.3. Milk yield and milk composition

THI and time per day temperature was below 21°C on d−1 affected milk yield (Table 4). As shown in Figure 1B, milk yield increased with THI up to THI 60 and then decreased.

Utilisation of DMI for milk production was affected by THI and time below 21°C on d_{-1}, yet, adjusted r^2 was low (0.02) indicating a very weak relation (Table 4). Milk protein content was affected nearly linearly by THI on d_{-1} (Table 4 and Figure 1C), yet the impact was very weak. Milk protein yield was affected by THI on d_{-1}, where protein yield decreased beyond THI 60 similar to milk yield. Milk protein content was not affected by time per day temperature was below 21°C on d_{-1}. Milk fat content was affected by THI on d_{-1} in a linear manner; milk fat yield was not affected. Subsequently, FCM-yield, which was affected by THI on d_{-1}, followed a similar pattern as milk yield (Table 4 and Figure 1E).

4. Discussion

In 2010–2012, the climate in Germany can be considered to represent mild heat stress. Average daily THI exceeded THI 72 on several days which is considered to be a threshold for mild heat stress (Hahn 1985; Chase 2006). Recent publications, however, indicate that heat stress thresholds need to be re-evaluated and that thresholds might differ between hot and temperate regions (Zimbelman et al. 2009; Brügemann et al. 2012).

4.1. Dry matter and water intake

In accord with the present results, decreases in DMI due to increased ambient temperature were reported for lactating dairy cows (Wayman et al. 1962; West et al. 1999; Bouraoui et al. 2002) and were termed “a survival strategy” (Baumgard and Rhoads 2012). Reductions in feed intake due to heat stress were also observed for heifers (Olbrich et al. 1972; Bernabucci et al. 1999) and small ruminants (Maloiy et al. 2008).

In the present trials, animals reduced intake of forage and concentrate to the same extent because they received a TMR and were thus not able to select feedstuffs (except for Trial 2, Period 2). Under this conditions, reducing total DMI is the only strategy for the cow to reduce heat increment from feed intake, which obviously was the case in the present trials. In the present study, a decrease of DMI was observed beyond THI 60, indicating that a heat stress threshold was reached.

Cows raised water consumption slightly with increasing THI beyond THI 30 which corresponds to a temperature of −5°C and a RH of 65%. Others have observed sharp increases in water consumption by dairy cows (McDowell et al. 1969) and heifers (Bernabucci et al. 1999). These authors, however, used climatic chambers to maintain controlled conditions with constantly high temperatures in their heat stress treatment (McDowell et al. 1969: 32.3°C, 60% RH, (i.e. THI 83) for 14 d; Bernabucci et al. 1999: 33°C 60% RH, (i.e. THI 84) for 40 d). These experimental designs are in contrast to the present trials where continuous changes of THI were evaluated and animals were able to cool down at night. Thus, it can be expected that heat stress was much lower and therefore the increase in water intake was less pronounced.

4.2. Milk yield

In accord with the present findings, other researchers have also reported that heat stress lowers milk yield in dairy cows (Wayman et al. 1962; Moody et al. 1967; Bouraoui et al. 2002; Gantner et al. 2011). Reduced DMI due to heat stress was thought to be the main factor accounting for reduced milk yield, with a minor effect of heat stress per se (Wayman et al. 1962). Recent studies, in contrast, showed that reduced DMI might explain only 35–50% of milk yield reduction in heat-stressed animals (Rhoads et al. 2009; Wheelock et al. 2010; Baumgard and Rhoads 2012). The remainder may be a result of changes in post-absorptive energy and nutrient partitioning. Heat-stressed cows seem to have a reduced ability to mobilise adipose tissue and therefore rely on glucose as energy source for the peripheral tissue (Rhoads et al. 2009; Wheelock et al. 2010; Baumgard and Rhoads 2012). In consequence, energy available for milk production is reduced (Baumgard and Rhoads 2012). Maintenance requirement of heat-stressed animals might, furthermore, be increased by 11–32% at temperatures of 30–40°C (NRC 1981) which corresponds to a THI of 78 at a RH of 50%. Severity of heat stress (actual thresholds yet have to be identified), however, has to be considered. For example, for broiler chicken it was shown that severe heat stress indeed increases muscle protein turnover but mild heat stress even led to a decrease (Yunianto et al. 1997) which might largely affect energy requirement for and heat production from maintenance.

In the present trials, animals were likely not in a negative energy balance as they were already 100–200 d in milk. Although animals were able to cover their energy demand from DMI at thermoneutral conditions, metabolic inflexibility as described above, may adulterate the energy deficiency for milk production arising from reduced DMI and increased maintenance energy requirement in heat stress situations. However, it remains unclear in how far mild heat stress affects post-absorptive nutrient partitioning, as most of the studies were conducted under conditions of severe heat stress, for example, Rhoads et al. (2009) and Wheelock et al. (2010) tested conditions at 20°C versus 29.4–38.9°C. Therefore, the impact of mild heat stress on energy requirement for maintenance has to be evaluated.

Also, in the present trials, the decrease in DMI accounted only partly for reduced milk yield. From the decrease in kg milk yield per kg DMI (Table 4) it appears that heat stress has an impact on milk yield beyond DMI, yet, the impact of THI on milk yield per kg DMI was very weak ($r^2 = 0.02$). Whether this impact is associated with maintenance requirements or changes in energy metabolism and to which extent these factors contribute to the decrease in milk yield cannot be clarified from the available data set. The threshold of THI 60, which indicated a decrease in milk yield in the present study, can be confirmed by the findings of Brügemann et al. (2012), who reported substantial declines in milk yield if THI exceeded a value of 60 calculated by the equation of Bohmanova et al. (2005).

4.3. Milk composition

In accord with the present results, declining milk protein concentrations in response to heat stress were also reported by Moody et al. (1967), Knapp and Grummer (1991) and Gantner et al. (2011). Accordingly, milk protein yield was also decreased. A decreased protein content along with a decreased milk yield might be a result of the reduced energy intake due to lower

DMI (Emery 1978). The present findings are supported by Brügemann et al. (2012), who have shown that the milk protein percentage decreases continuously with increasing THI. However, the impact of THI on milk protein content was very weak ($r^2 = 0.177$); therefore, the decrease in protein yield for THI over 60 can mainly be attributed to the decrease in milk yield. Milk fat content was affected similarly, which is in line with the results of Moody et al. (1967), Bouraoui et al. (2002) and Gantner et al. (2011) and it was concluded that this was a result of decreased forage intake (Bouraoui et al. 2002; Gantner et al. 2011). For the present trials, changes in forage to concentrate ratio due to selection were not possible, because TMR were fed (except for Trial 2, Period 2). Therefore, feeding TMR should have alleviated negative effects of heat stress on milk fat content, by maintaining the intended forage to concentrate ratio. Indeed, it remains unclear why fat yield was not affected by the reduction of fat percentage.

5. Conclusion

Heat stress thresholds derived from trials in hot climates or climatic chambers are not appropriate for a temperate climate as in Germany. Threshold values of THI below 72 seem to be justified. The present results indicate that THI 60 seems to be a threshold denoting declines in DMI as well as in milk yield. Milk protein and milk fat percentage were negatively affected by THI linearly; therefore, no general thresholds can be derived from the present data. However, these initial investigations have to be substantiated by further research, especially as regional differences may exist. The mode of action of heat stress on the regulation of post-absorptive energy and nutrient (re-)partitioning should be investigated in more detail. Reduced DMI in heat-stressed cows is not sufficient to explain the total THI-induced decline in performance. Furthermore, the impact of mild heat stress requires particular attention because knowledge about it is scarce, yet it affects dairy production. Especially, as different degrees of heat stress might lead to non-linear responses of metabolism and production.

Acknowledgements

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 the experiments is gratefully acknowledged. The authors, furthermore, thank the “Deutscher Wetterdienst, Zentrum für Agrarmeteorologische Forschung Braunschweig” for providing meteorological data and Prof. Gertheiss, Department of Animal Sciences, University of Göttingen, for statistical advice.

Funding

The authors acknowledge financial support provided by the “Niedersächsisches Ministerium für Wissenschaft und Kultur” within the research network “KLIFF” (Climate Impact and Adaptation Research).

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Chapter 7 General discussion

1. Brown-midrib mutations in maize

1.1. Implications on dry matter intake, digestion events, efficiency of nutrient utilisation, and performance of dairy cows and sheep

Dry matter intake and efficiency of nutrient and energy utilisation

Previous studies have shown that the higher ruminal degradation of neutral detergent fibre (NDF) of brown-midirb (Bm) allowed for an increase in dry matter (DM) intake (DMI) due to lower ruminal fill (Oba and Allen, 1999; Qiu et al., 2003; Gehman et al., 2008). Oba and Allen (2000) assumed that ruminal fill from diets high in NDF may not limit DMI in animals with a low energy demand but if energy demand is high even diets low in NDF will do so.

Even though DMI capacity might have been higher for Bm due to a higher rate and extent of fibre degradation (Gorniak et al., 2013) and increased rate of passage (Chapter 4, Table 4), animals did not increase DMI. From the positive energy balance in the present trial (Chapter 3) and the moderate energy demand due to advanced stage of lactation, it can be concluded that ruminal fill did not limit DMI. Likely animals did not use their DMI potential because metabolic satiety terminated DMI before maximum ruminal capacity was reached.

The Bm hybrid had a higher efficiency of nutrient and energy utilisation (expressed as kg milk yield per kg DMI and kg milk yield per MJ net energy for lactation [NEL]; Table 1).

Table 1. Efficiency of dry matter and energy utilisation of maize silage-based diets (LSmeans with standard errors).

Milk yield [kg/kg DMI]	Con	Bm
Chapter 3 trial one[§]	1.48 ± 0.041	1.63 ± 0.041
Chapter 3 trial two[§]	1.30 ± 0.029	1.49 ± 0.028
Chapter 4	1.44 ± 0.101	1.54 ± 0.101
Milk yield [g/MJ NEL]		
Chapter 3 trial one[§]	209 ± 5.8	229 ± 5.8
Chapter 3 trial two[§]	195 ± 4.3	223 ± 4.2
Chapter 4	235 ± 18.1	252 ± 18.1

Notes: Con, Control; Bm, Brown-midrib; Chapter 3: Con, n=30; Bm n=31; Chapter 4: Con and Bm n=6; [§]Treatments differ, $p < 0.05$; Chapter 3: *Ad libitum* intake, Chapter 4: Restricted intake.

The differences between trials reported in Chapters 3 and 4 are likely due to restricted feed intake and the lower number of animals used in Chapter 4. Especially cows early in lactation respond to Bm (Chapter 1, Table 2). Animals with a lower milk yield or in advanced stages of lactation seem to be less responsive.

Energy content of silages estimated in vivo and in vitro

Differences in estimated values of energy content of the Control (Con) and Bm silages and diets were observed (Table 2). In the *in vivo* trials with sheep (Chapter 5) higher contents of metabolisable energy (ME) were observed for Bm, which was in line with *in vitro* results (Table 2). Contrastingly, no differences were observed for ME content between Con and Bm in the *in vivo* trials reported in Chapter 3. This difference might, however, be a result of silage preservation. Silages were ensiled in bunker silos (Chapter 3), or in big bales and sealed with stretch foil (Chapters 4 and 5).

Table 2. Overview of energy content (MJ ME/kg DM) of Con and Bm, estimated *in vivo* and *in vitro* (LSmeans).

	Con		Bm
Chapter 3			
Silages *in vivo*[*]	10.6		10.5
Chapter 4			
Silages *in vitro*[#a]	10.5		10.9
Concentrate *in vitro*[§] (same for both diets)		12.9	
Whole diet *in vivo*	10.2		10.2
Intact kernels [%]	5		5
Ensilage quality[$]	very good		very good
Chapter 5[&]			
Silages *in vivo*[*a]	9.6		10.4

Notes: Con, Control; Bm, Brown-midrib; [a]Treatments differ, $p < 0.05$; [*]Estimated according to GfE (1991, 2001): [ME (MJ/kg DM) = 0.0312 · DEE + 0.0136 · DCF + 0.0147 · (DOM–DEE–DCF) + 0.00234 · CP]; [#]Estimated from ESOM according to VDLUFA (2007, Method No. 6.6.1) and regression equation of GfE (2008): [ME (MJ/kg DM) = 7.15 + 0.00580 · ESOM – 0.00283 · NDFom + 0.03522 · EE]; [§]Estimated from Hohenheim gas test (Menke and Steingass 1988) and regression equation of GfE (2009): [ME (MJ/kg DM) = 7.17 – 0.01171 · Ash + 0.00712 · CP + 0.01657· EE + 0.002 · starch – 0.00202 · ADFom + 0.06463 · GP]; where: ADFom, acid detergent fibre expressed without residual ash; CP, Crude protein; DCF, Digestible crude fibre; DEE, Digestible ether extract; DOM, Digestible organic matter; EE, Ether extract; ESOM, Enzyme soluble organic matter; GP, 24 h gas production; [$]According to DLG (2004); Silages were prepared in bunker silos (Chapter 3) or in big bales and sealed with stretch foil (Chapter 4 and 5); [&]Values are means of three Con and three Bm treatments.

The ME content of the whole diet estimated *in vivo*, surprisingly, was lower than the energy content of the pure silages. This discrepancy might be explained by silage quality and method of energy estimation. In terms of fermentation, silage quality was good (DLG, 2004) but 5% of the total kernels of each silage were not cracked (estimated from visual evaluation, according to Sächsische Landesanstalt für Landwirtschaft, 2008), which is most likely due to technical problems during harvesting.

Small ruminants are able to chew maize kernels when ruminating (Ørskov, 1986); *in vitro* methods also do not consider intact kernels because these methods require grinding of the sample material. Cattle, in contrast, are not able to utilise intact maize kernels completely (Ørskov, 1986). Therefore, energy content of the whole diet estimated *in vivo* with dairy cows might have been lower. Pieper et al. (2005) summarized that digestibility values obtained from sheep and cattle do not necessarily reveal the same results. Pex et al. (1996) and Südekum et al. (2000) observed that cattle are superior in digesting maize silage compared to sheep. Flachowsky et al. (2004) in contrast showed that OM digestibility was lower in dairy cows, when fed at increased plane of nutrition (two times maintenance requirement). Therefore, lower energy contents estimated *in vivo* might be a combined result of intact kernels and the per se higher OM digestibility in small ruminants.

Milk fat content

It was also observed that Bm maize silage decreased milk fat content and/or milk fat yield (Table 3). The Bm maize silage led to a considerable drop in milk fat content (Chapter 3, Table 4). Changes in ruminal fermentation might have accounted for these effects. Milk fat content was the same for Con and Bm and Bm did neither affect ruminal concentration nor molar proportion of short chain fatty acids (SCFA; Chapter 4, Table 3), although Con and Bm differed considerably in *in situ* degradation of amylase pre-treated, ash free NDF (aNDFom) and ash free acid detergent fibre (ADFom; Gorniak et al., 2013).

Ruminal SCFA production may affect milk fat synthesis. Ruminal SCFA concentrations as well as molar proportions, however, do not reflect SCFA production rates (Dijkstra et al., 1993). Thus, conclusions from these figures are limited. Especially, a reduced proportion of acetate is not necessarily related to a reduced acetate production rate, but might also be due to an increased propionate production rate (Bauman et al., 1971). Particularly in low fibre diets, ruminal SCFA proportions do not depict SCFA production rates, as low fibre diets reduce ruminal pH, which in turn alters rate of absorption of SCFA (Dijkstra et al., 1993).

Oba and Allen (2000) concluded that the decrease in ruminal pH due to feeding Bm maize silage might have affect duodenal flow of trans-$C_{18:1}$ fatty acids, but did not find changes in milk fat yield and, therefore, ruled out the impact of *trans*-fatty acids on milk fat content. Lower physical effectiveness of diets containing Bm maize silage may also account for the lower milk fat concentration (Holt et al., 2010). It remains, however, unclear how Bm affects milk fat content in detail. Yet, it is widely accepted that milk fat content can easily be manipulated by feeding, but knowledge about the detailed mechanisms is scarce.

From the present results it can be concluded that Bm had a distinct effect on milk fat synthesis. Inconsistency of literature and inconsistency of the present results (Chapters 3 and 4) as well as the tendency of Bm to interact with other dietary components (Oba and Allen, 2000; Taylor and Allen, 2005; Castro et al., 2010), however, make clear that further research is warranted to clarify the detailed impact of Bm on milk fat production.

Table 3. Milk fat content as affected by feeding Bm maize silage.

	Con	Bm
Chapter 3		
Trial one		
Milk fat [%][a]	3.8	3.3
Milk fat [kg/d][a]	1.26	1.14
Trial two		
Milk fat [%][a]	4.4	4.0
Milk fat [kg/d]	1.12	1.17
Chapter 4		
Milk fat [%]	2.9	3.1
Milk fat [kg/d]	0.65	0.73
Oba and Allen (2000)[§a]		
Milk fat [%][a]	3.79	3.57
Milk fat [kg/d]	1.22	1.27
Taylor and Allen (2005) [§a]		
Milk fat [%][a]	3.57	3.47
Milk fat [kg/d]	1.43	1.44
Holt et al. (2010) [§a]		
Milk fat [%][a]	2.94	2.56
Milk fat [kg/d][a]	1.24	1.09
Oba and Allen (1999)		
Milk fat [%]	3.46	3.44
Milk fat [kg/d][a]	1.33	1.42
Sommerfeldt et al. (1979)		
Milk fat [%]	3.88	3.79
Milk fat [kg/d]	0.99	0.95
Castro et al. (2010) [§]		
Milk fat [%]	3.39	3.33
Milk fat [kg/d]	1.33	1.35

Notes: Con, Control; Bm, Brown-midrib; [a]Treatments differ, $p < 0.05$; [§]Values are means of different Con and Bm treatments; Chapter 3: Con, n= 30, Bm n=31; Chapter 4: Con and Bm n=6.

1.2. Nitrogen utilisation

The results concerning the nitrogen (N) utilisation and the N balance in Chapter 4 do not appear logical. It was shown that total N balance and ruminal N balance (RNB = [crude protein (CP) intake – utilisable CP (uCP) at the duodenum] / 6.25) were unrealistically high (Chapter 4, Table 5. The N balances of 36.2 and 35.5 g N per day for Con and Bm cannot be explained by deposition of N. About 36 g N would mean 225 g protein. That corresponds with an estimated deposit of about 750 g of body tissue per day (assuming a protein content of about 30%), which might be realistic in rapidly growing animals but not in adult dairy cows. The high N balances cannot be fully explained but it has to be taken into account that N balances in adult ruminants are often greater than expected (Spanghero and Kowalski, 1997; Reynolds and Kristensen, 2008). Nitrogen balances over a whole lactation might be zero, measurements over short periods often yield positive or negative N balances (Reynolds and Kristensen, 2008). Marginal losses might sum up to appreciable errors, thus, high methodological accuracy is necessary (Spanghero and Kowalski, 1997; Reynolds and Kristensen, 2008). Furthermore, N losses from coat and scurf are generally not considered and might partially contribute to misjudgement.

Martin (1966), who investigated N losses during N balance trials very extensively, including losses from faeces, urine, expired air, wool and suint, could explain only a fractional amount of lost N. Ammonia losses from urine might be considerable even if urine is acidified during collection (Martin 1966). Labile N pools or protein reserves, might contribute to high N balances in short term trials (Paquay et al., 1972; Biddle et al., 1975). For the first time, labile N pools were discussed by Voit (1866). Plasma protein and urea (Biddle et al., 1975), as well as liver, other viscera, and newly synthesised muscles (Paquay et al., 1972) might contribute to such labile N pools. These studies, however, were undertaken with growing cattle or dry non pregnant cattle, whilst the cows in the present trials were adult, pregnant animals. Furthermore, such labile N pools or protein reserves are not clearly identified yet (Waterlow, 1999). The very high N balances cannot be explained but it seems to be most likely that several marginal losses, as mentioned above, as well as the short period of N balance measurement contributed to these results. Lack of knowledge about N balances and body protein reserves, which was summarized by Waterlow (1999) as “The mysteries of nitrogen balance”, however, shows that further research is necessary.

The RNB reported are also remarkable, especially as the whole diets were calculated to have a balanced RNB (Chapter 4). Two reasons might contribute to the unexpectedly high RNB. At first, the silages contained about 5% intact kernels, which might have reduced energy available for ruminal microorganisms and, therefore, might have negatively affected microbial crude protein (MCP) synthesis and subsequently increased RNB. The second reason might be methodological problems. The RNB is calculated as follows:

$$\text{RNB [g/d]} = (\text{CP intake [g/d]} - \text{uCP [g/d]}) / 6.25$$

where:

$$\text{uCP [g/d]} = \text{CP flow at the duodenum [g/d]} - NH_3\text{-N [g/d]} \cdot 6.25 \\ - \text{endogenous CP (ECP) [g/d]}$$

and

$$\text{ECP [g/d]} = 3.6 \cdot \text{kg duodenal DM flow (DMF)} \cdot 6.25$$

Crude protein flow and NH_3-N flow at the duodenum were estimated from DMF and the corresponding analyses. Therefore, an underestimation of DMF would result in an underestimation of uCP and, thus, an overestimation of RNB. Indeed an underestimation of DMF would underestimate ECP and, hence, overestimate uCP. However, ECP affects uCP and thus RNB only marginally. Losses of DM during feeding and changes of nutrient content of the diet during preparation, storing and feeding (e.g. aerobe fermentation in the troughs) might contribute to an overestimation of CP intake and subsequently to an overestimation of RNB. The latter two, however, are probably marginal. Therefore, it is likely that an underestimation of DMF is the main reason for the overestimation of RNB. Nonetheless any errors were the same for both treatments. Even though absolute values cannot be derived, treatments can be compared.

2. Heat stress in ruminants

2.1. Estimating critical climatic conditions for ruminants in temperate climates

A temperature-humidity index (THI) of 70 or 72 was generally accepted to be a heat stress threshold (Hahn, 1985; Johnson, 1985; Dupreez et al., 1990; Armstrong, 1994; Chase, 2006; Figure 1). The THI, however, is a value to physically describe the animals' environment and, thus, indirectly describes heat stress. This method is very easy to adopt on farm but it has to be considered that a validation is needed. Especially certain thresholds may be valid for a certain breed in a certain environment only. For Northern America for example it was shown that a THI threshold of 72 calculated according to Ravagnolo and Misztal (2000) from daily maximum temperature [°C] and daily minimum relative humidity (RH) [%] or Bohmanova et al. (2005) from hourly means of temperature [°C] and RH [%] using the following equation was appropriate to predict milk yield losses.

$$\text{THI} = (1.8 \cdot \text{temperature} + 32) - (0.55 - 0.0055 \cdot \text{RH}) \cdot (1.8 \cdot \text{temperature} - 26)$$

Though, thresholds are different for Lower Saxony, Germany. A THI of 60 calculated according to Hahn et al (1999) or Bohmanova et al. (2005) can be accepted to be a threshold denoting a decline in milk yield for Holstein cows in Lower Saxony, Germany (Chapter 6; Brügemann et al., 2012). A THI of 70 might also denote a threshold if the equation adapted by Ravagnolo and Misztal (2000) is applied (Brügemann et al., 2012).

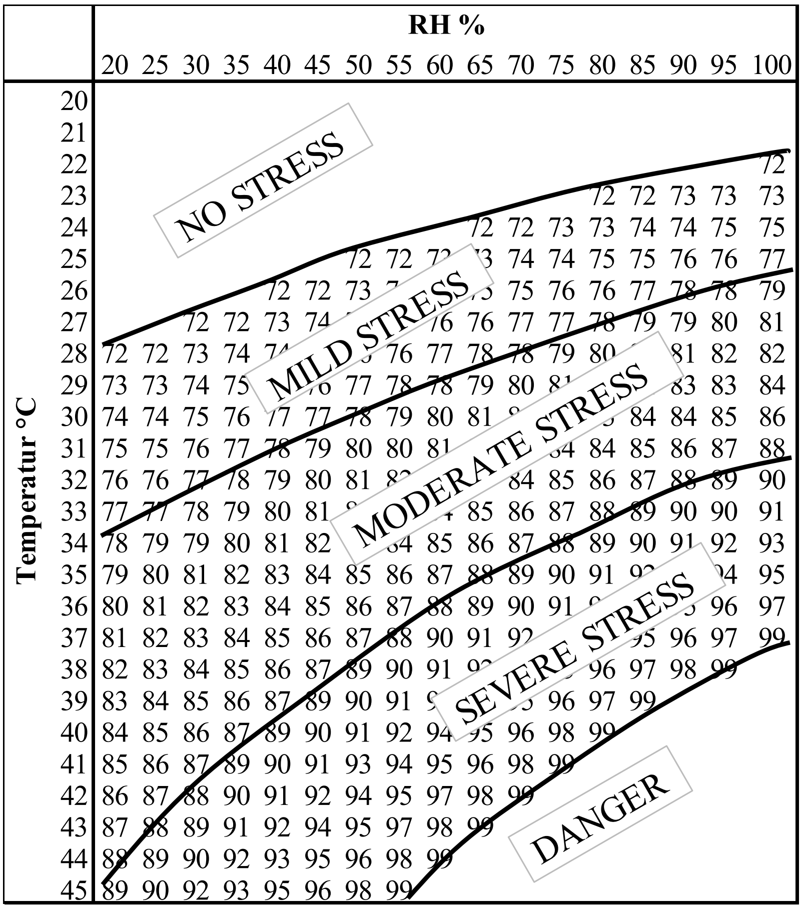
Contrastingly, Ammer et al. (2013) reported that milk yield begins to decline above a THI of 55 for dairy farms using automatic milking system in Lower Saxony, Germany. Zimbelman et al. (2009), furthermore, observed that milk yield declines above THI 68. However, different stages of lactation [Brügemann et al. (2012): whole lactation; Chapter 6: 100-200 days in milk], or different production systems [Zimbelman et al. (2009): insulated, environmentally controlled tie stall barn; Brügemann et al. (2012): indoor housing in an intensive crop production region vs. pasture based system vs. maritim region; Ammer et al. (2013): automatic milking systems in insulated or non-insulated barns; Chapter 6: insulated free stall barn with focus on mid-lactation cows]. Therefore, it can be concluded that THI thresholds do not only differ among climatic regions but even on a very small scale within a region differences might occur and certain management systems make dairy cows more or less susceptible to changes in THI.

Another problem might be to categorise heat stress. Terms like “severe heat stress”, “moderate heat stress” and “mild heat stress” were used in literature and it was shown that a kind of moderate or mild heat stress exists (Hofman and Riegle, 1977; Ominski et al., 2002; Odongo et al., 2006; Lohölter et al., 2012). The definitions of moderate/mild heat stress, however, varied widely (Table 4). Odongo et al. (2006) actually used the term “mild heat stress” exclusively in their title and missed to give any definition of the expression “mild”.

Table 4. Definitions of mild heat stress used in literature.

Reference	Animals	Definition of heat stress	
Hofman and Riegle (1977)	Shorn and unshorn Dorset ews	25, 30, 35 or 40°C, RH[*] 40% for 120 minutes	
Ominski et al. (2002)	Lactating Holstein cows	Thermoneutral:	from 07:00 to 18:00h, 24°C from 18:00 to 07:00 h 20°C
		Moderate heat stress:	07:00 to 10:00 h increase from 20°C to 32°C 10:00 to 18:00 h 32°C 18:00 to 07:00 h 20°C
Odongo et al. (2006)	Unshorn Canadian Arcott lambs	Thermoneutral: Mild heat stress:	18 to 20°C, RH 30% for 24 h/d 35°C, RH 40% for 9 h/d; 20°C, RH 40% for 15 h/d
Lohölter et al. (2012)	Castrated male German Blackheaded Mutton sheep	Temperate: Mild heat: Severe heat:	THI[#] 57 to 63, 24 h/d THI 68 to 71, 24 h/d THI 75 to 80, 24 h/d

Notes: [*]RH, Relative humidity; [#]THI, Temperature-humidity index.



Temperatur °C	RH % 20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
20																	
21																	
22																	72
23													72	72	73	73	73
24										72	72	73	73	74	74	75	75
25							72	72	[illegible]	[illegible]	74	74	75	75	76	76	77
26					72	72	73	[illegible]	[illegible]	[illegible]	75	76	76	77	78	78	79
27			72	72	73	74	[illegible]	[illegible]	76	76	77	77	78	79	79	80	81
28	72	72	73	74	74	[illegible]	[illegible]	76	77	78	78	79	80	[illegible]	81	82	82
29	73	73	74	75	[illegible]	76	77	78	78	79	80	81	[illegible]	[illegible]	83	83	84
30	74	74	75	76	77	77	78	79	80	81	[illegible]	[illegible]	[illegible]	84	84	85	86
31	75	75	76	77	78	79	80	80	81	[illegible]	[illegible]	[illegible]	84	85	86	87	88
32	76	76	77	78	79	80	81	[illegible]	[illegible]	[illegible]	84	85	86	87	88	89	90
33	77	77	78	79	80	81	[illegible]	[illegible]	[illegible]	85	86	87	88	89	90	90	91
34	78	79	79	80	81	82	[illegible]	[illegible]	85	86	87	88	89	90	91	92	93
35	79	80	81	82	83	84	85	86	87	88	89	90	91	[illegible]	[illegible]	[illegible]	95
36	80	81	82	83	84	85	86	87	88	89	90	91	[illegible]	[illegible]	[illegible]	96	97
37	81	82	83	84	85	86	87	88	90	91	92	[illegible]	[illegible]	95	96	97	99
38	82	83	84	85	86	87	89	90	91	[illegible]	[illegible]	[illegible]	96	97	98	99	
39	83	84	85	86	87	89	90	91	[illegible]	[illegible]	[illegible]	96	97	99			
40	84	85	86	87	89	90	91	92	94	95	96	98	99				
41	85	86	87	89	90	91	93	94	95	96	98	99					
42	86	87	88	90	91	92	94	95	97	98	99						
43	87	88	89	91	92	94	95	97	98	99							
44	88	89	90	92	93	95	96	98	99								
45	89	90	92	93	95	96	98	99									

Figure 1. Classification of heat stress using the temperature-humidity index according to Armstrong (1994) and Chase (2006), adapted from Armstrong (1994).

The work of Ominski et al. (2002) and Odongo et al. (2006) show that diurnal variations can be taken into account, by cooling down the rooms at night, whereas Lohölter et al. (2012) used a constant ambient temperature for their digestibility trials. Contrastingly, Hofman and Riegle (1977) used very short heat stress periods of about 120 minutes. One has to consider that these authors had very different aims. Lohölter et al. (2012) investigated the feeding value of maize silages. Ominski et al. (2002) and Odongo et al. (2006) investigated physiological and production responses considering diurnal temperature fluctuations. Hofman and Riegle (1977) examined short term changes in thermoregulation. Thus, it becomes obvious that the definition of (mild/moderate) heat stress depends on the question that is to be examined.

With regard to production responses of dairy cattle it can be defined that occurrence heat stress and, therefore, graduation of heat stress depends on the impact of daily means of an indicator (i.e. temperature or THI) on DMI and milk yield (as mediated by DMI or physiological reactions to increased ambient temperature/THI). Daily fluctuation might be considered in terms of minimum and maximum values or for example by deriving thresholds that should not be exceeded for a given time per day to alleviate animals from heat stress (Igono et al., 1992; Chapter 6). For the derivation of thresholds it also has to be taken into account that production responses might be delayed in relation to the occurrence of a stressor for one or more days (Collier et al., 1981; West et al., 2003; Chapter 6).

2.2. Implications on digestion events, feed intake, and performance of dairy cows and sheep

Even mild or moderate heat stress (yet, a generally accepted and acceptable definition does not exist, see 2.1.) affects digestion events, feed intake, and performance of dairy cows and sheep (Chapters 5 and 6). Climate change will, however, affect animal production on two levels, animals will be affected directly, and forage quality might be impaired.

Climate change will lead to an increase in severity and frequency of heat waves, but it will also lead to an increase in mean ambient temperature. The former being the main factors affecting heat stress in cattle, whilst the latter likely will also affect forage quality. Especially lignification impairs forage quality and is known to be more problematic in warmer regions than in temperate regions (Van Soest, 1994).

Several authors have addressed diet formulation for heat-stressed cattle and small ruminants, namely the fibre content of the diet (Stott and Moody, 1960; Coppock et al., 1964; Tsai et al., 1967; Webster et al., 1975; Cummins, 1992; West et al., 1999), CP concentration and ruminal degradation (Hassan and Roussel, 1975; Higginbotham et al., 1989a, b; Ames et al., 1980; Taylor et al., 1991; Chen et al., 1993; Huber et al., 1994) and dietary fat supplements (Moody et al., 1967; O'Kelly, 1987; Saunders et al., 1990; Knapp and Grummer, 1991). With regard to Bm maize, the latter might be of less importance and will, therefore, not be discussed in detail.

The major problem under heat stress conditions might be reduced DMI, reduced performance and increased maintenance requirements. Reduced DMI might be a reaction of the animal to reduced heat increment of feed intake. Therefore, processes associated with heat production have to be addressed when formulating rations for heat stress conditions.

Processes of heat production related to dry matter intake and fibre.

Processes of heat production related to DMI and dietary fibre are 1. energy expenditure for ingestion (Osuji et al., 1975; Susenbeth et al., 1998; Susenbeth et al., 2004), 2. heat of ruminal fermentation (Webster et al., 1975; Czerkawski, 1980; Reynolds et al., 1991) and 3. heat increment of utilisation of fermentation products (mainly acetate; MacRae and Lobley, 1982). Heat production from feed ingestion (including rumination) might contribute to total heat production to 5-12% of ME intake (Susenbeth et al., 1998; Susenbeth et al., 2004). Heat of fermentation accounts for up to 10% of total heat production (Czerkawski, 1980). Heat production from metabolic use of acetate, furthermore, is also of importance but difficult to quantify (MacRae and Lobley, 1982). The underlying process is the utilisation of acetate from fibre fermentation (e.g., its conversion into fatty acids and finally fat), which depends on the availability of reduced nicotinamide adenine dinucleotide phosphate (NADP). Reduced NADP itself is derived from glucose metabolism (MacRae and Lobley, 1982). Thus, propionate which is an precoursor of glucose might limit biosynthesis of fatty acids and, therefore, acetate utilisation. As low-roughage diets have a lower heat production from ingestion, fermentation, and acetate utilisation, reducing forage to concentrate ratio seems appropriate for heat-stressed dairy cows

Decreasing fibre content of the diet, however, is limited in high yielding dairy cows, due to the risk of acidosis and laminitis (Nocek, 1997; Kleen et al., 2003). Particularly buffering of the rumen seems to be reduced under heat stress conditions, which might be due to a decrease of alkaline reserves (Niles et al., 1980; Collier et al., 1982). Furthermore, animals seem to benefit from an increas of concentrate proportion only up to 60-65% concentrate in the diet (Coppock, 1985). Therefore, it can be concluded that maintaining an adequate forage to concentrate ratio rather than simply reducing fibre concentration is the essential strategy to formulate rations for heat-stressed dairy cows.

From ruminal temperature measurements it can be concluded that heat of fermentation was not affected by silage maize variety in the present study, (Table 5). The Bm diet, however, showed a trend for a lower ruminal temperature. Heat emerging from ingestion was not measured in the present study but total time ruminating was not different between Con and Bm (Chapter 3). Therefore, it can be concluded that time for feed intake, and subsequently heat production from ingestion was similar for Con and Bm.

Table 5. Mean, maximum, and minimum ruminal temperature of animals fed Con or Bm maize silage-based diets (LSmeans with SEM[†]).

	Con	Bm	SEM	*p*-values[‡]		
Temperature [°C]				Hybrid	Period	Hybrid x· period
Chapter 3						
Mean	39.4	39.2	0.07	0.058	-	-
Maximum	40.7	40.6	0.09	0.335	-	-
Minimum	36.0	35.4	0.28	0.174	-	-
Chapter 4						
Mean	39.0	39.3	0.46	0.571	0.179	0.536
Maximum	42.8	45.9	2.69	0.314	0.130	0.418
Minimum	34.4	35.2	1.09	0.596	0.196	0.710

Notes: SEM, [†]Standard error of the means; Con, Control; Bm, Brown-midrib; [‡]Effects of maize hybrid, experimental period, and maize hybrid × period interaction.

Under heat stress conditions DMI might also be affected by changes in ruminal motility, mean retention time and ruminal fill. Reduced motility might, however, also decrease absorption and, therefore, hamper utilisation of fermentation products because they will not reach the rumen wall.

Wethers and steers had an increased ruminal retention time in heat stress situations (Weniger and Stein, 1992; Miaron and Christopherson, 1992). Frequency and amplitude of rumen contractions was reduced when animals were exposed to 38°C for five days (cattle; Attebery and Johnson, 1969) or 41.8°C for 12 hours (goats; Cąkała, 1965). Therefore, reduced DMI might not only be an adaptation to metabolic heat production but also mediated by ruminal fill. Ruminal fill might limit DMI especially for high-yielding dairy cows but also in lower yielding animals fed high-fibre diets (West et al., 1999). Furthermore, ruminal volume was increased in beef cows (Silanikove and Tadmor, 1989) and swamp buffalo (Chaiyabutr et al., 1987) because of increased water intake in heat stress situations, which might also lower DMI.

As reduced DMI is a problem in heat-stressed dairy cattle (even though heat stress was mild/moderate, Chapter 6), Bm maize may contribute to a feeding strategy for heat-stressed cows. It was shown that animals on Bm maize silage-based diets had the same milk yield as animals fed Con maize silage-based diets, whilst DMI was lower for Bm. Furthermore, Bm maize silage had a higher ruminal particle outflow rate (Chapter 5). Thus, DMI capacity was higher because of a higher ingesta efflux. In high-yielding animals with a limited DMI capacity, diets high in ruminal DM degradation and low in ruminal retention time might be advantageous. Especially heat-stressed dairy cows might benefit from Bm maize silage in such a manner.

Processes of heat production related to dietary crude protein.

Crude protein utilisation has to be taken into account when feeding heat-stressed ruminants. It was assumed that an increase in CP content increases DMI and milk yield under heat stress conditions (Hassan and Roussel, 1975). Increased dietary CP content, however, also increased plasma and milk non-protein nitrogen (Hassan and Roussel, 1975), which might be interpreted as excess protein. Ames et al. (1980) found increased utilisation of protein when CP content of the diet was reduced under heat stress conditions in feedlot cattle without a decline in average daily gain. Huber et al. (1994) summarized that excess protein in heat-stressed dairy cows decreased milk yield due to energy cost for urea formation. Urea formation consumes about 23 kJ per gram of N (Martin and Blaxter, 1965) and burdens animals with further heat production.

More protein was available to the animals fed Bm maize silage because of increased efficiency of MCP synthesis (Chapter 4, Table 5). Milk N and faecal N excretion were increased. From the lower milk urea concentration and the trend for a lower urinary N excretion it can be concluded that urea formation was lower for animals fed Bm maize silage. Therefore, energy expenditure, and subsequently heat production, would be reduced.

A lower protein requirement was observed in heat-stressed animals than in animals under thermoneutral conditions (Ames et al., 1980). Increased energy requirement for maintenance reduces energy available for protein utilisation, therefore, CP content of the diet for heat-stressed animals might be reduced to increase efficiency of protein utilisation without a further decrease in performance (Ames et al., 1980). However, according to Higginbotham et al. (1989a, b) hot and moderate temperatures might lead to different reactions and CP content as well as CP degradability might have to be adjusted depending on severity of heat stress.

3. Brown-midrib maize and climate change

Regarding forage production, Bm maize might be a strategy to overcome negative effects on forage quality associated with climate change. Recent studies have shown that climate change in Lower Saxony, Germany will not severely affect forage production, if technological progress keeps up with climate change. However, the recent investigations have addressed phytopathology (von Tiedemann and Juroszek, 2013), effects of atmospheric carbon dioxide concentration (Ebeling et al., 2013; Manderscheid et al., 2013), and nutrient composition and *in vitro* degradation regarding drought during growth (Lindig et al., 2013). Forage quality, will, however, be impaired by temperature with regard to fibre digestibility. Especially lignification was enhanced with increasing temperature in Bermuda grass, Guinea grass, perennial ryegrass, and lucerne (Wilson et al., 1991; Van Soest, 1994), and maize (Deinum, 1976; Cone and Engels, 1990).

Temperature, light, water, fertilisation, and soil affect forage quality in descending order (Van Soest, 1994). With regard to climate change, temperature and water availability might be the most important factors. Reduced water availability, however, might reduce maturity and thus increase digestibility, whereas DM yield is reduced (Van Soest, 1994). Irrigation is not common all over Germany, but it is widely used in some regions, particularly in intensive horticulture. Therefore, drought stress might be of less importance than temperature, which cannot be controlled in any case. Especially water scarcity (at least partly) might be compensated for by the increase in atmospheric carbon dioxide concentration, (Drake et al., 1997; Manderscheid et al., 2013).

Therefore, Bm maize could be useful within the context of climate change. Additionally to its potential for feeding heat-stressed dairy cows it might also serve as an option to counteract negative effects of climate change on forage quality. Van Soest (1994) concluded that the Bm gene could be valuable in regions where lignification is of major importance (e.g. warm, tropical countries). But bearing climate change and its effects on average temperature as well as frequency and intensity of heat waves in mind, Bm maize would also be a potential strategy for temperate climates. In addition to the above mentioned positive effects on animal nutrition, Bm maize has the potential to compensate for negative effects of climate change on forage quality. However, agronomic performance of Bm maize is still unsatisfactory (Chapter 1), therefore, progress in plant breeding is necessary to gain practical relevance in animal nutrition.

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Chapter 8 Conclusion

In contrast to the hypothesis, dry matter intake (DMI) was not higher in animals fed diets based on brown-midrib (Bm) maize silage. Milk yield and efficiency of nutrient utilisation, however, were improved (Chapter 3 and 4). Digestibility and metabolisable energy content estimated from digestibility trials with sheep were higher for Bm (Chapter 5) but not when included in a diet for dairy cows (Chapter 4). In fact, Bm maize silage-based diets had a higher ruminal rate of passage and efficiency of microbial crude protein (MCP) synthesis in comparison with the control (Con) maize silage-based diets. Ruminal fermentation characteristics (molar proportion of short chain fatty acids, NH_3 concentration, and pH) were not affected. Therefore, it can be concluded that Bm maize silage is superior for dairy cows in comparison to Con silage, if agronomic performance is satisfactory. Further investigations characterising Bm hybrids in comparison to non-Bm hybrids according to organic matter (OM) yield, digestible OM yield and milk yield per hectare are necessary.

In accord with the hypothesis, DMI, milk and protein yield were lowered in heat-stressed dairy cows. In contrast to the hypothesis, higher ambient temperatures did not affect nutrient digestibility in sheep. Interactions of ambient temperature and silage maize variety, however, were found. From increased respiration rate and rectal temperature in sheep and reduced DMI and milk yield in dairy cows it can be concluded that graduations of heat stress can be identified by physiological and performance parameters. Thus, ***mild*** heat stress exists in sheep and dairy cows. The definition of ***mild*** heat stress, however, is still ambiguous. Distinct thresholds and more precise definitions of ***mild*** heat stress have to be evaluated and determined. Particularly with regard to inconsistency of literature, further research is necessary to describe heat stress and its impact on digestion events in ruminants. Especially, graduation of heat stress has to be investigated, as linear effects cannot be expected.

Reasonable feeding strategies exist to compensate for (at least partly) the negative effects of heat stress. From the results of Chapter 3 to 6 it can be concluded that Bm maize silage has advantages under heat stress conditions in comparison to the Con silage. The higher efficiency of nutrient utilisation and the higher rate of ruminal passage of particles of Bm silage might help to maintain DMI during periods of heat stress. Increased efficiency of MCP synthesis might counteract crude protein deficiency due to reduced DMI without the negative effects of increasing dietary crude protein concentration. The lower lignin content of Bm maize, furthermore, is an advantage with regard to climate change, as increasing temperature increases lignification. Indeed, these properties are not unique for Bm maize. However, the aim of the present thesis was not to draw general conclusions on Bm maize but to give a first insight into the potential of Bm maize silage under climate conditions in Germany.

Regarding the impact of heat stress on dairy production, it is obvious that nutrition cannot be the sole answer. Appropriate strategies to cope with heat stress require a combination of nutritional, breeding, and management adaptations. Hence, further research is justified to describe the possibilities and limitations of nutrition but also management and breeding (including plant breeding) to cope with a changing climate in a more comprehensive way.

Danksagung

Mein besonderer Dank gilt Herrn Prof. Dr. Karl-Heinz Südekum für die Betreuung der vorliegenden Arbeit und die Übernahme des ersten Gutachtens.

Des Weiteren danke ich Herrn PD Dr. Jürgen Schellberg für die Übernahme des zweiten Gutachtens.

Herrn Prof. Dr. Dr. Sven Dänicke danke ich für die Möglichkeit am Institut für Tierernährung in Braunschweig die vorliegende Arbeit im Rahmen des Projektes ***KLIFF – Klimafolgenforschung in Niedersachsen*** anzufertigen, für die Betreuung der vorliegenden Arbeit und die Übernahme des dritten Gutachtens.

Ich danke Dr. Ulrich Meyer, Dr. Liane Hüther und Dr. Peter Lebzien für die Betreuung der vorliegenden Arbeit.

Gottinga, Anno Domini MMXV