Institut für Tierwissenschaften
Rheinische Friedrich-Wilhelms-Universität Bonn

Lucerne silage for high yielding dairy cows – evaluation of the nutritional value using chemical and in vivo methods

Dissertation
zur
Erlangung des Grades
Doktor der Agrarwissenschaften
(Dr. agr.)
der
Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität
Bonn

vorgelegt im September 2017

von

M. Sc. Julia Trautwein

aus

Ludwigsburg

Referent: **Prof. Dr. Karl-Heinz Südekum**

Korreferent: **Prof. Dr. Wolfgang Büscher**

Korreferent: **Prof. Dr. Georg Dusel**

Tag der mündlichen Prüfung: **17. November 2017**

Erscheinungsjahr: **2018**

Meiner Familie

SUMMARY

Lucerne silage for high yielding dairy cows – evaluation of the nutritional value using chemical and in vivo methods

Lucerne has a high yield potential and can provide ruminants with significant amounts of energy and protein as well as having excellent structural properties. This thesis focussed on the response (dry matter intake (DMI), milk yield, milk composition and chewing activity) of high yielding dairy cows on a feeding with different combinations of forages offered as total mixed rations (TMR) with an average forage to concentrate ratio of 55:45 (dry matter (DM) basis). The forage component of the control (CON) ration was a 50:50 mixture of grass and maize silages (DM basis). The forage component of the three lucerne-based diets comprised (DM basis) either lucerne and grass silage (GL, 50:50), lucerne and maize silage (ML, 50:50) or equal proportions of grass, maize and lucerne silage (GML). A seven-phase feeding trial was conducted with a herd of 60 lactating cows. The whole herd received each diet *ad libitum* for six weeks. Diets with lucerne silage were only compared to the control diet. All diets (TMR) were formulated to contain the same utilisable crude protein at the duodenum (uCP) but differed in the energy, starch, fibre as well as physically effective NDF (peNDF) content. A digestibility study with sheep, determined a lower digestibility of lucerne silage of organic matter (OM) and energy content compared to grass and maize silage and a lower digestibility of fibre fractions (aNDFom and ADFom) compared to grass silage. DMI ranged from 20.8 kg (CON) to 22.3 kg (ML) and was increased for cows fed diets with lucerne silage ($P<0.05$). The lower energy density common to all the lucerne diets was only fully compensated for by higher DMI in the case of the ML diet. Despite higher feed intake the energy corrected daily milk yield (31.7 (GML) to 33.6 kg/day (CON)) was lower if cows were fed diets with lucerne silage. All lucerne diets resulted into a lower milk protein content compared to CON. The milk fat content of cows fed diet GL (highest $peNDF_{>1.18}$ and lowest starch content) was higher (4.31%) compared to CON (3.92%). Chewing activity, determined with a chewing halter, and feeding behaviour using the data from the feed bins were examined with six dairy cows. The rumination and total chewing activity as well as the eating behaviour were not affected by diets. Cows fed ML showed the highest FI and longest eating time and spent 37.3% of total chewing time on eating and 62.7% on rumination and therefore spent more time in eating and less time on rumination compared to CON ($p<0.05$). A similar tendency ($p<0.10$) was observed for cows fed GL compared with CON. In conclusion, overall effect of forage type on feeding behaviour and chewing activity was small. Lucerne has the potential to improve the protein and fibre supply from domestic farmland to dairy cows if harvested at the optimal stage of maturity. Depending on harvest condition and post-harvest treatment lucerne silage is recommended as component for dairy rations. Maize silage is able to compensate for the lower energy content of lucerne silage and is thus well suited as a further ration component.

ZUSAMMENFASSUNG

Luzernesilage für hochleistende Milchkühe-
Bestimmung des Futterwertes mit Hilfe labor- und tierbasierter Methoden

Die Luzerne kann eine sehr ertragreiche Futterpflanze sein und einen wichtigen Beitrag zur Energie-, Protein-, sowie Strukturversorgung in der Wiederkäuerernährung leisten. In dieser Studie wurde der Einfluss unterschiedlicher Grobfutterkombinationen, die als Gesamtmischration (total mixed ration, TMR) mit einem Verhältnis von Grobfutter zu Kraftfutter von 55:45 (auf Trockenmasse (DM)-Basis) angeboten wurde, auf die Trockenmasseaufnahme (DMI), die Milchleistung und Milchzusammensetzung sowie die Kauaktivität untersucht. Die Grobfutterkomponenten der Kontrollration (CON) bestanden zu je 50% aus Gras- und Maissilage (auf DM-Basis). Die Grobfutterkomponenten der Rationen mit Luzerne setzten sich auf DM-Basis entweder aus je 50% Gras- und Luzernesilage (GL), Mais- und Luzernesilage (ML) oder zu gleichen Anteilen aus Gras-, Mais- und Luzernesilage (GML) zusammen. Es wurde ein Fütterungsversuch mit sieben Phasen und einer Herde von 60 laktierenden Kühen durchgeführt. Dabei erhielt die gesamte Herde jede Ration zur *ad libitum*-Aufnahme über einen Zeitraum von sechs Wochen. Rationen mit Luzernesilage wurden nur mit der Kontrollration verglichen. Alle Versuchsrationen waren so konzipiert, dass das nutzbare Rohprotein am Duodenum (uCP) ähnlich war, sich aber hinsichtlich ihrer Gehalte an Nettoenergie, Stärke, Faser und physikalisch effektiver Faser (peNDF) unterschieden. In einer Verdaulichkeitsstudie am Hammel wurde eine geringere Verdaulichkeit der organischen Masse und ein geringerer Gehalt an umsetzbarer Energie im Vergleich zu Gras- und Maissilage bestimmt, sowie gegenüber Grassilage auch eine geringere Verdaulichkeit der Faserfraktionen. Die mittlere tägliche DMI variierte zwischen 20,8 kg (CON) und 22,3 kg (ML) und zeigte sich bei den Kühen signifikant erhöht, die mit Luzernesilage in der Ration gefüttert wurden ($p<0,05$). Alle Rationen mit Luzerne wiesen eine geringere Energiedichte auf, die nur in der Kombination ML durch die höhere DMI kompensiert werden konnte. Trotz der höheren Futteraufnahme war der energiekorrigierte Milchertrag (31,7 (GML) bis 33,6 kg/Tag (CON)) niedriger, wenn Kühe mit Luzerne gefüttert wurden. Alle Luzernerationen führten im Vergleich zur CON zu einer Abnahme des Milchproteingehaltes. Der Milchfettgehalt lag bei Fütterung mit GL (höchster peNDF- und niedrigster Stärkegehalt) am höchsten (4,31%) im Vergleich zur CON (3,92%). Die Kauaktivität wurde mit einem Kauhalfter an sechs Kühen je Rationsvariante bestimmt und das Fressverhalten über die Futterwiegetröge erfasst. Das Wiederkauen und die Kauaktivität sowie das Futteraufnahmeverhalten unterschied sich auf Grund der Versuchsrationen nicht. Die Fütterung mit Mais- und Luzernesilage führte zur höchsten Futteraufnahme und längsten Fresszeit. Die Kauzeit teile sich in 37,3% Fresszeit und 62,7% Wiederkauzeit auf. Damit war im Vergleich zur Kontrolle die anteilige Fresszeit signifikant erhöht und im Gegenzug die Wiederkauzeit vermindert. Eine ähnliche Tendenz ($p<0,1$) wurde auch für die Fütterung mit GL bestimmt. Insgesamt ist jedoch der Einfluss der Grobfutterkombinationen auf das Fressverhalten und die Kauaktivität gering. Luzerne besitzt das Potential, die Protein- und Faserversorgung ‚vom heimischen Acker‘ für Milchkühe aufzuwerten, wenn sie im optimalen Vegetationsstadium geerntet wird. In Anhängigkeit von den Ernte- und Konservierungsbedingungen kann Luzernesilage als Rationskomponente für Milchkühe empfohlen werden. Maissilage kann den geringeren Energiegehalt der Luzerne ausgleichen und ist damit gut als weiterer Rationsbestandteil geeignet.

TABLE OF CONTENTS

FIGURES

Chapter 3

Chapter 4

TABLES

Chapter 1

Chapter 3

Chapter 4

Chapter 5

ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
ADFom	Acid detergent fibre expressed exclusive of residual ash
ADL	Acid detergent lignin
aNDFom	Neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash
ANOVA	Analysis of variance
BFT	Backfat thickness
BW	Body weight
CF	Crude fibre
CFA	Continuous Flow Analyzer
CL	Crude lipid
CON	Control ration with grass and maize silage (50 : 50) as forages
CON-1	First group (phase 1 to 5) of cows equipped with chewing halters and fed with grass and maize silage during phase five
CON-2	Second group (phase 5 to 7) of cows equipped with chewing halters and fed with grass and maize silage during phase five
CP	Crude protein
d	Day
DCF	Digestible crude fibre
DCL	Digestible crude lipid
DFI	Daily feed intake
DM	Dry matter
DMI	Dry matter intake
DOM	Digestible organic matter
ECM	Energy corrected milk
ESOM	Enzyme soluble organic matter
FI	Feed intake
FTIR	Fourier transform infrared spectroscopy
GE	Gross energy
GL	Ration with grass and lucerne silage (50 : 50) as forages

GML	Ration with grass, maize and lucerne silage (equal proportions) as forages
GP	Gas production
HSD	Honestly significant difference
IPCC	International Panel of Climate Change
ME	Metabolisable energy
ML	Ration with maize and lucerne silage (50 : 50) as forages
N	Nitrogen
N_2	2-atomic nitrogen
NDF	Neutral detergent fibre
NEL	Net energy for lactation
NIRS	Near infrared spectroscopy
OM	Organic matter
peNDF	Physically effective neutral detergent fibre
$peNDF_{>1.18}$	Physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet
$peNDF_{>8}$	Physically effective neutral detergent fibre representing particles > 8 mm multiplied with NDF content of the total diet
SARA	Subacute ruminal acidosis
SAS	Statistical Analysis System
TMR	Total mixed ration
uCP	Utilisable crude protein at the duodenum
UDP	Undegraded dietary protein (bypass protein)

Chapter 1

General introduction

Characteristics of lucerne

Lucerne (*Medicago sativa* L.) is an erect (up to 1.2 meter if not cut), flowering and perennial plant with alternate trifoliate leaves in the pea family Fabaceae. Lucerne is the most widely used and the highest yielding forage legume in temperate zones of the world, e.g. United States of America, southern Canada, Europe, China, southern Latin America and South Africa. Dry matter (DM) yields up to 20 t/ha can be achieved under optimal conditions such as deep, well-drained fertile soils having a high pH between 6.0 and 6.5. Lucerne can be used over 4 - 6 consecutive years and is often preserved as hay but can also be ensiled. Also grazing is possible especially if lucerne cultivars with improved tolerance to grazing and/or mixed lucerne-grass swards are used under controlled grazing intensity to prevent selective overgrazing. During preservation of lucerne as hay or silage particular care must be taken to prevent losses of the nutritious leaf fraction because leaf shatter and loss is major hazard during drying. Rapid and sufficient acidification during ensilage is hampered due to the high crude protein (CP) content, low water-soluble carbohydrate content and high buffering capacity of lucerne. To achieve high quality lucerne wilting, chopping and the application of an effective additive have been recommended (Sheaffer *et al.*, 2003, Frame, 2005).

Lucerne has a deep tap root system (2 - 4 m or more in deep) and can thus reach moist deep soil layers for water uptake. Therefore lucerne is one of the most drought-tolerant crops making this legume particularly attractive in summer-dry regions, which are becoming more widespread caused by global warming (Frame, 2005, IPCC, 2007). Infection of the roots by rhizobia (*Rhizobium meliloti*) that lead to a plant-bacterial symbiosis enables lucerne to fix nitrogen (N) from the air and convert it into a plant nutrient. The annual nitrogen fixations rates of lucerne may range widely from 85 to 360 kg N/ha (Frame, 2005). This ability can reduce mineral fertilizer application without yield reduction, making lucerne particularly valuable for organic farming (Pietsch *et al.*, 2007).

Lucerne is considered to be an high-quality forage because of its high CP concentration and digestibility as well as favourable structural components for ruminants compared to many other forages (Mirzaei-Aghsaghali *et al.*, 2008). This statement is valid for immature plants at vegetative stages of development, whereas nutrient content and digestibility decline during maturation (Engels and Jung, 2005). During maturation the stem fraction increases at expense of the leaf fraction. While the cell contents (CP and other easily digestible nutrients) in leaves changes little with respect to content and in-vitro degradability, stems undergo drastic changes with maturation. The majority of stem dry matter (DM) is cell wall material that becomes more lignified and therefore less digestible with age (Nordkvist and Åman, 1986). Südekum *et al.* (1995) reviewed that lignin is the major factor limiting the digestibility of feeds because core lignin hardly undergoes any digestion under anaerobic conditions as in the digestive tract of ruminants. The changes in CP content and content of fibre fractions with increasing age of lucerne forage is exemplary shown in Table 1. The degradability of DM and CP decreases with increasing maturity from early budding to full blooming lucerne.

Table 1 Chemical analyses and in situ degradability of freshly cut and dried lucerne harvested at different stages of maturity (Balde *et al.* (1993)

	Lucerne			
Maturity	Early bud	Early bloom	Mid bloom	Late bloom
Harvest date (1985)	29 April	10 May	14 May	20 May
Chemical analysis (% of DM)				
CP	25.2	23.2	18.7	18.3
NDF	39.7	42.4	47.7	50.8
ADF	29.9	31.6	35.0	37.7
ADL	4.8	5.2	5.7	7.1
Effective ruminal degradability (%)				
DM	72.9	70.5	65.1	61.9
CP	84.8	83.8	80.5	80.4

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin

Hence it becomes obvious that the maturity stage at harvest is crucial for the composition and the digestibility of DM and its constituents. The stage of development at harvest date is suitable as predictor of CP content in lucerne (Nordkvist and Åman, 1986).

Lucerne as forage source in rations for high yielding dairy cows

Numerous studies conducted in North America (USA, Canada) and in Northern and Eastern Europe have demonstrated that lucerne is as favourable forage for dairy cows. Frequently an increased DM intake (DMI) has been observed when lucerne replaced grass and maize forages but this did not always translate into a better performance, i.e. higher milk yield (Dewhurst, 2013). Dewhurst *et al.* (2003a) reported that forage legume silages (lucerne and white and red clover) lead to greater DMI and higher milk yields than ryegrass silage (mixture of perennial, Italian and hybrid ryegrass), with little effect on milk composition. Similar results were obtained in US studies by Hoffman *et al.* (1998) and Broderick *et al.* (2002). Some data are also available from Germany. Bulang *et al.* (2006) compared three rations for high yielding dairy cows (all numbers DM basis): maize (41.5% maize silage and 12% grass silage), grass (29% grass silage and 18% maize silage) and lucerne (29% lucerne silage and 18% maize silage). The DMI was significant higher for the lucerne diet (23.2 kg/d) than for the grass (21.2 kg/d) and maize (22.6 kg/d) diets. The milk yield was higher for the diet based on maize silage (41.7 kg/d) than for grass (37.3 kg/d) and lucerne diets (39.5 kg/d). Ettle *et al.* (2011) evaluated the effect of a grass- and maize silage-based diet (DM basis: 30.6% grass silage and 39.0% maize silage) compared to a diet based on lucerne and maize silages (30.9% lucerne silage and 39.0% maize silage). The dairy cows consumed significantly more DM of the lucerne-maize diet than of the grass-maize diet (22.1 versus 20.3 kg) but had similar milk yields (28.8 and 28.2 kg) and milk composition. Both studies (Bulang *et al.*, 2006, Ettle *et al.*, 2011) reported a lower digestibility – determined in sheep using the standard protocol as suggested by GfE (1991) – of organic matter (OM) and fibre and thus, a lower net energy of lactation content of lucerne silage compared with grass silage that was just compensated by the greater DMI such that performance of cows was the same.

Legume silages generally lead to higher intakes compared with grass silages of comparable digestibility (Dewhurst, 2013). The higher feed intake of lucerne forage compared to grass was often described as a consequence of more rapid degradation and faster passage from the rumen (Waghorn *et al.*, 1989, Dewhurst *et al.*, 2003b, Krizsan *et al.*, 2010). Lucerne silage particles break down rapidly leading to a high proportion of particles less than 2 mm (particles passes 2 mm sieve apertures by wet-sieving) in the rumen (Dewhurst *et al.*, 2003b). The passage of digesta is influenced by the upper limit to the size of particles able to leave the rumen. Therefore the faster particle breakdown of lucerne compared to grass results into a faster clearance in the rumen that facilitates high feed intake (Waghorn *et al.*, 1989, Dewhurst *et al.*, 2003b).

A challenge in feeding high yielding dairy cows is to provide a high-energy ration without compromising the ruminal ecosystem, animal welfare, production and reproductive performance (Zebeli *et al.*, 2007). Preventing cows from ruminal disorders requires – among other factors – an adequate amount of fibre from forage (NRC, 2001, GfE, 2014). With fibre all cell wall compounds are summarized. The most common method of analysis is the detergent fibre procedure, that allows a separation of the most important cell wall consists of cellulose, hemicelluloses and lignin (Van Soest, 1994). Besides representing chemical entities, physically effective fibre stimulates chewing activity and influences rumen pH by increasing salivary buffer production and release, which stabilizes rumen function (‘rumen health’) and reduces the risk of subacute rumen acidosis (SARA; (Mertens, 1997)). The parameter physically effective neutral detergent fibre (peNDF) was created to amalgamate information on fibre content based on chemical analysis and particle size of feedstuffs (Zebeli *et al.*, 2012). When coupled with starch proportions of the diet and dry matter intake, the peNDF system is a useful and accurate tool to predict ruminal pH, which is an advisable indicator of rumen function (GfE, 2014). Especially in areas where cropland dominates, energy-rich rations based on maize silage need an appropriate peNDF source. Lucerne integrated in a crop rotation can thus be a useful and valuable alternative (Bulang *et al.*, 2006). In addition to its structural value, lucerne as a legume can make an important contribution to CP, and finally, amino acid supply from regional sources given that the maturity stage at harvest is properly controlled.

Chewing and feed intake behaviour as a measure for the evaluation of ruminant rations

Chewing stimulates saliva secretion. Saliva contains bicarbonate and phosphate buffers that neutralize acids produced by fermentation of OM in the rumen. The production of fermentation acid and the buffer secretion influences ruminal pH. Low ruminal pH may decrease DMI, fibre digestibility and microbial yield and thus decreases milk production, increases feed costs and promotes ruminal disorders. Diets should be formulated to maintain adequate and stable mean ruminal pH (GfE, 2014). The fermentation of carbohydrates (e.g. degradable starch) leads to the greatest amount of acid production in the rumen and therefore lowers ruminal pH. The peNDF is the fraction of feed that stimulates chewing activity, increases saliva secretion and act as antagonist (Allen, 1997). A proportion of degradable starch in the diet increases the amount of peNDF (Silveira *et al.*, 2007). Chewing activity does not only stimulate saliva secretion but is also intimately associated with solubilisation of feed DM and physical breakdown of feed particles, which facilitate the rumen fermentation process and passage of digesta from the rumen (Ulyatt *et al.*, 1986). Particle breakdown during rumination causes an enlargement of the surface and provides nutrients for the rumen microbes (Reid *et al.*, 1979). Chewing during eating and ruminating is the most important factor for the reduction of particle size, accounting for more than 80% of total reduction in size. Thus, 17% of the total reduction in particle size was attributed to digestion and detrition (McLeod and Minson, 1988). Differences between forages exist in regard to particle size reduction. Waghorn *et al.* (1989) determined that eating reduces 46% of freshly cut ryegrass and 61% of lucerne forage to a size able to pass a 2-mm sieve aperture and lucerne was more rapidly cleared from the rumen than ryegrass.

Low-fibre diets require less chewing time per day than high-fibre diets. However, this must not negatively affect the ruminal environment as long as a critical duration is not reached. For lucerne silage-based diets, Beauchemin *et al.* (1994) reported that 360 min rumination time and 600 min total chewing time per day were sufficient to maintain healthy ruminal condition without adversely affecting DMI. Mertens (1997) related the time cows should spent chewing to peNDF and suggested that a chewing activity of 150 min/kg peNDF intake is required to preserve an optimal rumen function. A (too) low fibre content in the diet causes a low milk fat content, thus milk fat percentage is an important variable reflecting animal well-being and performance (Mertens, 1997). To maintain a normal milk fat percentage of dairy cows, Sudweeks *et al.* (1979) proposed that 30 min of chewing activity per kg of DM are necessary, whereas Woodford and Murphy (1988) reported moderately lower values, namely 24 min/kg DMI. Based on literature evaluations, Mertens (1997) and, more recently, Zebeli *et al.* (2006) suggested that a mean chewing time of 744 min/day or 36.1 min/kg DMI and 797 min/day or 36.5 min/kg DMI, respectively, are needed to achieve a milk fat content of 3.6%.

Physical and chemical characteristics of dietary ingredients and their interactions, e.g. fibre content, ease of hydrolysis of starch and fibre, particle size, particle fragility, silage fermentation products, concentration and characteristics of fat and the amount and ruminal degradation of CP can have large effects on DMI of lactating cows (Allen, 2000). The feed intake per time unit is a function of the number, the length and the size of a meal or intake rate during meals (Vasilatos and Wangsness, 1980, Dado and Allen, 1994). Vasilatos and Wangsness (1980) determined that characteristics such as frequency of meals and time spent eating are unique for individual cows whereas eating rate and meals size were fairly consistent among cows.

Eating behaviour can also be affected by dietary factors such as silage type. Deswysen *et al.* (1993) described a shorter eating time and fewer meals with a greater intake rate within meals with maize silage compared to grass silage that finally translated into a higher maize silage DMI. A high eating rate because of lower insalivation of feed as well as large meals because of the greater amount of acid production per period of time are associated with low ruminal pH. The proportion of roughage in the diet associated with the particle size and the NDF and starch content, respectively are the main factors affecting feeding behaviour (Dulphy *et al.*, 1980, Gonzalez *et al.*, 2012).

REFERENCES

Allen MS 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. Journal of Dairy Science 80, 1447-1462.

Allen MS 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83, 1598-1624.

Balde AT, Vandersall JH, Erdman RA, Reeves JB and Glenn BP 1993. Effect of stage of maturity of alfalfa and orchardgrass on in-situ dry-matter and crude protein degradability and amino-acid-composition. Animal Feed Science and Technology 44, 29-43.

Beauchemin KA, Farr BI, Rode LM and Schaalje GB 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. Journal of Dairy Science 77, 1326-1339.

Broderick GA, Koegel RG, Walgenbach RP and Kraus TJ 2002. Ryegrass or alfalfa silage as the dietary forage for lactating dairy cows. Journal of Dairy Science 85, 1894-1901.

Bulang M, Kluth H, Engelhard T, Spilke J and Rodehutscord M 2006. Zum Einsatz von Luzernesilage bei Kühen mit hoher Milchleistung. Journal of Animal Physiology and Animal Nutrition 90, 89-102.

Dado RG and Allen MS 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. Journal of Dairy Science 77, 132-144.

Deswysen AG, Dutilleul P, Godfrin JP and Ellis WC 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. Journal of Animal Science 71, 2739-2747.

Dewhurst R 2013. Milk production from silage: comparison of grass, legume and maize silages and their mixtures. Agricultural and Food Science, 57-69.

Dewhurst RJ, Fisher WJ, Tweed JK and Wilkins RJ 2003a. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. Journal of Dairy Science 86, 2598-2611.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ 2003b. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. Journal of Dairy Science 86, 2612-2621.

Dulphy JP, Remond B and Theriez M 1980. Ingestive behaviour and related activities in ruminants. In Digestive physiology and metabolism in ruminants (eds. Y Ruckebush and P Thivend), pp. 103-122, AVI Publishing Co., Westport, CT.

Engels FM and Jung H-JG 2005. Alfalfa stem tissues: Impact of lignification and cell length on ruminal degradation of large particles. Animal Feed Science and Technology 120, 309-321.

Ettle T, Obermaier A, Weinfurtner S and Spiekers H 2011. Luzernsilage im Austausch gegen Grassilage bei der Milchkuh. In VDLUFA-Schriftreihe, Speyer, pp. 588-594.

Frame J 2005. Forage legumes for temperate grasslands. Science Publishers, Inc., Enfield, New Hamshire.

GfE 1991. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. Journal of Animal Physiology and Animal Nutrition 65, 229-234.

GfE 2014. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Communications of the committee for requirement standards of the society of nutrition physiology: Evaluation of structural effectiveness of mixed rations for dairy cows − status and perspectives. Proc. Soc. Nutr. Physiol. 23, 165-179.

Gonzalez LA, Manteca X, Calsamiglia S, Schwartzkopf-Genswein KS and Ferret A 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Animal Feed Science and Technology 172, 66-79.

Hoffman PC, Combs DK and Casler MD 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. Journal of Dairy Science 81, 162-168.

IPCC 2007.(Intergovernmental Panel on ClimateChange) Summary for Policymakers. In Climate Change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)],Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., Krizsan SJ, Ahvenjärvi S and Huhtanen P 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. Journal of Dairy Science 93, 5890-5901.

McLeod MN and Minson DJ 1988. Large particle breakdown by cattle eating ryegrass and alfalfa. Journal of Animal Science 66, 992-999.

Mertens DR 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science 80, 1463-1481.

Mirzaei-Aghsaghali A, Maheri-Sis N, Mirza-Aghazadeh A, Safaei AR and Aghajanzadeh-Golshani A 2008. Nutritional value of alfalfa varieties for ruminants with emphasis on different measuring methods: A review. Research Journal of Biological Sciences 3, 1227-1241.

Nordkvist E and Åman P 1986. Changes during growth in anatomical and chemical composition and in-vitro degradability of lucerne. Journal of the Science of Food and Agriculture 37, 1-7.

NRC 2001. (National Research Council) Nutrient requirements of dairy cattle. National Academy Press, Washington, D.C.

Pietsch G, Friedel JK and Freyer B 2007. Lucerne management in an organic farming system under dry site conditions. Field Crops Research 102, 104-118.

Reid CSW, John A, Ulyatt MJ, Waghorn GC and Milligan LP 1979. Chewing and the physical breakdown of feed in sheep. Annales De Recherches Veterinaires 10, 205-207.

Sheaffer CC, Ehlke NJ, Albrecht KA and Peterson PR 2003. Forage legumes - Clovers, birdsfoot, trefoil, cicer milkvetch, crownvetch and alfalfa. Second edition. Station Bulletin 608-2003, Minesota Agricultural Experiment Station. University of Minnesota.

Silveira C, Oba M, Beauchemin KA and Helm J 2007. Effect of Grains Differing in Expected Ruminal Fermentability on the Productivity of Lactating Dairy Cows. Journal of Dairy Science 90, 2852-2859.

Südekum K-H, Oestmann A and Stangassinger M 1995. Zur Rolle von Lignin und phenolischen Monomeren in Futtermitteln für Wiederkäuer II. Einfluss auf die Verdauung pflanzlicher Gerüstsubstanzen. Übersichten zur Tierernährung 23, 229-260.

Sudweeks EM, Law SE, Ely EO, McCullough ME and Sisk LR 1979. Development and application of a roughage value index for formulating dairy rations. Georgia Agric. Exp. Stn. Res. Bull. 238, Univ. Georgia, Athens.

Ulyatt MJ, Dellow DW, John A, Reid CSW and Waghorn GC 1986. Contribution of chewing during eating and rumination to the clearance of digesta from the reticulorumen. In The control of digestion and metabolism in ruminants (eds LP Milligan, WL Grovum, and A Dobson), pp. 488-517, Prentice-Hall (Englewood Cliffs, N.J.).

Van Soest PJ 1994. Nutritional ecology of the ruminant, 2nd ed.. Cornell University Press, Ithaca, N.Y.; USA.

Vasilatos R and Wangsness PJ 1980. Feeding behavior of lactating dairy cows as measured by time-lapse photography. Journal of Dairy Science 63, 412-416.

Waghorn GC, Shelton ID and Thomas VJ 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. British Journal of Nutrition 61, 409-423.

Woodford ST and Murphy MR 1988. Effect of forage physical form on chewing activity, dry matter intake, and rumen function of dairy cows in early lactation. Journal of Dairy Science 71, 674-686.

Zebeli Q, Tafaj M, Steingass H, Metzler B and Drochner W 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of Dairy Science 89, 651-668.

Zebeli Q, Tafaj M, Weber I, Dijkstra J, Steingass H and Drochner W 2007. Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cow. Journal of Dairy Science 90, 1929-1942.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN and Drochner W 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science 95, 1041-1056.

Chapter 2

Scope of the thesis

Lucerne is increasingly being used in Germany as forage in feed rations for dairy cows. Depending on the local conditions grass and maize silage are the most common forages in diets for lactating cows. A feeding trial with approx. 60 high yielding dairy cows (German Holstein) was conducted to investigate the effect of different forage combinations.

The results are presented in chapters 3 and 4, which comprise manuscripts that will be submitted for publication in scientific journals.

The third chapter focuses on the impact on feed intake, milk yield and milk composition (in particular milk fat, milk protein and milk urea content) depending on different forage combinations in the experimental diets. Seven diets fed as total mixed rations were fed to the whole herd over six weeks. A control diet (50:50 mixture of grass and maize silage) alternated with three different forage combination with lucerne silage (50:50 mixture of grass and lucerne silage, 50:50 mixture of maize and lucerne silage and equal proportion of grass, maize and lucerne silage). Each diet with lucerne silage was only compared with the control diet.

In order to sufficiently characterize the nutritional value of the forages, a digestibility study with sheep for each silage was conducted.

In the fourth chapter the effects of the same feed rations as used in chapter 3 on chewing activity and feeding behaviour were examined. Changes in the forage composition resulted into varying nutrients in particular fibre fractions, which are able to influence feeding and chewing behaviour. From the whole herd six cows were selected and equipped with a chewing halter. Within each trial period the chewing und rumination activity was recorded over 24 hours. In the same period the feed intake data from these cows recorded from the feed bins were used to determine the feed intake behaviour (feed intake per meal, meals per day, length of meals, eating rate).

In chapter 5 the results of chapter 3 and 4 are discussed together and a general conclusion of the impact of diets containing lucerne silage for lactating cows is given in chapter 6.

Chapter 3

Comparative evaluation of lucerne, grass and maize silage-based diets for lactating dairy cows: Feed intake, milk yield and milk composition

J. Trautwein[1,2], K.-H. Südekum[1], G. Dusel [2], H. Steingaß[3], F.-J. Romberg[4], C. Koch[5] and K. Landfried[5]

[1] *University of Bonn, Institute of Animal Science, 53115 Bonn, Germany*

[2] *University of Applied Sciences Bingen, 55411 Bingen/Rhein, Germany*

[3] *University of Hohenheim, Institute of Animal Nutrition, 70599 Stuttgart, Germany*

[4] *Dienstleistungszentrum ländlicher Raum Westpfalz, 67728 Münchweiler/A., Germany*

[5] *Lehr- und Versuchsanstalt für Viehhaltung Hofgut Neumühle, 67728 Münchweiler/A., Germany*

To be submitted

ABSTRACT

Lucerne (*Medicago sativa L.*), grass and whole-crop maize silages were offered to lactating dairy cows in various combinations and feed intake, milk yield and milk composition were determined. Cows were fed total mixed rations (TMR) with an average forage to concentrate ratio of 55:45 (dry matter (DM) basis). The forage component of the control (CON) ration was a 50:50 mixture of grass and maize silages (DM basis). The forage component of the three lucerne-based diets comprised (DM basis) either lucerne and grass silage (50:50), lucerne and maize silage (50:50) or equal proportions of the three silage types. TMR were formulated to have the same protein content, expressed as utilisable crude protein at the duodenum (uCP) but rations differed with regard to energy, fibre and starch concentrations and physically effective NDF. In a digestibility study in sheep, lucerne silage was found to be less digestible in terms of organic matter, crude lipid and gross energy than grass or maize silages; the fibre fractions were also less digestible than those of grass silage. A seven-phase feeding trial was conducted with a herd of 60 lactating cows. The whole herd received each diet *ad libitum* for six weeks; data were collected over the last four weeks and then the diet was changed. The CON diet was fed during phases 1, 3, 5 and 7. Daily DM intake (DMI) ranged from 20.8 kg to 22.3 kg and was greater ($P<0.05$) when cows were fed the lucerne silage diets. Cows were only able to compensate for the lower energy density of the lucerne diets when they were fed the combination of maize and lucerne silages; the greater DMI of the maize and lucerne diets relative to the CON diet allowed them to achieve the same net energy intake per day. The average energy-corrected milk yield ranged from 31.7 and 33.6 kg/day across treatments. Including lucerne silage in the TMR reduced milk yield, milk protein concentration and milk protein yield. Only the combination of lucerne and grass silage increased milk fat content relative to the CON diet (4.31 vs. 3.89%). In this study lucerne silage did not improve dairy cow performance although there was an improvement in DMI; this indicates that the maturity of lucerne at harvest is critical to the nutritional value of lucerne silage and its potential contribution to improving the protein and fibre supplied to dairy cows.

***Keywords*:** lucerne silage, protein, fibre, digestibility, maturity stage

IMPLICATIONS

Lucerne has a high yield potential and can provide ruminants with significant amounts of energy and protein as well as having excellent structural properties. This study shows that feeding a combination of lucerne silage and grass or maize silages increases feed intake, however the lower digestibility of the nutrients in lucerne silage resulted in a decreased milk yield relative to maize and grass silage-based diets. Maize and lucerne silage complement each other and therefore represent a potentially beneficial forage combination in diets for dairy cows.

INTRODUCTION

Lucerne (*Medicago sativa* L.*)* is globally the oldest, most important perennial legume; it is cultivated on arable land and is well adapted to a wide range of environmental conditions (Frame, 2005, Radovic *et al.*, 2009). Its high yield potential up to 20 t dry matter (DM)/ha per annum in Northern Europe; (Halling *et al.*, 2004) is noteworthy. Lucerne makes more efficient at using water than other forage species (Frame, 2005) and is therefore suitable for cultivation in spring- or summer-dry regions; there has been a revival of interest in its use in the light of climate change. Climate change may include extreme weather events, warmer and more frequent hot days and an increase in the area affected by drought, heavy rainfall and strong wind (IPCC, 2007). This makes forage species with demonstrable tolerance for climate change more attractive. As a N_2-fixing plant lucerne can be grown without N-fertilizer and has a high crude protein (CP) content (Frame, 2005). Lucerne has potential as a drought-tolerant, high yielding forage and could contribute to local CP supply in organic as well as conventional farming systems in areas where it has been underused for decades, such as Central and Western Europe. As findings based on genotypes that have been bred in and adapted to particular environmental conditions may not generalise to other environmental conditions, research is needed to evaluate the performance of lucerne forage as a component of ruminant rations.

Several studies demonstrated that lucerne silage-based diets result in greater DM intake (DMI) than diets based on perennial ryegrass (Hoffman *et al.*, 1998), a mixture of ryegrass silages (Dewhurst *et al.*, 2003a) or grass silage (Bulang *et al.*, 2006).

As well as providing energy and protein for high-yielding dairy cows, lucerne silage may also provide worthwhile amounts of physically effective neutral detergent fibre (peNDF). It has a beneficial impact on buffer capacity as a result of the greater chewing activity and may help to prevent acidosis. Over-acidification of the rumen is a common reaction in dairy cows fed diets high in energy and starch (NRC, 2001). Additionally, the cation exchange capacity of lucerne stabilises the ruminal pH and thus ensures optimal microbial activity in the rumen (McBurney *et al.*, 1983).

In comparative studies the effects of lucerne silage on milk yield have been inconsistent. Dewhurst *et al.* (2003a) demonstrated in cows that lucerne resulted in greater DMI and higher fat-corrected milk yields than grass silage. Bulang *et al.* (2006) observed no effect on milk yield although lucerne silage did result in greater DMI than grass silage. In the same study, cows fed lucerne silage had lower milk yields than cows fed a maize silage-based diet, although DMI was similar.

Lucerne silage has demonstrated its potential as a ration ingredient; however in practice the benefits are critically influenced by forage characteristics, ration composition, and other factors. The objectives of this study were to measure digestibility by sheep of grass, maize and lucerne silages and to evaluate DMI, milk production and milk composition for lactating dairy cows fed diets based on the three silage types in different combinations.

MATERIAL AND METHODS

Forages and diets

The only substantial difference between the various rations in this study was the forage mixture. The composition of the concentrate component was adapted slightly, to minimize differences in key diet characteristics, e.g. amount of utilisable CP at the duodenum (uCP; Lebzien and Voigt, 1999; GfE, 2001), a precursor to metabolisable protein. The composition of the diets is shown in Table 1.

Lucerne (*Medicago sativa* L. *cv.* Franken Neu) silage was prepared from a first- and a second cut. The first cut was harvested on June 4, 2009 at the late bud stage (estimated according to Kalu and Fick (1983), the second cut was harvested between early and late flowering (July 15, 2009). The lucerne was field-wilted for one day and then chopped. The first cut was ensiled in a clamp silo and the second cut in round bales, silage additive was not used in either case.

Lucerne silage (50:50 first- and second-cut mixture) was fed in combination with grass and/or maize silage. Maize silage from the 2009 harvest was fed during phases 1 to 3 (explanation below), whereas a silage from the 2010 harvest was fed in the other phases. Grass silage was from the first and second cuts in 2010; the first-cut silage was fed during phases 1 to 5 and the second-cut silage during the other two phases.

Table 1 Ingredient composition of the experimental diets

	Total mixed ration						
	CON	GL	CON	ML	CON	GML	CON
Composition (g/kg of DM)							
Grass silage	286	297	286		283	222	316
Maize silage	286		286	295	293	191	279
Lucerne silage		251		250		162	
Grass hay	41	43	41	42	41	41	39
Soybean meal	47	26	47	26	46	34	44
Rapeseed meal	47	26	47	26	46	34	44
Barley/wheat grain (1:1)	138	174	138	173	137	151	131
Maize grain	138	174	138	173	137	151	131
Urea	5	-	5	5	5	3	5
Minerals and vitamins[a]	12	9	12	10	12	11	11

[a] Mineral and vitamin premix, sodium chloride (NaCl), calcium carbonate ($CaCO_3$)
CON = control diet (50/50 forage mixture of grass and maize silage)
GL = 50/50 forage mixture of grass and lucerne silage
ML = 50/50 forage mixture of maize and lucerne silage
GML = forage mixture (equal proportion) of grass, maize and lucerne silage
DM = dry matter

Animals and trial design – sheep

This trial was conducted at the University of Applied Sciences, Bingen. Forage digestibility was determined with eight Merino wether sheep per forage following the standardized procedure described by GfE (1991). All silages fed to the dairy cows were assessed in the digestibility study: grass silage, first- and second-cut 2010; lucerne silage, first- and second-cut 2010; maize silage harvest 2009 and 2010. Each forage was offered to eight wethers as the sole feed in two meals per day. A 14-day adaptation period was followed by a 7-day collection period in which all faeces were collected on a daily basis and aliquots were stored at -18°C. At the end of each collection period a composite sample spanning the entire 7-day period was prepared and freeze-dried for later analysis. The gross energy concentration and chemical composition of feeds and faeces was analysed as described below for the dairy cow rations.

Animals and trial design – dairy cows

The trial was carried out at the Educational and Research Centre for Animal Husbandry Hofgut Neumühle (Münchweiler an der Alsenz, Germany). The herd comprised approximately 60 primiparous and multiparous dairy cows (German Holstein) in early to mid lactation. Average days in milk in the various phases ranged from 94 days (phase 3) to 131 days (phase 7). The cows calved continuously throughout the year and healthy cows were integrated into the herd 5 days after calving. The experimental design involved continual inflow and outflow of cows. All cows were housed in a loose-housing system and milked in a side-by-side or herring-bone milking parlour twice a day at 0500 h and 1530 h. The body weight of all cows was measured at the beginning and at the end of each phase. Every second week the backfat thickness (BFT) of the cows was measured by ultrasonography (Aloka SSD 500, 48 mm linear transducer, frequency 5 MHz; Aloka, Meerbusch, Germany) according to the method described by Schröder and Staufenbiel (2006).

Cows were offered their feeds as total mixed ration (TMR) using an automated system with troughs (Typ RIC, Insentec B.V. Marknesse, The Netherlands) equipped with scales. The cows were equipped with transponders at the collar that allowed cows to be identified at each visit and thus enabled recording of individual daily feed intakes. The TMR was prepared in the morning and supplied in the morning and evening in amounts which allowed *ad libitum* intake. Fresh water was freely available. Each diet was given to the whole herd for a period of six weeks. The first two weeks of the period were an adaptation period and data were collected only during the last four weeks. After six weeks the diet was changed to the next treatment over 3 to 4 days. During phases 1, 3, 5 and 7 the cows were fed with the control (CON) diet, with a forage component consisting of a 50:50 (DM basis) grass silage to maize silage mix. In phase 2 the maize silage was replaced by lucerne silage (GL diet). In phase 4 the grass silage was replaced by lucerne silage in the same way (ML diet). In phase 6 the forage component consisted of equal proportions (by DM) of lucerne, grass and maize silage (GML diet). All lactating cows received the diets in the same order over the same period. The trial design is shown in Figure 1 which also summarises the composition of the various diets fed to the dairy cows.

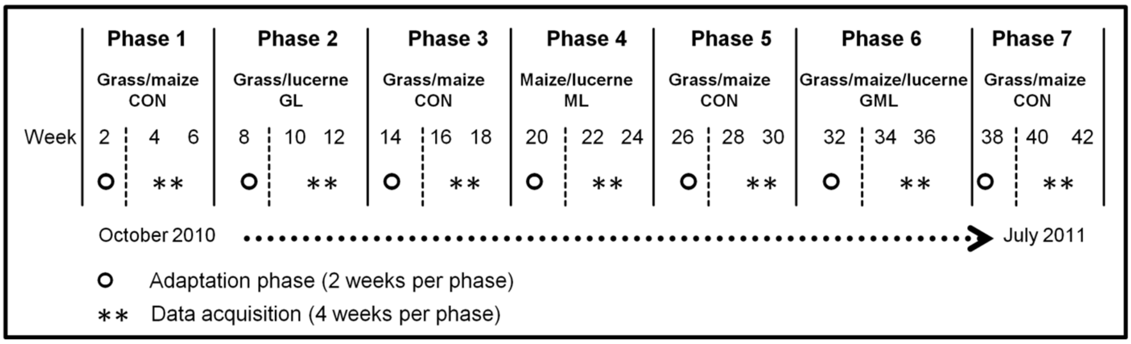

Figure 1 Trial design: schematic showing the ordering and organisation of the experimental diets during the feeding trial

Data collection and chemical analysis

Daily samples of the TMR were taken during morning feeding and were used to confect one composite sample per week for DM determination. Daily DMI was calculated for each cow on the basis of the automatically determined TMR intake and the DM content of the diet. Forages and concentrates were sampled weekly during the final four weeks of each phase, stored frozen and blended to give one composite sample per phase. Chemical analyses of these samples and silages and faeces from the digestibility trial on sheep were carried out using the protocols of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2007) and method numbers are given below. The DM content of silages was determined using a two-step procedure involving pre-drying samples at 60°C, followed by oven-drying at 105°C, the one-step procedure used for all air-dry feedstuffs (3.1). Analyses of ash and crude lipid (CL) were carried out using methods 8.1 and 5.1.1 respectively; CP was determined by Dumas combustion (4.1.2). *In vitro* gas production (GP, 25.1); enzyme-soluble organic matter (ESOM; maize silage only, 6.6.1) and starch (7.2.1) were analysed using established procedures. Analysis of the hay, grass and lucerne silages also included determination of their water-soluble carbohydrate (‘sugar’) content (7.1.1). Neutral detergent fibre content, which was assayed with a heat stable amylase (6.5.1) and acid detergent fibre content (6.5.2) were expressed exclusive of residual ash and therefore denoted aNDFom and ADFom respectively. Acid detergent lignin (ADL, 6.5.3) was only analysed in forages. Gross energy concentration of silages and faeces from the digestibility trial on sheep was determined using conventional bomb calorimetry.

Once a week a representative sample of TMR was collected from several troughs after morning feeding and used to estimate the $peNDF_{>1.18}$ (physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet) content of the TMR. A modified Penn State Particle Separator was used to determine the proportion of the TMR retained on a 1.18-mm sieve, as described by Kononoff *et al.* (2003), multiplied by the NDF proportion (DM basis) of the TMR.

Daily milk yield was automatically recorded at each milking (twice a day). Aliquot milk samples were collected once a week from two consecutive milkings in accordance with German guidelines (method AS42 (ADR, 2001)) and analysed at an official milk control laboratory (Landeskontrollverband Rheinland-Pfalz e.V., Bad Kreuznach, Germany). Milk fat and protein were analysed using Fourier transform infrared spectroscopy and somatic cells were counted with flow cytometry (CombiFoss^{TM}, Foss, Hillerød, Denmark). The urea content of milk was analysed using a continuous flow analyzer (San, Skalar Analytical B.V., AA Breda, The Netherlands).

Calculations

The uCP concentrations of diet ingredients were estimated by two different approaches. The uCP of the silages was determined from the equation (GfE, 2001):

uCP (g/kg DM) = [187.7 - (115.4 · (UDP (g/kg DM)/CP (g/kg DM)))] · DOM (kg/kg DM) + 1.03 · UDP (g/kg DM),

where UDP is ruminally undegraded feed CP and DOM is digestible organic matter as determined in sheep in the digestibility trial. For concentrates and hay the following equation was used:

uCP (g/kg DM) = [11.93 - (6.82 · (UDP (g/kg DM)/CP (g/kg DM)))] · ME (MJ/kg DM) + 1.03 · UDP (g/kg DM),

where ME is metabolisable energy which was estimated as outlined below and CP values are based on from chemical analyses of diet ingredients in each phase. The UDP proportions of CP were assumed to be 15%, 20%, 22.5%, 25%, 30% and 50% for grass silage, lucerne silage and hay, barley and wheat grain, maize silage, soybean and rapeseed meals, and maize grain respectively. The uCP concentrations of the feedstuffs were then used to calculate the uCP content of the TMR (Table 3).

The ME content of the diets was calculated in the same way. The ME content of the silages was determined using the following equation (GfE, 2001):

$\text{ME (MJ)} = 0.0312 \cdot \text{DCL} + 0.0136 \cdot \text{DCF} + 0.0147 \cdot (\text{DOM} - \text{DCL} - \text{DCF}) + 0.00234 \cdot \text{CP}$,

where DCL is digestible crude lipid, DCF is digestible crude fibre and DOM is digestible organic matter (all expressed as g/kg DM). The concentrates were proportionally assembled to a pooled sample and the energy content was estimated according to the following equation (GfE, 2009):

$\text{ME (MJ/kg DM)} = 7.17 - 0.01171 \cdot \text{ash} + 0.00712 \cdot \text{CP} + 0.01657 \cdot \text{CL} + 0.002 \cdot \text{starch} - 0.00202 \cdot \text{ADFom} + 0.06463 \cdot \text{GP}$ (ash, CP, CL, starch and ADFom, g/kg DM; GP, ml/200mg DM).

Net energy for lactation (NEL) values were estimated from ME according to GfE (2001):

$\text{NEL (MJ/kg DM)} = 0.6 \cdot (1 + 0.004 \cdot (q - 57)) \cdot \text{ME (MJ)}$,

where $q = \text{ME/GE} \cdot 100$.

The energy corrected milk yield (ECM) was calculated as follows (Spiekers and Potthast, 2004):

$\text{ECM (kg/day)} = \text{milk yield (kg/day)} \cdot (([0.38 \cdot \text{fat (\%)} + 0.21 \cdot \text{protein (\%)}] + 1.05)/3.28)$.

Feed efficiency is defined as ECM (kg/d) per DMI (kg/d) and energy efficiency as ECM (kg/d) per 10 MJ NEL/d intake.

The energy balance was calculated as the energy intake from TMR (MJ NEL/d) minus the maintenance requirement and the energy output in milk, all using the individual cow as the experimental unit.

Statistical analysis

Performance data from the feeding trial with dairy cows were analysed using the mixed model procedure in SAS with the maximum likelihood method (Statistical Analysis System Institute, 2009)

The following model was used:

$Y_{ijkl} = \mu + P_i + A_j + N_k + M_l(N)_k + \varepsilon_{ijkl}$

where Y_{ijkl} is the observed response; μ is the overall mean; P_i is the fixed effect of trial phase i (1 to 7); A_j is the random effect of animal j; N_k is the fixed effect of lactation number k (1 to 6); $M_l(N)_k$ is the fixed effect of month of lactation l (1 to 11) nested within lactation number k and

ε_{ijkl} is the residual error. All cows for whom at least two complete consecutive weeks of data were available were included in the statistical analysis. Treatment effects were evaluated by comparing phases with lucerne TMR with the average of the preceding and subsequent phases in which no lucerne was fed. The results of the digestibility study were analysed using one-way ANOVA and Tukey's Honestly Significant Difference test (Tukey-HSD) was used for *post hoc* comparisons.

RESULTS

Forages and diets

The chemical composition of the forages used in the feeding trial is reported in Table 2. The maize silages from the 2009 and 2010 harvests had a similar chemical composition. NEL (MJ/kg DM) was slightly below 7 in 2009 (6.9) and slightly above in 2010 (7.2). Grass silage from the second harvest, which was used in phases 6 and 7, had lower CP (155 versus 174 g/kg DM) and lower NEL values (6.0 versus 6.6 MJ NEL/kg DM) than the grass silage used in the other phases, but higher fibre content. Lucerne silages from the first and second harvest were always used as a mixture; CP and NEL values were lower for the second-cut silage.

Table 3 summarizes the chemical composition and feeding value variables for the TMR fed to dairy cows. The lucerne TMR had a greater DM and ADFom content than the other diets, whilst diets without maize silage had a lower starch content. As intended the uCP values (range 141 (GML) to 149 (CON) g/kg DM) and the ruminal N balance (RNB**,** range -0.2 (ML) to 1.4 (GL) g/kg DM) of the various TMR showed little variation. Diets containing lucerne silage had a lower energy content. The range of observed $\text{peNDF}_{>1.18}$ values was probably due to variation in the forage portion of the TMR.

Table 2 Chemical composition of the forages used in the dairy cow trial (means over the diets and s.d.)

	Maize silage				Grass silage				Lucerne silage			
	Harvest 2009		Harvest 2010		First cut		Second cut		First cut		Second cut	
Phase	1, 3		4, 5, 6, 7		1, 2, 3, 5		6, 7		2, 4, 6		2, 4, 6	
Harvest dates												
	09/09/2009		20/09/2010		22/05/2010		01/07/2010		04/06/2010		15/07/2010	
Chemical composition (g/kg DM unless stated)												
DM (g/kg)	337	7.8	330	7.7	329	4.4	407	8.5	407	6.5	404	38.3
CP	76	3.0	81	2.7	174	5.7	155	3.7	175	5.1	160	5.3
ADFom	190	8.6	177	6.5	280	11.0	317	24.7	365	12.1	399	19.7
aNDFom	339	16.2	317	12.5	454	19.5	496	31.4	467	7.5	495	7.8
Energy value (MJ/kg DM)												
NEL	6.9	0.2	7.2	0.8	6.6	1.9	6.0	4.0	5.3	1.1	4.8	1.7

DM = dry matter, CP = crude protein, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, NEL = net energy for lactation

Table 3 Nutrient composition of the experimental diets

	Total mixed ration						
	CON	GL	CON	ML	CON	GML	CON
Analysed (g/kg DM)							
Dry matter (g/kg)	459	492	455	481	460	502	491
CP	150	156	151	141	153	145	150
Starch	294	204	287	315	283	259	280
ADFom	167	221	171	182	172	207	191
aNDFom	298	339	302	297	306	329	329
Calculated (g/kg DM unless stated)[a]							
uCP[b]	148	147	149	142	148	141	147
RNB[c]	+0.3	+1.4	+0.3	-0.2	+0.8	+0.6	+0.5
NEL (MJ/kg DM)[d]	7.18	6.76	7.10	6.73	7.02	6.71	6.96
peNDF$_{>1.18}$ (%)[e]	26.3	31.1	26.6	26.3	27.3	29.0	28.5

[a] Calculated values for the TMR based on the analysed components

[b] Utilisable crude protein at the duodenum according to GfE (2001) (see material and methods)

[c] Ruminal Nitrogen Balance = (CP – uCP) / 6.25

[d] Energy (MJ) for lactation calculated according to GfE (2001) and GfE (2009) (see material and methods)

[e] Physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet (Kononoff *et al.*, 2003)

CON = control diet (50/50 forage mixture of grass and maize silage)

GL = 50/50 forage mixture of grass and lucerne silage

ML = 50/50 forage mixture of maize and lucerne silage

GML = forage mixture (equal proportion) of grass, maize and lucerne silage

DM = dry matter, CP = crude protein, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash

Forage digestibility – sheep

Although maize silages from the 2009 and 2010 harvest had similar digestibility coefficients (Table 4) the calculated energy value of the 2010 maize silage (based on the determined digestibility coefficients) (6.6 MJ NEL/kg DM) was lower than that of the 2009 silage (6.9 MJ NEL/kg DM). There were no differences between the first- and second-cut grass silages of 2010 in terms of either nutrient digestibility or calculated energy value (6.6 and 6.5 MJ NEL/kg DM respectively).

Table 4 Chemical composition, digestibility coefficients and energy value of forages

	Maize silage				Grass silage				Lucerne silage			
	Harvest 2009		Harvest 2010		First harvest		Second harvest		First harvest		Second harvest	
Chemical composition (g/kg DM unless stated)												
DM (g/kg)	342		309		351		448		358		352	
Ash	35		42		94		88		87		97	
CP	76		84		168		154		170		176	
CL	38		36		37		33		22		26	
ADFom	184		181		285		300		366		392	
aNDFom	328		330		385		490		475		474	
ADL	18		16		25		33		56		68	
Digestibility coefficients (%; means (s.d.), n = 8)												
OM	76^{AB}	(1.9)	74^{B}	(1.7)	77^{A}	(1.5)	77^{A}	(0.9)	66^{C}	(1.1)	58^{C}	(1.6)
CP	53^{A}	(3.0)	52^{A}	(2.4)	72^{B}	(1.5)	71^{BC}	(2.1)	70^{BC}	(0.8)	68^{C}	(1.0)
CL	82^{A}	(3.6)	75^{A}	(4.4)	68^{B}	(3.3)	63^{B}	(4.0)	50^{C}	(4.3)	39^{D}	(4.1)
ADFom	46^{A}	(3.5)	46^{A}	(5.3)	75^{B}	(1.5)	74^{B}	(1.6)	55^{C}	(1.7)	47^{A}	(2.5)
aNDFom	50^{A}	(4.8)	49^{AD}	(4.5)	78^{B}	(1.9)	77^{B}	(1.4)	55^{C}	(1.5)	45^{D}	(2.5)
Gross energy	73^{AB}	(1.9)	72^{A}	(1.6)	73^{AB}	(1.7)	75^{B}	(1.2)	62^{C}	(1.3)	56^{D}	(1.5)
Energy value (MJ/kg DM)[a]												
ME	11.3^{A}	(0.3)	10.9^{B}	(0.2)	10.8^{B}	(0.2)	10.8^{B}	(0.1)	9.2^{C}	(0.2)	8.2^{D}	(0.2)
NEL	6.9^{A}	(0.2)	6.6^{B}	(0.2)	6.6^{B}	(0.2)	6.5^{B}	(0.1)	5.4^{C}	(0.1)	4.6^{D}	(0.1)

[a]ME (MJ) = 0.0312 + DCL + 0.0136 * DCF + 0.0147 * (DOM – DCL – DCF) + 0.00234 * CP (where DCL is digestible (dig.) crude lipid, DF is dig. crude fibre and DOM is dig. organic matter, all in g/kg DM), conversion into NEL (MJ) according to GfE (2001)

DM = dry matter, CP = crude protein, CL = crude lipid, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, ADL = acid detergent lipid, OM = organic matter, ME = metabolisable energy, NEL = net energy for lactation

[A, B, C, D] Means within a row with different superscripts are different (P<0.01)

There were differences between the first- and second-cut lucerne silages. The first-cut silage had higher digestibility coefficients (P<0.001) for CL (50% vs. 39%), all fibre fractions and gross energy. In consequence there was also a difference between calculated NEL values for the first and second cut ($P<0.001$).

The differences in nutrient digestibility between the silage types were as expected. Lucerne had lower digestibility values for OM and gross energy than grass or maize silages. Grass silage had the highest digestibility of fibre fractions (aNDFom, ADFom); values for maize and lucerne silage were much lower. The resulting NEL values were lower ($P<0.001$) for lucerne silage than for grass or maize silage.

Dairy cows

The results of the feeding trial in lactating dairy cows are shown in Table 5. Inclusion of lucerne silage in the mixed diets (GL, ML and GML), increased daily DMI compared to the respective controls. Intakes of aNDFom and ADFom were greater with lucerne diets due to the higher fibre content in lucerne silage. Less uCP in the GL diet resulted in lower uCP intake per day compared to control diet (CON). The lower energy (NEL) content of the diets containing lucerne could be fully compensated by higher feed intake when cows consumed the ML diet. In contrast, the GL as well as the GML diets resulted in lower energy intakes per day in relation to the control.

Lucerne silage as a part of TMR reduced milk ECM yields ($P<0.05$ to $P<0.001$). The milk fat content for dairy cows fed the GL diet (4.3%) was greater than for those fed the CON diet (3.9%), but daily milk fat yield was not affected as the milk yield was lower. Milk from cows fed lucerne silage had a lower protein content; protein production per day and the urea content were also affected by treatments. In line with the calculated RNB values milk from cows fed the GL diet had a higher urea content than that of cows fed the CON diet; however when cows consumed the ML or GML diets their milk had a lower urea content than when they were fed the CON diet. Lucerne silage diets resulted in a lower daily ECM yield despite the higher DMI, which reflects the lower feed and, to a lesser extent, lower energy efficiency. The GL and GML diets were similar in energy efficiency to the CON diet, but the ML diet was less energy efficient. The cows had a positive energy balance across all diets and there were only slight differences between diets. The ML diet was the only diet with a significantly greater energy density than the CON diet. Body weight and BFT remained approximately constant throughout the trial, dropping only when cows were fed GL. Backfat thickness was higher on the GML diet than the CON diet.

Table 5 Comparison of feed intake, milk production and composition, efficiency and body characteristics of dairy cows fed diets containing lucerne and grass-maize based diets (least squares means and s.e.)

	Experimental diets							s.e.		
Item	CON	GL	CON	ML	CON	GML	CON	GL v. CON	ML v. CON	GML v. CON
Days of lactation	123	105	94	117	126	122	131			
Intake (kg/day)										
Dry matter	21.6	21.6*	20.8	22.3***	21.3	22.3*	22.4	0.195	0.195	0.191
aNDFom	6.44	7.33***	6.29	6.62***	6.51	7.34***	7.37	0.062	0.062	0.061
ADFom	3.62	4.76***	3.57	4.07***	3.66	4.62***	4.29	0.038	0.038	0.037
uCP	3.33	3.17***	3.21	3.22	3.24	3.26	3.37	0.029	0.029	0.029
Energy (MJ NEL/day)	155	146***	148	150	150	149**	156	1.354	1.352	1.321
Production (kg/day)										
Milk	34.0	32.0***	34.4	33.9*	35.2	33.0***	34.4	0.364	0.362	0.354
Energy corrected milk	33.1	32.1**	33.6	32.7*	33.5	31.7***	33.1	0.386	0.380	0.372
Milk fat	1.31	1.35	1.32	1.30	1.32	1.25**	1.30	0.003	0.020	0.019
Milk protein	1.13	1.00***	1.14	1.07***	1.12	1.04***	1.09	0.012	0.012	0.012
Fat/protein ratio	1.14	1.34***	1.16	1.21**	1.18	1.20	1.18	0.014	0.014	0.014
Milk composition (%)										
Milk fat	3.89	4.31***	3.94	3.95	3.91	3.83	3.83	0.042	0.042	0.041
Milk protein	3.42	3.26***	3.39	3.26***	3.31	3.20***	3.24	0.019	0.019	0.018
Milk urea (mg/l)	182	219***	206	193***	205	185***	190	2.431	2.400	2.342
Feed efficiency (kg ECM/DM)[a]	1.53	1.50***	1.64	1.48***	1.58	1.43***	1.48	0.021	0.021	0.020
Energy efficiency (kg ECM/10 MJ NEL)[b]	2.14	2.22	2.30	2.20*	2.25	2.14	2.12	0.030	0.030	0.029
Energy balance (MJ NEL/d)[c]	11.9	7.8	3.0	7.6*	4.1	9.2	11.8	1.607	1.595	1.558
Body weight (kg)	678	633***	660	669	679	686	686	3.281	3.251	3.175
Back fat thickness (mm)	11.4	10.2***	13.2	11.7	10.7	11.4***	10.1	0.030	0.030	0.029

[a]Energy corrected milk yield (kg) per kg DM intake, [b]Energy corrected milk yield (kg) per 10 MJ NEL intake, [c]Energy intake minus energy required for maintenance and milk production, CON = control diet (50/50 forage mixture of grass and maize silage), GL = 50/50 forage mixture of grass and lucerne silage, ML = 50/50 forage mixture of maize and lucerne silage, GML = forage mixture (equal proportion) of grass, maize and lucerne silage, * $P<0.05$; ** $P<0.01$; *** $P<0.001$: significant differences between diets containing lucerne (GL, ML, GML) compared with the surrounding control diets (CON)

DISCUSSION

The NEL values of the TMR fed to the dairy cows reflected the differences between forages as measured in the digestibility trial in sheep (Table 4). The results of the digestibility trial were in line with tabulated digestibility values in Germany (Universität Hohenheim - Dokumentationsstelle (Ed.) (1997) and a report by Bulang *et al.* (2006), so the forages used in this study can be considered typical grass, maize and lucerne silages.

Other studies which have compared grass (species not specified) and lucerne silages directly have also reported that lucerne silage has lower digestibility with respect to OM, CL and fibre fractions (NDF and ADF) (Bulang *et al.,* 2006, Ettle *et al.,* 2011). Similar results were reported from a comparison of lucerne silage with ryegrass silage (Hoffman *et al.*, 1998). The lower digestibility is probably due to the lower potential ruminal degradability, shorter retention time and hence greater passage rate of lucerne silage (Waghorn *et al.*, 1989, Flachowsky *et al.*, 1994, Hoffman *et al.*, 1998). It remains possible that a negative relationship between passage rate and nutrient digestibility could enhance feed intake. The most pronounced negative effect of increased intake on digestibility occurs with feeds with a high fibre (NDF) content (El Khidir and Thomsen, 1983, Südekum *et al.*, 1995b). Our analysis indicated that the fibre components of lucerne silage had considerably lower digestibility than the fibre components of grass silage. Lignin is the major limiting factor on the digestibility of feeds. Inhibition of cell wall digestion is influenced by lignin content, but also by the composition of monomeric lignin and the presence of strong intramolecular bonds and linkages to other cell wall components (Südekum *et al.*, 1995a). In our study lignin (ADL) content was highest for the lucerne silages (first-cut: 56 g/kg DM, second-cut: 68 g/kg DM; Table 4). Lignification is closely correlated with plant maturity (Südekum *et al.*, 1995a). The composition of lucerne changes as the plant ages, protein concentration decreases and the stem-to-leaf ratio increases; this is reflected in an increase in cell wall mass and lignification (Nordkvist and Åman, 1986). As ruminants are incapable of digesting core lignin (Südekum *et al.*, 1995a) the digestibility of ADFom is negatively correlated with the ADL content of the diet. This is reflected in the energy values of the various types of silage, lucerne silage (4.6 to 5.4 MJ NEL/kg DM) had lower values than maize silages (6.6 to 6.9 MJ NEL/kg DM) and grass silages (6.5 to 6.6 MJ NEL/kg DM; Table 4). Differences in the energy content of the silages were reflected in the energy densities of the TMR (Table 3); lucerne silage had a diluting effect on the energy content of TMR.

Our results are consistent with reports that use of lucerne silage in dairy cow diets results in increased DMI (Hoffman *et al.*, 1998, Dewhurst *et al.*, 2003a, Bulang *et al.*, 2006, Ettle *et al.*, 2012). When fed in a mixture with grass or maize silages lucerne silage resulted in higher DMI than grass or maize silage-based diets (Table 5). The largest increase relative to the CON diet (+1.5 kg DM/day) was observed for the lucerne-maize silage combination. Bulang *et al.* (2006) and Ettle *et al.* (2011) fed two diets containing lucerne and maize silages at ratios (DM basis) of 40:60 and 45:55 respectively; both diets resulted in greater DMI than a grass-maize silage-based diet. It has been reported that feed intake is higher (8.2%) for maize silage than grass silage due to the shorter eating time (Deswysen *et al.* (1993). The lower fibre (aNDFom) content of the ML diet (297 g/kg DM) compared to the GL diet (339 g/kg DM) probably meant that the eating time per kg DM was lower for the ML diet and this has been associated with a higher feed intake (Beauchemin, 1991).

Lucerne silage improved feed intake regardless of the other forage in the diet. Voluntary DMI of forages is limited by ruminal distension and therefore highly dependent on factors which affect the rate of ruminal digestion and flow from the reticulo-rumen (Allen, 2000). In a study in sheep Flachowsky *et al.* (1994) found that the degradation rate of lucerne hay was much higher (11.2%/h) than that of artificially dried Italian ryegrass (4.7%/h). Dewhurst *et al.* (2003b) argued that the high intake of lucerne silage was due to rapid ruminal particle breakdown and high passage rate. Hoffman *et al.* (1998) also reported that the passage rate was higher for cows consuming lucerne silage rather than perennial ryegrass, and that this resulted in a higher feed intake. This corroborated previous research by Waghorn *et al.* (1989) who reported faster breakdown, clearance and fermentation in the rumen of cows fed fresh lucerne compared with those fed perennial ryegrass. In their study 61% of lucerne DM, but only 46% of ryegrass DM, was reduced by eating to a size capable of passing through a 2 mm sieve; they concluded from this that ryegrass was cleared more slowly from the rumen than lucerne. The rate of particle breakdown was similar, however, for lucerne stem (higher proportion of lignin and lower proportion of hemicelluloses) and whole-crop ryegrass. Rapid physical breakdown of lucerne leaves to a size which can pass out of the rumen might enable high feed intake. Bulang *et al.* (2004) demonstrated *in vitro* that lucerne leaves fermented faster than grass silage leaves. These observations demonstrate the importance of avoiding leaf losses during harvest and subsequent processing.

Milk and ECM yields were lower when cows consumed the lucerne diets. Milk composition was also influenced by diet. As expected, the milk fat content was higher with the GL diet, probably because of the positive association between the peNDF content of the diet and the milk fat content (Zebeli *et al.*, 2008). The peNDF content of the GL diet (31.1%) was higher than that of the other diets, for which it ranged between 26.3% (CON) and 29.0% (GML). Milk fat content can be predicted more accurately if the starch or rumen-fermentable OM concentration of the diet are included in the model (Zebeli *et al.*, 2006). Sutton and Morant (1989) have already demonstrated that ADF concentration and starch intake account for more than half the variation in milk fat concentration; they also demonstrated that milk fat content increased with dietary ADF content. These findings suggest that the lower starch content of the GL diet, which was associated with higher fibre concentration, both ADF and peNDF, was responsible for the greater milk fat content for cows fed this diet. The lower milk protein content for the lucerne diets was obviously a consequence of the lower energy density of these diets which, in our study, was not fully compensated for by increased DMI. These data corroborate previous findings (Sutton and Morant, 1989, Yang and Beauchemin, 2007) and confirm the importance of energy supply for protein utilisation (Huhtanen and Hristov, 2010).

Milk urea content is an established indicator of relative N supply to ruminal microbes and is, therefore, a potential marker of feeding imbalances (Steinwidder and Gruber, 2000, Geerts *et al.*, 2004). In a meta-analysis Steinwidder and Gruber (2000) described several variables which influence milk urea content. They reported a correlation of r=0.43 between RNB and milk urea content. The correlation was highest between digestible CP intake and energy intake (r=0.5). Cows fed the GL diet had the highest milk urea concentration. This diet had greater CP (156 g) and RNB (+0.6 g/kg DM) concentrations and contained less energy than the other diets. Although there were significant differences between diets they were of little biological significance because the milk urea values ranged only between 182 and 219 mg/l. In a review of the literature Lebzien *et al.* (2008) concluded that estimates of energy and protein supply to the microbes in the rumen on the basis of milk urea content may be inaccurate owing to the large and variable influence of a number of known factors (e.g., genetics, feed intake, body mass, fat corrected milk yield, stage of lactation) and unknown factors. The values for milk urea content found in this study are around the lower boundary of the range given by Lebzien *et al.* (2008) for a well-balanced diet, 200 to 260 mg/l milk urea. This indicates that all the diets fed in this study, provided rumen microbes with sufficient CP and energy.

A comprehensive evaluation of the experimental diets would include the physical appearance of the cows throughout the study, as well as their milk output. The experimental design involved continual inflow and outflow of cows, so absolute body weights and BFT have to be interpreted with caution. Body weight and BFT were lower when cows were fed GL than CON; there was no obvious reason for this. The increase in BFT when cows were fed the GML diet was not reflected in the calculated energy balance, which was similar for cows on all diets. The highest positive energy balance was associated with the ML diet. The lower energy density common to all the lucerne diets was only fully compensated for by higher DMI in the case of the ML diet. Energy efficiency was, however, lower for the ML diet (kg ECM/10 MJ NEL intake). It is possible that the lower CP and uCP content of the ML diet induced a slight protein (amino acid) deficiency relative to milk production. Although the energy efficiency of the GL and GML diets was similar, both resulted in lower daily energy intake than the CON diet which was reflected in lower milk production.

CONCLUSIONS

Lucerne silage in mixed diets for lactating dairy cows increased DMI but decreased milk yield compared with diets whose forage portion consisted of grass or maize silages. The aim should be to harvest lucerne at an earlier stage of development in order to achieve a higher CP content, less lignification and hence higher nutrient digestibility. Improving the CP and digestibility of lucerne forage would further reduce the need to include soybean meal and rapeseed meal in diets including lucerne, relative to a control diet based on maize and grass silages. In combination with maize silage, lucerne silage improves the structural value of mixed diets for lactating dairy cows and the optimal proportion in a mixed diet for lactating dairy cows depends on its nutrient composition and digestibility which are influenced mainly by harvest date, harvest conditions and post-harvest treatment.

ACKNOWLEDGEMENTS

This study was supported by funds allotted to the Dienstleistungszentrum ländlicher Raum (DLR) Westpfalz, Educational and Research Centre for Animal Husbandry Hofgut Neumühle (Münchweiler an der Alsenz, Germany) and the University of Applied Sciences, Bingen, and was partly conducted in the Centre for Integrated Dairy Research (CIDRe), University of Bonn (Bonn, Germany).

REFERENCES

ADR 2001. (Arbeitsgemeinschaft Deutscher Rinderzüchter): Empfehlungen und Richtlinien, 1. Milchleistungsprüfungen und Milchqualitätsprüfungen. ADR, Bonn.

Allen MS 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83, 1598-1624.

Beauchemin KA 1991. Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. Journal of Dairy Science 74, 3140-3151.

Bulang M, Boghun J, Engelhard T, Kluth H and Rodehutscord M 2004. Studies with alfalfa silage as a forage source for high yielding dairy cows. Proceedings of the Society of Nutrition Physiology 13, 242.

Bulang M, Kluth H, Engelhard T, Spilke J and Rodehutscord M 2006. Zum Einsatz von Luzernesilage bei Kühen mit hoher Milchleistung. Journal of Animal Physiology and Animal Nutrition 90, 89-102.

Deswysen AG, Dutilleul P, Godfrin JP and Ellis WC 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. Journal of Animal Science 71, 2739-2747.

Dewhurst RJ, Fisher WJ, Tweed JK and Wilkins RJ 2003a. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. Journal of Dairy Science 86, 2598-2611.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ 2003b. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. Journal of Dairy Science 86, 2612-2621.

El Khidir OA and Thomsen KV 1983. Effect of intake on digestibility of plant cell walls and cell contents of complete diets for ruminants. Animal Feed Science and Technology 9, 197-204.

Ettle T, Obermaier A, Weinfurtner S and Spiekers H 2011. Luzernesilage im Austausch gegen Grassilage bei der Milchkuh. VDLUFA-Schriftenreihe 67, 588-594.

Flachowsky G, Grün M, Polzin S and Kronemann H 1994. In sacco dry matter degradability and Ca, Mg and P disappearance from italian ryegrass, alfalfa hay and wheat straw in sheep and goats. Journal of Animal Physiology and Animal Nutrition 71, 57-64.

Frame J 2005. Forage legumes for temperate grasslands. Science Publishers, Inc., Enfield, New Hampshire.

Geerts NE, De Brabander DL, Vanacker JM, De Boever JL and Botterman SM 2004. Milk urea concentration as affected by complete diet feeding and protein balance in the rumen of dairy cattle. Livestock Production Science 85, 263-273.

GfE 1991. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. Journal of Animal Physiology and Animal Nutrition 65, 229-234.

GfE 2001. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt am Main.

GfE 2009. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) New equations for predicting metabolisable energy of compound feeds for cattle. Proceedings of the Society of Nutrition Physiology 18, 143-146.

Halling MA, Topp CFE and Doyle CJ 2004. Aspects of the productivity of forage legumes in Northern Europe. Grass and Forage Science 59, 331-344.

Hoffman PC, Combs DK and Casler MD 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. Journal of Dairy Science 81, 162-168.

Huhtanen P and Hristov AN 2010. Effects of energy and protein supply on milk protein yield responses in dairy cows. In Energy and Protein Metabolism and Nutrition: EAAP publication no. 127, Parma, pp. 287-298. Wageningen Academic Publishers, Wageningen, Netherlands.

IPCC 2007. (Intergovernmental Panel on Climate Change) Summary for Policymakers. In Climate Change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)],Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., Kalu BA and Fick GW 1983. Morphological stage of development as a predictor of alfalfa herbage quality Crop Science 23, 1167-1172.

Kononoff PJ, Heinrichs AJ and Buckmaster DR 2003. Modification of the Penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. Journal of Dairy Science 86, 1858-1863.

Lebzien P, Brade W and Flachowsky G 2008. Der Milchharnstoffgehalt: Ein Indikator für die Energie- und Proteinversorgung, aber auch für die M-Emissionen in der Milcherzeugung. Übersichten zur Tierernährung 36, 59-74.

Lebzien P and Voigt J 1999. Calculation of utilisable crude protein at the duodenum of cattle by two different approaches. Archives of Animal Nutrition 52, 363-369.

McBurney MI, Van Soest PJ and Chase LE 1983. Cation exchange capacity and buffering capacity of neutral-detergent fibres. Journal of the Science of Food and Agriculture 34, 910-916.

Nordkvist E and Åman P 1986. Changes during growth in anatomical and chemical composition and in-vitro degradability of lucerne. Journal of the Science of Food and Agriculture 37, 1-7.

NRC 2001. (National Research Council) Nutrient requirements of dairy cattle. National Academy Press, Washington, D.C.

Radovic J, Sokolovic D and Markovic J 2009. Alfalfa most important perennial forage legume in animal husbandry. Biotechnology in Animal Husbandry 25, 465-475.

Schröder UJ and Staufenbiel R 2006. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. Journal of Dairy Science 89, 1-14.

Spiekers H and Potthast V 2004. Erfolgreiche Milchviehfütterung. DLG-Verlag, Frankfurt am Main.

Statistical Analysis System Institute 2009. SAS/IML 9.2: user's guide. SAS Publ., Cary, NC.

Steinwidder A and Gruber L 2000. Fütterungs- und tierbedingte Einflußfaktoren auf den Harnstoffgehalt der Milch von Kühen. Die Bodenkultur 51, 49-57.

Südekum K-H, Oestmann A and Stangassinger M 1995a. Zur Rolle von Lignin und phenolischen Monomeren in Futtermitteln für Wiederkäuer. II. Einfluss auf die Verdauung pflanzlicher Gerüstsubstanzen. Übersichten zur Tierernährung 23, 229-260.

Südekum K-H, Röh H, Brandt M, Rave G and Stangassinger M 1995b. Comparative digestion in cattle and sheep fed wheat silage diets at low and high intakes. Journal of Dairy Science 78, 1498-1511.

Sutton JD and Morant SV 1989. A review of the potential of nutrition to modify milk fat and protein. Livestock Production Science 23, 219-237.

Universität Hohenheim – Dokumentationsstelle (Ed.) 1997. DLG-Futterwerttabellen für Wiederkäuer, 7th ed. DLG-Verlag, Frankfurt/Main, Germany.

VDLUFA 2007. (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Waghorn GC, Shelton ID and Thomas VJ 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. British Journal of Nutrition 61, 409-423.

Yang WZ and Beauchemin KA 2007. Altering physically effective fiber intake through forage proportion and particle length: Digestion and milk production. Journal of Dairy Science 90, 3410-3421.

Zebeli Q, Tafaj M, Steingass H, Metzler B and Drochner W 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of Dairy Science 89, 651-668.

Zebeli Q, Dijkstra J, Tafaj M, Steingass H, Ametaj BN and Drochner W 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. Journal of Dairy Science 91, 2046-2066.

Chapter 4

Comparative evaluation of lucerne, grass and maize silage-based diets for lactating dairy cows: Feeding behaviour and chewing activity

J. Trautwein[1,2], K.-H. Südekum[2], G. Dusel [1], H. Steingaß[3], F.-J. Romberg[4], C. Koch[5] and K. Landfried[5]

[1] *University of Bonn, Institute of Animal Science, 53115 Bonn, Germany*

[2] *University of Applied Sciences Bingen,55411 Bingen/Rhein, Germany*

[3] *University of Hohenheim, Institute of Animal Nutrition, 70599 Stuttgart, Germany*

[4] *Dienstleistungszentrum ländlicher Raum Westpfalz, 67728 Münchweiler/A., Germany*

[5] *Lehr- und Versuchsanstalt für Viehhaltung Hofgut Neumühle, 67728 Münchweiler/A., Germany*

To be submitted

ABSTRACT

Lucerne (*Medicago sativa L.*), grass and whole-crop maize silages were offered to lactating German Holstein dairy cows in various combinations and feeding behaviour and chewing activity were evaluated. Cows were fed total mixed rations (TMR) with an average forage to concentrate ratio of 55:45 (dry matter (DM) basis). The forage component of the control (CON) ration was a 50:50 mixture of grass and maize silages (DM basis). The forage component of the three lucerne-based diets comprised (DM basis) either lucerne and grass silage (50:50), lucerne and maize silage (50:50) or equal proportions of the three silage types. TMR were formulated to have the same protein content, expressed as utilisable crude protein at the duodenum (uCP) but rations differed with regard to energy, fibre and starch concentrations. Chewing activity, determined with a chewing halter, and feeding behaviour were examined with six early to mid lactation cows. This group was not changed during the first five diets but a new group of six cows was used for the trial diets 5 to 7.

Total chewing time (620 to 748 min/day), eating time (189 to 288 min/day) as well as rumination time (395 to 529 min/day) were not affected by forage type, also not concerning on DM, aNDFom or peNDF intake. On average, 5.9 to 8.5 meals with an average DM intake (DMI) of 2.9 to 3.6 kg per meal within 29.6 to 38.7 min were consumed per day. The meal pattern with a meal criterion of 30 min did not vary with forage type. Also the eating activity (min/kg DMI) and the number of chews during rumination per kg DMI were not affected. However, the allocation of eating and rumination was influenced by forage type. Cows fed lucerne and maize silages spent 37.3% of total chewing time on eating and 62.7% on rumination and therefore spent more time in eating and less time on rumination compared to cows fed grass and maize silages ($p<0.05$). A similar tendency ($p<0.10$) was observed for cows on lucerne and grass silages compared with those on the grass and maize silage based diet. Compared with the grass and maize silage-based diet, chewing frequency (chews/min) during rumination was lower ($p<0.05$) if cows were fed lucerne and grass silages and a mixture of all three forage types. In conclusion, overall effect of forage type on feeding behaviour and chewing activity was small and it seems that lucerne silage expedites more eating than rumination activities.

Keywords: Eating behaviour, fibre, lucerne, meal pattern, rumination

IMPLICATIONS

Lucerne, grass and maize silages differ in chemical composition and structural properties. Fibre generally stimulates chewing, which plays an important role at maintaining healthy rumen and digestive functions. Chewing can be divided into chewing during eating and chewing during rumination. The lowest fibre content was analyzed for maize silage, followed by grass and lucerne silages. Cows fed the combination of maize and lucerne silage had the highest feed intake and spent proportionally more of the total daily chewing time with eating compared with cows fed a grass and maize silage-based diet.

INTRODUCTION

Maize and grass silage are the most common forages in dairy cow diets in Germany. Lucerne (*Medicago sativa L.)* is gaining interest as productive and drought-tolerant legume that can increase the yield of home-grown proteins (Frame, 2005). Chewing plays a key role in intake and digestion of forages (Beauchemin and Iwaasa, 1993). A high chewing activity stimulates salivary buffer secretion which leads to a lower risk of a drop in rumen pH and digestive disorders (Allen, 1997, Mertens, 1997). Furthermore chewing during eating as well as during rumination, in addition to microbial action and detrition, is responsible for breakdown of particulate DM in the rumen, which affects the rate of clearance of digesta from the rumen and hence voluntary feed intake (FI) (Ulyatt *et al.*, 1986, McLeod and Minson, 1988). Daily dry matter (DM) intake (DMI) is also affected by the number of meals per day, the length of meals and the intake rate during meals which can be summarized as feeding behaviour (Dado and Allen, 1994).

Allen (1997) defined physically effective fibre as the fraction of feed that stimulates chewing activity. To assess the adequacy of dietary fibre in dairy cattle diets, the concept of physically effective neutral detergent fibre (peNDF) was developed to amalgamate information on both chemical fibre content and particle size of feed (Zebeli *et al.*, 2012).

Lucerne, grass and maize forage differ in chemical fibre content, which also depends on the stage of maturity (Van Soest, 1994, Frame, 2005). Differences in chewing activity and feeding behaviour between forage sources have frequently been reported. Izumi and Unno (2010) observed that rumination and total chewing time per day and per kg DMI were shorter for lucerne hay than for grass hay. Feeding maize silage resulted in fewer meals and shorter daily eating und rumination times compared with grass silage (Deswysen *et al.*, 1993). Abrahamse *et al.* (2008) showed similar results, i.e. a longer eating time per day and a lower intake rate (g DM/min) when maize silage was substituted by grass silage. Replacing lucerne hay with maize silage increased daily chewing und rumination times (Kowsar *et al.*, 2008).

A physiological functionality of the rumen can be described with different variables related to chewing activity. Mertens (1997) suggested that diets for dairy cows should contain 22.3% peNDF to assure a chewing activity of 150 min/kg of peNDF to maintain optimal rumen function. To achieve 150 min/kg peNDF$_{>1.18}$ Zebeli *et al.* (2006) determined a necessary peNDF$_{>1.18}$ (particles retained on sieves > 1.18 mm) content of approximately 20% of DM in the diet. Later, Zebeli *et al.* (2012) recommended an optimal peNDF$_{>1.18}$ content (physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet) for dairy cow diets of 31.2% to stabilize rumen pH. To achieve a milk fat content of 3.6% cows needed to chew 36.1 min per kg of DMI (Mertens, 1997). Chewing records may therefore provide an important facet to the evaluation of the suitability of rations appropriate for ruminants.

The objective of this study was to evaluate the effect of lucerne, grass and whole-crop maize silages in various combinations in TMR fed to high yielding dairy cows on feeding behaviour and chewing activity.

MATERIAL AND METHODS

Forages and diets

The only substantial difference between the various rations in this study was the forage mixture. The composition of the concentrate component was adapted slightly, to minimize differences in key diet characteristics, e.g. amount of utilisable CP at the duodenum (uCP; Lebzien and Voigt, 1999, GfE, 2001), a precursor to metabolisable protein. The composition of the diets is shown in Table 1.

Lucerne (*Medicago sativa* L. *cv.* Franken Neu) silage was prepared from a first- and a second cut. The first cut was harvested on June 4, 2009 at the late bud stage (estimated according to Kalu and Fick (1983), the second cut was harvested between early and late flowering (July 15, 2009). The lucerne was field-wilted for one day and then chopped. The first cut was ensiled in a clamp silo and the second cut in round bales, silage additive was not used in either case.

Lucerne silage (50:50 first- and second-cut mixture) was fed in combination with grass and/or maize silage. Maize silage from the 2009 harvest was fed during phases 1 to 3 (explanation below), whereas a silage from the 2010 harvest was fed in the other phases. Grass silage was from the first and second cuts in 2010; the first-cut silage was fed during phases 1 to 5 and the second-cut silage during the other two phases.

Table 1 Ingredient composition of the experimental diets

	Total mixed ration						
	CON	GL	CON	ML	CON	GML	CON
Composition (g/kg of DM)							
Grass silage	286	297	286		283	222	316
Maize silage	286		286	295	293	191	279
Lucerne silage		251		250		162	
Grass hay	41	43	41	42	41	41	39
Soybean meal	47	26	47	26	46	34	44
Rapeseed meal	47	26	47	26	46	34	44
Barley/wheat grain (1:1)	138	174	138	173	137	151	131
Maize grain	138	174	138	173	137	151	131
Urea	5	-	5	5	5	3	5
Minerals and vitamins[a]	12	9	12	10	12	11	11

[a]Mineral and vitamin premix, sodium chloride (NaCl), calcium carbonate ($CaCO_3$)
CON = control diet (50/50 forage mixture of grass and maize silage)
GL = 50/50 forage mixture of grass and lucerne silage
ML = 50/50 forage mixture of maize and lucerne silage
GML = forage mixture (equal proportion) of grass, maize and lucerne silage
DM = dry matter

Animals and trial design

The trial was carried out at the Educational and Research Centre for Animal Husbandry Hofgut Neumühle (Münchweiler an der Alsenz, Germany). The herd comprised approximately 60 primiparous and multiparous dairy cows (German Holstein) in early to mid lactation. The cows calved continuously throughout the year and healthy cows were integrated into the herd 5 days after calving. The experimental design involved continual inflow and outflow of cows. All cows were housed in a loose-housing system and milked in a side-by-side or herring-bone milking parlour twice a day at 0500 h and 1530 h.

Cows were offered their feeds as total mixed ration (TMR) using an automated system with troughs (Typ RIC, Insentec B.V. Marknesse, The Netherlands) equipped with scales. The cows were equipped with transponders at the collar that allowed cows to be identified at each visit and thus enabled recording of individual daily feed intakes. The TMR were prepared in the morning and supplied in the morning and evening in amounts which allowed *ad libitum* intake. Fresh water was freely available. Each diet was given to the whole herd for a period of six weeks. The first two weeks of the period were an adaptation period and data were collected only during the last four weeks. After six weeks the diet was changed to the next treatment over 3 to 4 days. During phases 1, 3, 5 and 7 the cows were fed with the control (CON) diet, with a forage component consisting of a 50:50 (DM basis) grass silage to maize silage mix. In phase 2 the maize silage was replaced by lucerne silage (GL diet). In phase 4 the grass silage was replaced by lucerne silage in the same way (ML diet). In phase 6 the forage component consisted of equal proportions (by DM) of lucerne, grass and maize silage (GML diet). All lactating cows received the diets in the same order over the same period. The trial design is shown in Figure 1 which also summarises the composition of the various diets fed to the dairy cows.

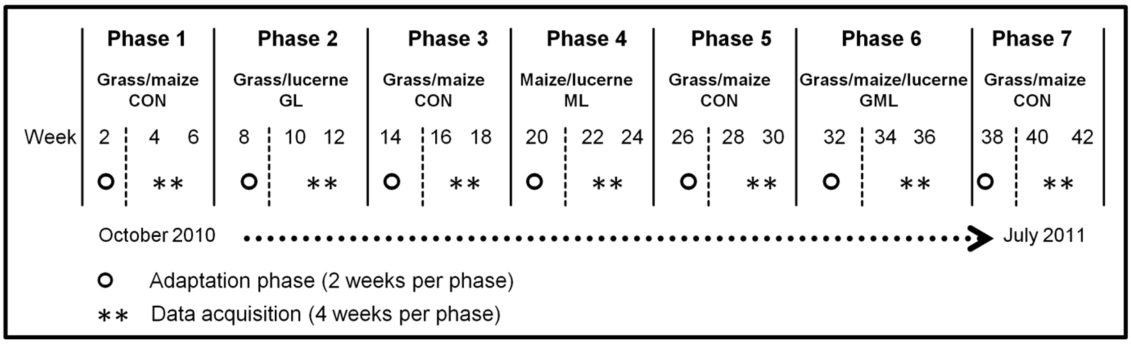

Figure 1 Trial design: schematic showing the ordering and organisation of the experimental diets during the feeding trial

Six out of the 60 cows were randomly selected to determine chewing activity and feeding behaviour. The selected healthy cows were retained for the first five trial periods. In trial period five the chewing activities of six additional cows were measured, because the first group of six animals left the herd after period five. For the following periods (5, 6 and 7) a new group of cows was selected and remained the same until the end of the trial.

Chewing activity and feeding behaviour

The chewing activity was determined on the selected group of cows over a period of 24 h during each data acquisition period over the whole feeding trial.

The cows wore a halter with an integrated air-filled silicone hose that was compressed by each jaw movement. Changes of the air pressure due to jaw movement were registered by a pressure switch and recorded by a data logger. In order to distinguish between eating and rumination activities, data was analysed computer-controlled and compared with the records of the feed intake from the automated system with troughs. As described by Zebeli *et al.* (2007), jaw movements were recorded every 6 seconds and appropriated as rumination chews if 4 to 7 chews were recorded within a 6-second time interval and if 5 to 12 successive intervals occurred followed by a pause. This is the assumptive time needed for mastication of a bolus. Within a chewing period a pause was considered when no jaw movement occurred within 4 s. This is the presumed time needed for swallowing the masticated bolus and regurgitating the next one. Chewing was regarded as a rumination period if at least three consecutive boli occurred separated by pauses. The jaw movements that were not assignable to a rumination period were caused by eating, drinking or licking. Therefore, the time spent eating at the single troughs was used to determine the eating time. The eating behaviour was ascertained with the aid of the feed intake within this 24 h period that was registered by the automated system with troughs, which recorded each feeding occurrence (start and end times of feed intake and consumed quantity of feed). From this, the feed intake rate (eating minutes per kg DM, NDF or peNDF) could be calculated. Visits of several troughs were summarized to one meal if the feed intake was not interrupted by a rumination period. The evaluation of the feed intake behaviour associated with the chewing activity resulted in a meal criterion (that is the longest non-feeding interval accepted as a part of a meal) of 30 minutes. This meal criterion was also used for the description of feed intake behaviour. The total chewing activity summarized the time spent eating at the troughs and the rumination time.

Data collection and chemical analysis

Daily samples of the TMR were taken during morning feeding and were used to confect one composite sample per week for DM determination. Daily DMI was calculated for each cow on the basis of the automatically determined TMR intake and the DM content of the diet. Forages and concentrates were sampled weekly during the final four weeks of each phase, stored frozen and blended to give one composite sample per phase. Chemical analyses of these samples and silages and faeces from the digestibility trial on sheep were carried out using the protocols of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2007) and method numbers are given below. The DM content of silages was determined using a two-step procedure involving pre-drying samples at 60°C, followed by oven-drying at 105°C, the one-step procedure used for all air-dry feedstuffs (3.1). Analyses of ash and crude lipid (CL) were carried out using methods 8.1 and 5.1.1 respectively; CP was determined by Dumas combustion (4.1.2). *In vitro* gas production (GP, 25.1); enzyme-soluble organic matter (ESOM; maize silage only, 6.6.1) and starch (7.2.1) were analysed using established procedures. Analysis of the hay, grass and lucerne silages also included determination of their water-soluble carbohydrate (‘sugar’) content (7.1.1). Neutral detergent fibre content, which was assayed with a heat stable amylase (6.5.1) and acid detergent fibre content (6.5.2) were expressed exclusive of residual ash and therefore denoted aNDFom and ADFom respectively. Acid detergent lignin (ADL, 6.5.3) was only analysed in forages.

Once a week a representative sample of TMR was collected from several troughs after morning feeding and used to estimate the $\text{peNDF}_{>1.18}$ content of the TMR. A modified Penn State Particle Separator was used to determine the proportion of the TMR retained on a 1.18-mm sieve, as described by Kononoff *et al.* (2003), multiplied by the NDF proportion (DM basis) of the TMR.

Calculations

The uCP concentrations of diet ingredients were estimated by two different approaches. The uCP of the silages was determined from the equation (GfE, 2001):

$$\text{uCP (g/kg DM)} = [187.7 - (115.4 \cdot (\text{UDP (g/kg DM)}/\text{CP (g/kg DM)}))] \cdot \text{DOM (kg/kg DM)} + 1.03 \cdot \text{UDP (g/kg DM)},$$

where UDP is ruminally undegraded feed CP and DOM is digestible organic matter as determined in sheep in the digestibility trial (see Chapter 3). For concentrates and hay the following equation was used:

$$\text{uCP (g/kg DM)} = [11.93 - (6.82 \cdot (\text{UDP (g/kg DM)}/\text{CP (g/kg DM)}))] \cdot \text{ME (MJ/kg DM)} + 1.03 \cdot \text{UDP (g/kg DM)},$$

where ME is metabolisable energy which was estimated as outlined below and CP values are based on from chemical analyses of diet ingredients in each phase. The UDP proportions of CP were assumed to be 15%, 20%, 22.5%, 25%, 30% and 50% for grass silage, lucerne silage and hay, barley and wheat grain, maize silage, soybean and rapeseed meals, and maize grain respectively. The uCP concentrations of the feedstuffs were then used to calculate the uCP content of the TMR (Table 3).

The ME content of the diets was calculated in the same way. The ME content of the silages was determined using the following equation (GfE, 2001):

$$\text{ME (MJ)} = 0.0312 \cdot \text{DCL} + 0.0136 \cdot \text{DCF} + 0.0147 \cdot (\text{DOM} - \text{DCL} - \text{DCF}) + 0.00234 \cdot \text{CP},$$

where DCL is digestible crude lipid, DCF is digestible crude fibre and DOM is digestible organic matter (all expressed as g/kg DM). The concentrates were proportionally assembled to a pooled sample and the energy content was estimated according to the following equation (GfE, 2009):

$\text{ME (MJ/kg DM)} = 7.17 - 0.01171 \cdot \text{ash} + 0.00712 \cdot \text{CP} + 0.01657 \cdot \text{CL} + 0.002 \cdot \text{starch} - 0.00202 \cdot \text{ADFom} + 0.06463 \cdot \text{GP}$ (ash, CP, CL, starch and ADFom, g/kg DM; GP, ml/200mg DM).

Net energy for lactation (NEL) values were estimated from ME according to GfE (2001):

$$\text{NEL (MJ/kg DM)} = 0.6 \cdot (1 + 0.004 \cdot (q - 57)) \cdot \text{ME (MJ)},$$

where $q = \text{ME}/\text{GE} \cdot 100$.

Statistical analysis

The data were analysed using the mixed model procedure in SAS with the maximum likelihood method (Statistical Analysis System Institute, 2009). The following model was used:

$$Y_{ijkl} = \mu + P_i + A_j + N_k + D_l + \varepsilon_{ijkl}$$

where Y_{ijkl} is the observed response; μ is the overall mean; P_i is the fixed effect of trial period i (1 to 7); A_j is the random effect of animal/cow j; N_k is the fixed effect of class of lactation number k (1 (lactation number 1 to 2), 2 (lactation number 3 to 4)); D_l is the fixed effect of class of lactation day l (1 (< 100 days), 2 (101-170 days) and 3 (>170 days)) and ε_{ijkl} is the residual error.

Treatment effects were evaluated by comparing phases with lucerne TMR with the average of the preceding and subsequent phases in which no lucerne was fed. After removal of incomplete data and validation of data, 36 observations were used for statistical data evaluation.

During period five, when the control diet was fed, the double number of cows, 6 from the first and 6 from the second group of cows was observed.

RESULTS

Forages and diets

The chemical composition of the forages used in the feeding trial is reported in Table 2.

Table 2 Chemical composition of the forages used in the dairy cow trial (means over the diets)

	Maize silage				Grass silage				Lucerne silage			
	Harvest 2009		Harvest 2010		First cut		Second cut		First cut		Second cut	
Phase	1, 3		4, 5, 6, 7		1, 2, 3, 5		6, 7		2, 4, 6		2, 4, 6	
Harvest dates												
	09/09/2009		20/09/2010		22/05/2010		01/07/2010		04/06/2010		15/07/2010	
Chemical composition (g/kg DM unless stated)												
DM (g/kg)	337	7.8	330	7.7	329	4.4	407	8.5	407	6.5	404	38.3
CP	76	3.0	81	2.7	174	5.7	155	3.7	175	5.1	160	5.3
ADFom	190	8.6	177	6.5	280	11.0	317	24.7	365	12.1	399	19.7
aNDFom	339	16.2	317	12.5	454	19.5	496	31.4	467	7.5	495	7.8
Energy value (MJ/kg DM)												
NEL	6.9	0.2	7.2	0.8	6.6	1.9	6.0	4.0	5.3	1.1	4.8	1.7

DM = dry matter, CP = crude protein, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, NEL = net energy for lactation

The maize silages from the 2009 and 2010 harvests had similar chemical composition. Grass silage from the second harvest, which was used in phases 6 and 7, had lower CP and lower NEL values than the grass silage used in the other phases, but higher fibre content. Lucerne silages from the first and second harvest were always used as a mixture; CP and NEL values were lower for the second-cut silage.

Table 3 summarizes the chemical composition and feeding value variables for the TMR fed to dairy cows. The lucerne TMR had a greater DM and ADFom content but a lower energy content than the other diets, whilst diets without maize silage had a lower starch content. As intended the uCP values and the ruminal N balance (RNB) of the various TMR showed little variation. The peNDF$_{>1.18}$ tended to be higher for the TMR with lucerne and grass silage and the TMR with the mixture of all three forage types.

Table 3 Nutrient composition of the experimental diets

	Total mixed ration						
	CON	GL	CON	ML	CON	GML	CON
Analysed (g/kg DM)							
Dry matter (g/kg)	459	492	455	481	460	502	491
CP	150	156	151	141	153	145	150
Starch	294	204	287	315	283	259	280
ADFom	167	221	171	182	172	207	191
aNDFom	298	339	302	297	306	329	329
Calculated (g/kg DM unless stated)[a]							
uCP[b]	148	147	149	142	148	141	147
RNB[c]	+0.3	+1.4	+0.3	-0.2	+0.8	+0.6	+0.5
NEL (MJ/kg DM)[d]	7.18	6.76	7.10	6.73	7.02	6.71	6.96
peNDF$_{>1.18}$ (%)[e]	26.3	31.1	26.6	26.3	27.3	29.0	28.5

[a] Calculated values for the TMR based on the analysed components

[b] Utilisable crude protein at the duodenum according to GfE (2001) (see material and methods)

[c] Ruminal Nitrogen Balance = (CP – uCP) / 6.25

[d] Energy (MJ) for lactation calculated according to GfE (2001) and GfE (2009) (see material and methods)

[e] Physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet (Kononoff *et al.*, 2003)

CON = control diet (50/50 forage mixture of grass and maize silage)

GL = 50/50 forage mixture of grass and lucerne silage

ML = 50/50 forage mixture of maize and lucerne silage

GML = forage mixture (equal proportion) of grass, maize and lucerne silage

DM = dry matter, CP = crude protein, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash

Chewing activity and feeding behaviour

Selected data of cows used for the determination of eating and rumination behaviour is shown in Table 4. In the first group of cows, the average daily milk yield was 38.8 kg at an average lactation day of 77 (diet 1) and declined to 28.0 kg at lactation day 238 (diet 5). The second group started with 42.9 kg milk per day at day 64 of lactation (diet 5) and decreased to 33.7 kg at lactation day 161 (diet 7). After validation of the rumination data 3 to 6 cows per experimental diet were included in the evaluation. The overall eating data and meal patterns are listed in Table 5. The eating behaviour was not influenced by forage type. The eating time per day ranged between 189 and 288 min/day. With progressive lactation stage the feeding rate (min/kg DMI) declined.

Table 6 presents the results of rumination and total chewing activity. Except for chews/second and chews/kg peNDF$_{>1.18}$ the rumination behaviour was not affected by feeding different silages. Time spent ruminating ranged between 395 to 529 minutes per day. Time spent ruminating per kg DMI was lowest if cows were fed the ML diet (15.9 min) and highest on the CON-2 diet (27.0 min). Chews per day during rumination ranged from 28,203 to 39,526. The chewing rate (chews per second) was significantly lower for cows on the GL ($p = 0.02$) and GML ($p = 0.03$) diets compared with CON. Across all diets 11 to 16 rumination periods per 24h were determined. Time spent ruminating during one period ranged from 26.6 to 39.5 min. The total chewing activity included eating and rumination varied between 620 and 748 min per day and was not influenced by forage type. However, a longer total chewing time per day was seen in the second group of cows (diet 5 to 7) compared to the first group (diet 1 to 5). A significant effect of forage source was detected in the allocation of eating and rumination. Cows fed ML spent 37.3% of total chewing time eating and 62.7% ruminating and therefore spent significantly more time eating and less time ruminating compared to cows on the control diet (31.9% vs. 68.1%). The same tendency ($p = 0.07$) was determined for animals fed the GL diet.

Table 4 Description of the selected cows used for the determination of the chewing and eating behaviour

	Experimental diets							
Item	CON	GL	CON	ML	CON-1	CON-2	GML	CON
Days of lactation	77	120	164	204	238	64	122	161
Lactation number	2.75	2.67	2.50	2.25	2.50	2.60	2.67	2.67
Milk yield (kg/day)	38.8	33.3	33.4	28.0	28.0	42.9	36.4	33.7
No. of cows (replications)	4	3	4	4	4	5	6	6

CON = control diet (50/50 forage mixture of grass and maize silage)
CON-1 = first group of cows (phase 1-5) fed CON
CON-2 = second group of cows (phase 5-7) fed CON
GL = 50/50 forage mixture of grass and lucerne silage
ML = 50/50 forage mixture of maize and lucerne silage
GML = forage mixture (equal proportion) of grass, maize and lucerne silage

Table 5 Comparison of milk yield and eating behaviour of dairy cows fed diets containing lucerne and grass-maize based diets (LSM and s.e.)

	Experimental diets								s.e.		
Item	CON	GL	CON	ML	CON-1	CON-2	GML	CON	GL v. CON	ML v. CON	GML v. CON
Days of lactation	77	120	164	204	238	64	122	161			
Lactation number	2.75	2.67	2.50	2.25	2.50	2.60	2.67	2.67			
Milk yield (kg/day)	38.8	33.3	33.4	28.0	28.0	42.9	36.4	33.7			
kg DM/d	21.1	21.8	23.3	27.3	24.9	19.5	22.0	24.7	1.735	1.574	1.283
kg aNDFom/d	6.3	7.3	7.1	8.1	7.6	6.0	7.2	8.1	0.545	0.494	0.404
kg peNDF/d	5.5	6.8	6.2	7.2	6.8	5.3	6.4	7.0	0.484	0.439	0.359
No. of cows (replications)	4	3	4	4	4	5	6	6			
Eating											
Min/d	189	221	216	252	205	216	268	288	21.80	20.10	15.80
Min/kg of DM	11.4	10.1	9.2	8.9	8.1	13.8	12.6	11.6	0.861	0.802	0.618
Min/kg of aNDFom	38.6	30.1	30.3	29.9	26.3	45.4	38.5	35.0	2.820	2.627	2.024
Min/kg of peNDF$_{>1.18}$	43.9	32.9	34.3	33.7	29.4	51.1	43.7	40.4	3.179	2.961	2.282
g DM/min	97.8	98.8	125.6	123.1	129.2	80.6	78.5	87.7	10.37	9.60	7.48
g aNDFom/min	29.4	34.1	37.7	36.3	39.3	25.0	26.0	28.7	3.140	2.907	2.266
g peNDF$_{>1.18}$/min	26.1	31.4	33.1	32.1	34.9	22.5	22.9	24.8	2.775	2.570	2.002
Meal pattern											
Meals/d	5.9	6.2	6.8	7.8	8.5	6.6	7.5	7.7	0.730	0.654	0.551
Min/meal	29.6	35.0	31.0	32.9	23.9	33.5	36.4	38.7	4.429	4.019	3.273
kg DM/meal	3.6	3.6	3.5	3.6	2.9	3.3	3.0	3.2	0.311	0.279	0.235
kg aNDFom/meal	1.08	1.24	1.05	1.05	0.89	1.01	0.98	1.04	0.099	0.089	0.074

LSM = least squares means, s.e. = standard error, CON = control diet (50/50 forage mixture of grass and maize silage), CON-1 = first group of cows (phase 1-5) fed CON, CON-2 = second group of cows (phase 5-7) fed CON, GL = 50/50 forage mixture of grass and lucerne silage, ML = 50/50 forage mixture of maize and lucerne silage, GML = forage mixture (equal proportion) of grass, maize and lucerne silage, DM = dry matter, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, peNDF$_{>1.18}$ = physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet, d = day, $P< 0.05$ ** $P< 0.01$ *** $P<0.001$: significant differences between diets containing lucerne (GL, ML, GML) compared with the surrounding control diets (CON)

Table 6 Comparison of rumination and chewing activity of dairy cows fed diets containing lucerne and grass-maize based diets (LSM and s.e.)

	Experimental diets								s.e.		
Item	CON	GL	CON	ML	CON-1	CON-2	GML	CON	GL v. CON	ML v. CON	GML v. CON
Rumination											
Min/d	429	395	446	428	433	529	469	446	27.83	24.96	21.01
Min/kg of DM	20.3	18.7	19.0	15.9	17.5	27.0	21.4	18.3	2.156	1.938	1.621
Min/kg of aNDFom	68.4	55.4	63.1	53.3	57.1	88.2	65.0	55.4	6.730	6.059	5.044
Min/kg of peNDF$_{>1.18}$	77.6	60.4	71.8	60.1	64.1	98.9	73.8	64.0	7.515	6.769	5.623
Chews/d	34117	28234	30139	28314	28203	39526	32852	30012	2127	1907	1606
Chews/sec.	1.26	1.18*	1.17	1.19	1.17	1.19	1.14*	1.14	0.014	0.013	0.010
Chews/min	75.7	70.9*	70.4	71.7	70.5	71.3	68.6*	68.5	0.821	0.774	0.584
Chews/kg of DM	1477	1319	1285	1062	1143	1981	1498	1232	147	132	111
Chews/kg of aNDFom	5250	3895	4281	3596	3753	6434	4556	3746	470	421	354
Chews/kg of peNDF$_{>1.18}$	5959	4247*	4871	4064	4218	7223	5167	4326	525	472	395
Ruminating periods/d	10.5	12.4	15.2	14.8	16.2	14.1	15.0	13.8	0.939	0.858	0.687
Min/ruminating period	39.5	32.1	29.7	27.7	26.6	37.6	32.1	33.6	2.403	2.204	1.751
Total chewing activity											
Min/d	623	620	660	672	635	748	739	733	38.84	35.33	28.61
Min/kg of DM	28.0	28.5	28.1	25.6	25.6	36.9	33.5	30.4	2.634	2.362	1.989
Min/kg of aNDFom	97	87	91	79	77	124	105	92	8.307	7.450	6.273
Min/kg of peNDF$_{>1.18}$	112	93	105	94	93	140	116	105	8.981	8.130	6.664
% eating	29.5	35.7	32.0	37.3*	31.8	27.5	36.3	39.5	2.513	2.305	1.832
% rumination	70.5	64.3	68.0	62.7*	68.2	72.5	63.7	60.5	2.513	2.305	1.832

LSM = least squares means, s.e. = standard error, CON = control diet (50/50 forage mixture of grass and maize silage), CON-1 = first group of cows (phase 1-5) fed CON, CON-2 = second group of cows (phase 5-7) fed CON, GL = 50/50 forage mixture of grass and lucerne silage, ML = 50/50 forage mixture of maize and lucerne silage, GML = forage mixture (equal proportion) of grass, maize and lucerne silage, DM = dry matter, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, peNDF$_{>1.18}$ = physically effective neutral detergent fibre representing particles > 1.18 mm multiplied with NDF content of the total diet, d = day, P< 0.05 ** P< 0.01 *** P<0.001: significant differences between diets containing lucerne (GL, ML, GML) compared with the surrounding control diets (CON)

Discussion

The replacement of grass or maize silage with lucerne silage changed the chemical composition of the TMR, especially aNDFom, ADFom, starch and $\text{peNDF}_{>1.18}$, indicating that physical characteristics of the TMR were also affected. An adequate amount of fibre in dairy cow diets stimulates chewing activity and reduces acid load (Mertens, 1997).

The total chewing activity was not affected by the different forages. Total chewing time is in agreement with Zebeli *et al.* (2006), who calculated a mean total chewing time of 691 min/day (between 425 and 969 min/day) from more than 20 published studies. Whereas the first group of cows, during experimental periods 1-5, had a total chewing time between 620 and 672 min/day, the second group of cows, during the periods 5 to 7, ranged from 733 to 748 min/day. Both the higher milk yield of the second group and the inter-individual variation could be the reason for this difference (Table 4). Dado and Allen (1994) found out that the total time spent chewing per day was, on the one hand, positively associated with milk production, and on the other hand differences between cows were the greatest source of variation for most variables describing chewing activity.

A significant effect appeared in the allocation of total chewing time to eating time and rumination time. The eating percentage varied among treatments between 27.5 and 39.5% and, vice versa, the rumination percentage between 72.5 and 60.5%. Cows fed the ML diet showed a higher proportion of eating (37.3%) compared to the control diet CON (31.9%) and a lower proportion of rumination (62.7% vs. 68.1%, $p=0.032$). This effect, among others, can be attributed to the trend for a higher DMI ($p = 0.058$) for cows fed the ML diet (27.3 kg) compared with the control (CON) diet (24.1 kg). Deswysen *et al.* (1987) determined a positive correlation between voluntary intake and daily eating time and also stated that a higher feed intake would not cause a longer rumination time per g DM. In another study, a greater daily DMI by sheep led to an increased proportion of eating time and an decreased proportion of rumination time with, at the same time, longer total chewing time per day (Kaske *et al.*, 2002). In our study, for cows on the GL diet the proportion of eating time of total chewing time tended also to be increased at similar DMI compared to the CON-TMR ($p = 0.067$), indicating a slight effect of lucerne silage on chewing activities. Kornfelt (2012) concluded from her studies that the structural particles from legumes increased the time spent comminuting during eating compared to grass and this can lead to faster passage of the legume particles out of the rumen. A higher rate of passage of cows consuming lucerne silage resulted in a higher feed intake (Waghorn *et al.*, 1989, Hoffman *et al.*, 1998). When the whole group of cows (app. 60 lactating cows) in our experiment are considered, a significantly higher feed intake of lucerne-silage diets was determined (Chapter 3).

The eating time per day ranged from 189 to 288 min and seemed to be influenced more by the amount of feed consumed per day than by forage source. Therefore, no effect on eating time (min) per kg DMI was seen if grass or maize silage was replaced with lucerne silage. Similarly, Beauchemin and Iwaasa (1993) concluded that the duration of eating is not affected by forage despite differences in chemical composition. A different peNDF content did not influence the eating time per kg DMI (Beauchemin and Yang, 2005). Kornfelt *et al.* (2013), however, showed a greater eating activity (min /kg DMI) when cows were fed late harvested compared to early harvested lucerne silage with lower NDF content. Thus, the higher aNDFom content especially in the GL diet should have affected eating activity. However, advancing lactation of cows associated with increasing feed intake as well as milk yield could have interfered with a possible impact of aNDFom content on eating activity. The eating rate increased with progressive lactation stage and increasing daily DMI. Animals with high DMI spent less time chewing per unit DM than at lower DMI (Welch and Hooper, 1988). Also Dado and Allen (1994) showed that high producing cows spent less time eating per unit DMI than lower producing cows. Comparable with our study, Kornfelt *et al.* (2013) found no differences in NDF eating rate (g NDF intake/min). Due to different methods of measurement, care must be taken to compare the absolute time spent eating per day (189 to 288 min, Table 5) with literature values. Some studies (Zebeli *et al.*, 2007, Kornfelt *et al.*, 2013) clearly overestimated the eating time (up to about 400 min), because it was calculated as the difference between the total chewing time and the rumination time and, therefore, eating also comprises other activities such as licking, grooming and drinking. In our study a 17% shorter eating time (data not shown) would result if the eating times were summarized from recordings of the automated system with troughs compared to the method by Zebeli *et al.* (2007) and Kornfelt *et al.* (2013), using chewing halters. On the other hand, summarizing the time spent eating at the trough likely underestimate the feed intake time slightly, because jaw movements that occurred during changing the trough are not recorded and therefore not accounted for as eating time. This underestimation becomes obvious when our results are compared with those of Beauchemin and Yang (2005). These authors declared jaw movements, detected with a halter, as eating time only if a meal (defined as an eating activity greater than 30 s and including more than 300 g of feed intake) took place and detected a mean eating time of 260 to 296 min/day for a mean DMI of about 20 to 21 kg. Results presented by Abrahamse *et al.* (2008) are in line with our findings, possibly due to a similar method of measurement. They observed similar eating durations at the feed bins of between 177 and 227 min/day for slightly lower daily DMI which ranged from 17.3 to 20.1 kg.

The meal pattern was not affected by forage type and the number of meals per day increased with advancing lactation. Visits of several troughs were summarized to one meal if the intake was not interrupted by a rumination period. A lot of different methods have been used to create a meal criterion (Tolkamp *et al.*, 2000). After analysing the feed intake and rumination data from the 36 cows of our study it became obvious that 30 min was the most appropriate meal criterion to split feed intakes into meals, because no cow started rumination earlier than 30 min after the last feed intake. Breaks during feed intake (without occurrence of a rumination period) were detected that lasted up to about 25 min. Independent of forage source in the diet, 5.9 to 8.5 meals occurred during 24 h and 2.9 to 3.6 kg of DM were eaten per meal during an average meal duration of 29.6 to 38.7 min. Similar values were reported by Tolkamp *et al.* (2000) at an average meal duration of about 45 min. A higher number of meals per day instead of a higher DMI per meal as well as longer meal duration is beneficial for stabilizing ruminal fluid pH and helps therefore preventing subacute ruminal acidosis (SARA) (Gonzalez *et al.*, 2012).

Results presented by Zebeli *et al.* (2006) convincingly indicate that dietary physically effective fibre, i.e. peNDF, is a more efficient parameter to assess effective fibre adequacy of a diet than aNDFom as single variable. The peNDF incorporates information on physical structure and chemical fibre content of the diet (Zebeli *et al.* 2012, GfE, 2014). Both the chemically measured concentration of fibre and the physical characteristics of fibre influence chewing activity and thereby salivary buffer secretion, which in turn affects ruminal pH (Mertens, 1997). The $\mathrm{peNDF}_{>1.18}$ content of the experimental diets ranged between 26.3% (CON and ML) and 31.1% (GL) (Table 3). Zebeli *et al.* (2012) recommend an average amount of 31.2% $\mathrm{peNDF}_{>1.18}$ in the diet to ensure prevention of

SARA through a stabilized ruminal pH. GfE (2014) recommends 22% physically effective neutral detergent fibre representing particles > 8 mm multiplied with NDF content of the total diet (peNDF$_{>8}$) to reach a daily average pH of 6.2 depending on dry matter intake (between 21 and 27 kg) as well as starch content in our diet (between 26% and 32%) for all diets except diet GL and could not be achieved in our study. Only diet GL could reach the recommendation (18% peNDF$_{>8}$) due to lower starch content (20.4%) and a higher peNDF$_{>8}$ content (20.8%) in the diet (Table 3 and 5). Zebeli *et al.* (2006) determined a poor, although slightly positive, correlation between peNDF$_{>1.18}$ content in the diet to daily duration of chewing and rumination. In our study we did not find differences between diets, neither for total chewing time per kg of DMI nor for rumination time per kg of DMI. Likely, the structural properties affecting chewing activities were sufficient in all diets and were well above the minimum requirements of the cows, such that no influence on chewing activity was observed. Tafaj *et al.* (2005) reported no differences among diets of total chewing time, rumination time and eating time per day as well as per unit of DMI of dairy cows in mid lactation fed diets with 24, 28 and 32% peNDF of DM. Only a further reduction to 19% peNDF reduced chewing activity. Mertens (1997) suggested that a chewing activity of 150 min/kg of peNDF intake is necessary to maintain optimal rumen function. This remark was seized in a meta-analysis by Zebeli *et al.* (2006) who demonstrated that the recommended 150 min of chewing per kg peNDF intake were achieved if a TMR contained 20% peNDF$_{>1.18}$. The total chewing time per kg peNDF$_{>1.18}$ in our study ranged between 93 and 140 min and did not achieve the recommended 150 min. Mertens (1997) and Zebeli *et al.* (2006) determined a mean chewing time of 744 min/day or 36.1 min/kg of DM and 797 min/day or 36.5 min/kg of DM, respectively, to achieve a milk fat content of 3.6%. In our study the chewing time per day ranged from 620 to 748 min and the time needed for 1 kg of DMI ranged from 25.6 to 36.9 min, and, therefore, was slightly lower than postulated. However, the milk fat content ranged between 3.8% and 4.3% (Chapter 3). The lower chewing time in our study compared to the above authors can be explained by the measurement method, which leads in our study to a slight underestimation of the eating and therefore chewing time.

The rumination time per day and the rumination time per kg intake of DM, aNDFom and peNDF$_{>1.18}$ were not affected by forage type. Cows spent between 395 and 529 min per day ruminating. These values are in line with the mean rumination time of 434 min (ranging from 151 to 632 min) determined from 24 different studies by Zebeli *et al.* (2006). Similar mean values for rumination time were reported by Beauchemin *et al.* (1994), who concluded that, for high-producing dairy cows, 360 min of rumination per day is sufficient to maintain healthy ruminal conditions. These authors also showed that an addition of long-stemmed lucerne hay to low-fibre diets increased rumination. For adequate-fibre diets the addition of structural fibre did not increase rumination, in contrast it decreased rumination. This decrease in rumination time was partially compensated by increased eating time (Beauchemin *et al.*, 1994). An effect occurred in our study when the GL diet (high peNDF$_{>1.18}$) was compared with the CON diet regarding the allocation of total chewing time per day to eating and rumination activities per day. Beauchemin and Yang (2005) concluded that varying peNDF content in the diet did not affect the number of chews per unit of DMI during rumination. This implies that cows efficiently reduced long particles by increasing chewing rate during eating. The average rumination time per unit of NDF intake decreased with increasing intake of NDF (Kornfelt, 2012). The highest aNDFom content (339 g/kg DM) was analysed in diet GL. Nevertheless, a significant reduction of rumination time per kg of aNDFom intake was not detected.

Chews per day during rumination were not significantly different between diets. The values, ranging between 28,234 and 39,526 chews during rumination are similar to results presented by Beauchemin and Yang (2005) (30,209 to 33,006 with increasing peNDF content in the diet at 20.0 to 21.1 kg DMI). Slightly lower number of chews per day during rumination were observed by Beauchemin *et al.* (1994) (25,330 to 27,610 at 20.1 to 21.7 kg DMI) and Yang and Beauchemin (2006) (25,412 to 29,511 at 23.7 to 24.8 kg DMI). Chews per kg of DMI did not differ between forages and fibre contents in the diets and did not resemble results of Tafaj *et al.* (2005) who showed a greater number of chews/kg DMI during rumination with an increased peNDF level (19 to 39% peNDF) in the diet. Opposed to that, Beauchemin and Yang (2005) did not detect an influence of peNDF content in the diet on the number of chews per unit DMI. Chews/kg DMI decreased with increasing feed intake (Table 5 and 6). The values (1,062 to 1,981 chews/kg DM) were similar to those presented by Beauchemin and Yang (2005), Zebeli *et al.* (2007) as well as Robinson and McQueen (1997). Due to the highest peNDF$_{>1.18}$ content in the GL diet, the amount of chews per kg peNDF$_{>1.18}$ intake (4,247 chews) was significantly lower than for the CON diet (5,959 and 4,871 chews, respectively).

Chewing frequency (chews per min) during rumination increased from 60.2 to 63.9 chews/min when peNDF content in the diet (from 19 to 39%) increased. However, when the peNDF content was between 24 and 32% of DM, the chewing frequency was not affected (Tafaj *et al.*, 2005). Therefore, with a peNDF content of 26.3 to 31.1 % in the diets of this study it is plausible that no effect on chewing frequency was observed. Also Robinson and McQueen (1997) detected neither an effect of concentrate level nor an effect of fermentability of forage fibre on chewing frequency, ranging from 59.7 to 62.6 chews/min. The feeding level also did not influence chewing frequency (Luginbuhl *et al.*, 1989). However, in our study the chews per min during rumination ranged from 68.5 to 75.7 chews per min and were significantly lower for the GL and GML (68.6) diets than for the CON diet. Domingue *et al.* (1991) concluded from their study with sheep and goats that a greater efficiency of chewing during rumination in breaking down the feed particles can be accounted for by a greater number of chews/min during rumination. Structural particles from legumes increases the time spent on comminuting during eating compared to grass and this can lead to a faster passage rate of the legume particles out of the rumen (Kornfelt, 2012). Overall eating seems to be the most important part of the total chewing activity when ruminants are consuming legumes. The chewing frequency during eating was not analysed in our study. Kornfelt (2012) showed that lucerne NDF was apparently chewed more thoroughly during ingestion than orchard grass NDF, which was indicated by eating times and chews per unit of NDF. During eating the resistance of the large particles to breakdown is lower in grass, conversely during ruminating higher compared to legume (McLeod *et al.*, 1990). Possibly this could result in a varied chewing frequency.

A precise, consist method to assess eating and rumination activities is not described in the literature. The determination of the feeding behaviour and rumination activity is labour-intensive and costly. Due to the great influence of inter-individual effect with coefficients of variation between 15 and 20%, for several variables even up to 40%, the experimental unit heterogeneity should be kept as small as possible (Dado and Allen, 1994). In our study we selected cows to create a representative group regarding days of lactation, lactation number and milk yield. Variations illustrated in Table 4 appeared due to low number of replications. In our study, obviously incomplete or incorrect measurements were immediately repeated. Nevertheless, inconsistency of the data of a cow was often detected only after the chewing data was compared with the feeding data stored at the troughs and, therefore, could only at that stage be omitted from the evaluation. Based on this experience, a thorough validation of the chewing data seems to be essential to prevent wrong conclusions. Results of evaluations, which are only based on technical methods without other independent validation mechanisms, should therefore be regarded with caution. Different methods (ranging from technical equipment to methods of evaluation) for determination of eating and rumination behaviour of cows hamper the comparability of the results. For the future, a standardization of methods would be highly desirable. Due to our decision to investigate the same group of individuals and therefore to minimize the inter-individual effect, the lactation stage proceeded which is associated with changes in feed intake and milk yield. This also influences feeding behaviour and chewing activity (Deswysen *et al.*, 1987, Welch and Hooper, 1988, Dado and Allen, 1994). Although the lactation day in addition to the number of lactations and the effect of the individual was incorporated into the statistical model, a statistical coverage is more difficult especially through the small number of replications (observed cows).

Conclusions

Lucerne silage in combination with grass and/or maize silage did not affect total chewing time per day compared to a grass and maize silage-based diet. Also no differences due to forage source were seen in eating and rumination time per unit DMI. Meal pattern as well as eating behaviour were similar across diets. Also the eating activity (min/kg DMI) was not affected by forage source in the diets. Cows fed ML showed the highest feed intake and spent more time of total chewing time eating and less time ruminating compared with control cows fed the CON diet. The same trend was observed when cows were fed the GL diet. Cows fed lucerne diets (GL and GML) showed a lower number of chews per minute during rumination compared with cows fed CON. In conclusion, overall effect of forage type on feeding behaviour and chewing activity was small and it seems that lucerne silage expedites more eating than rumination activities.

Acknowledgement

This study was supported by funds allotted to the Dienstleistungszentrum ländlicher Raum (DLR) Westpfalz, Educational and Research Centre for Animal Husbandry Hofgut Neumühle (Münchweiler an der Alsenz, Germany) and the University of Applied Sciences, Bingen, and was partly conducted in the Centre for Integrated Dairy Research (CIDRe), University of Bonn (Bonn, Germany).

References

Abrahamse PA, Vlaeminck B, Tamminga S and Dijkstra J 2008. The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. Journal of Dairy Science 91, 4778-4792.

Allen MS 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. Journal of Dairy Science 80, 1447-1462.

Beauchemin KA and Iwaasa AD 1993. Eating and ruminating activities of cattle fed alfalfa or orchard-grass harvested at 2 stages of maturity. Canadian Journal of Animal Science 73, 79-88.

Beauchemin KA and Yang WZ 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. Journal of Dairy Science 88, 2117-2129.

Beauchemin KA, Farr BI, Rode LM and Schaalje GB 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. Journal of Dairy Science 77, 1326-1339.

Dado RG and Allen MS 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. Journal of Dairy Science 77, 132-144.

Deswysen AG, Ellis WC and Pond KR 1987. Interrelationships among voluntary intake, eating and ruminating behavior and ruminal motility of heifers fed corn-silage. Journal of Animal Science 64, 835-841.

Deswysen AG, Dutilleul P, Godfrin JP and Ellis WC 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. Journal of Animal Science 71, 2739-2747.

Domingue BMF, Dellow DW and Barry TN 1991. The efficiency of chewing during eating and ruminating in goats and sheep. British Journal of Nutrition 65, 355-363.

Frame J 2005. Forage legumes for temperate grasslands. Science Publishers, Inc., Enfield, New Hamshire.

GfE 2001. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verl., Frankfurt am Main.

GfE 2009. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) New equations for predicting metabolisable energy of compound feeds for cattle. Proceedings of the Society of Nutrition Physiology 18, 143-146.

GfE 2014. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Communications of the committee for requirement standards of the society of nutrition physiology: Evaluation of structural effectiveness of mixed rations for dairy cows – status and perspectives. Proc. Soc. Nutr. Physiol. 23, 165-179.

Gonzalez LA, Manteca X, Calsamiglia S, Schwartzkopf-Genswein KS and Ferret A 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Animal Feed Science and Technology 172, 66-79.

Hoffman PC, Combs DK and Casler MD 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. Journal of Dairy Science 81, 162-168.

Izumi K and Unno C 2010. Effects of feeding ratio of beet pulp to alfalfa hay or grass hay on ruminal mat characteristics and chewing activity in Holstein dry cows. Animal Science Journal 81, 180-186.

Kalu BA and Fick GW 1983. Morphological stage of development as a predictor of alfalfa herbage quality. Crop Science 23, 1167-1172.

Kaske M, Beyerbach M, Hailu Y, Gobel W and Wagner S 2002. The assessment of the frequency of chews during rumination enables an estimation of rumination activity in hay-fed sheep. Journal of Animal Physiology and Animal Nutrition 86, 83-89.

Kononoff PJ, Heinrichs AJ and Buckmaster DR 2003. Modification of the penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. Journal of Dairy Science 86, 1858-1863.

Kornfelt LF 2012. Physical degradation of forage particles and digestion of structural fibre in alfalfa, red and white clover crops fed to cattle. PhD. Univ. of Copenhagen.

Kornfelt LF, Weisbjerg MR and Norgaard P 2013. Effect of harvest time and physical form of alfalfa silage on chewing time and particle size distribution in boli, rumen content and faeces. Animal 7, 232-244.

Kowsar R, Ghorbani GR, Alikhani M, Khorvash M and Nikkhah A 2008. Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. Journal of Dairy Science 91, 4755-4764.

Luginbuhl J-M, Pond KR, Burns JC and Russ JC 1989. Eating and ruminating behaviour of steers fed coastal bermudagrass hay at four levels. Journal of Animal Science 67, 3410-3418.

McLeod MN and Minson DJ 1988. Large particle breakdown by cattle eating ryegrass and alfalfa. Journal of Animal Science 66, 992-999.

McLeod MN, Kennedy PM and Minson DJ 1990. Resistance of leaf and stem fractions of tropical forage to chewing and passage in cattle. British Journal of Nutrition 63, 105-119.

Mertens DR 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science 80, 1463-1481.

Robinson PH and McQueen RE 1997. Influence of level of concentrate allocation and fermentability of forage fiber on chewing behavior and production of dairy cows. Journal of Dairy Science 80, 681-691.

Statistical Analysis System Institute 2009. SAS/IML 9.2: user's guide. SAS Publ., Cary, NC.

Tafaj M, Maulbetsch A, Zebeli Q, Steingass H and Drochner W 2005. Effects of physically effective fibre concentration of diets consisting of hay and slowly degradable concentrate on chewing activity in mid lactation dairy cows under constant intake level. Archives of Animal Nutrition 59, 313-324.

Tolkamp BJ, Schweitzer DPN and Kyriazakis I 2000. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. Journal of Dairy Science 83, 2057-2068.

Ulyatt MJ, Dellow DW, John A, Reid CSW and Waghorn GC 1986. Contribution of chewing during eating and rumination to the clearance of digesta from the reticulorumen. In The control of digestion and metabolism in ruminants (eds LP Milligan, WL Grovum, and A Dobson), pp. 488-517, Prentice-Hall (Englewood Cliffs, N.J.).

Van Soest PJ 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell University Press, Ithaca, NY, USA.

VDLUFA 2007. (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Waghorn GC, Shelton ID and Thomas VJ 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne L.*) and lucerne (*Medicago sativa L.*) fed to cows during a restricted feeding period. British Journal of Nutrition 61, 409-423.

Welch JG and Hooper AP 1988. Ingestion of feed and water. In The ruminant animal: digestive physiology and nutrition (ed. DC Church), pp. 108-116, Prentice-Hall, Englewood Cliffs, NJ.

Yang WZ and Beauchemin KA 2006. Physically effective fiber: Method of determination and effects on chewing, ruminal acidosis, and digestion by dairy cows. Journal of Dairy Science 89, 2618-2633.

Zebeli Q, Tafaj M, Steingass H, Metzler B and Drochner W 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of Dairy Science 89, 651-668.

Zebeli Q, Tafaj M, Weber I, Dijkstra J, Steingass H and Drochner W 2007. Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cow. Journal of Dairy Science 90, 1929-1942.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN and Drochner W 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science 95, 1041-1056.

CHAPTER 5

GENERAL DISCUSSION

An in-depth discussion of the results of a large range of variables was performed in Chapters 3 and 4. In the following, only particular results will be pointed out and a synopsis of the findings presented in the chapters three and four is made.

To characterize the nutritional value of the forages used in the feeding trial chemical analysis as well as digestibility studies with sheep were conducted and the results are presented and discussed in Chapter 3. The first and the second cut of lucerne in the harvest year 2009 were included in equal proportions in the experimental diets. The digestibility of organic matter (OM), ADFom and aNDFom of the second cut (58%, 47% and 45%) indicated advanced stage of maturity at harvest of the lucerne plant compared to the first cut (66%, 55% and 55%, respectively). Overall the digestibility of lucerne silage for OM, fibre fractions (ADFom, aNDFom) and energy was significantly lower than the grass silage values and confirmed the results presented by Hoffman *et al.* (1998), Bulang *et al.* (2006) and Ettle *et al.* (2011). The lower digestibility can be considered as a consequence of the lower potential ruminal degradability, shorter retention time in the rumen or, vice versa, higher passage rate from the rumen for lucerne silage (Waghorn *et al.*, 1989, Flachowsky *et al.*, 1994, Hoffman *et al.*, 1998). Thus, there is a negative relationship between passage rate and the digestibility of nutrients. An increased intake has the most negative effect on digestibility of diets or feeds with high fibre (NDF) content (El Khidir and Thomsen, 1983, Südekum *et al.*, 1995b). On the other hand, a rapid particle breakdown followed by a high passage rate results in high feed intake (Waghorn *et al.*, 1989, Hoffman *et al.*, 1998, Dewhurst *et al.*, 2003).

Regardless of the other forages (grass and/or maize silage), lucerne silage enhanced the feed intake of dairy cows in comparison with a grass and maize silage-based diet (Chapter 3). The largest increase (+1.5 kg DMI/day) could be achieved by feeding lucerne silage in combination with maize silage (ML). A higher feed intake (+8.2%) of maize silage versus grass silage due to a shorter eating time was observed by Deswysen *et al.* (1993). In this study, the feed intake of cows equipped with a chewing halter also tended to be increased ($p<0.1$) when the ML diet was fed (27.3 kg) compared with the control diet

(24.1 kg) (Chapter 4). Due to the higher feed intake the eating time per day (ML: 252 min, CON: 211 min) tended also to be longer ($p<0.1$). Deswysen *et al.* (1987) determined a positive correlation between voluntary intake and daily eating time. Feed intake behaviour can be used to estimate the risk of low ruminal pH. A higher number of meals per day instead of a higher feed intake per meal as well as longer meal duration is beneficial for ruminal fluid pH and therefore helps to prevent ruminal acidosis (Gonzalez *et al.*, 2012). The higher feed intake of cows fed ML did not result in longer meals. The DMI per meal remained constant independent of the diet which was fed and the DMI. The eating time per kg of DMI was not different between cows fed the ML or CON diet. Beauchemin and Iwaasa (1993) concluded from their study with lucerne and orchard-grass at two stages of maturity at harvest, that the duration of eating is not affected by forages with differences in chemical composition.

Despite no difference was observed between treatments for the eating rate, the cows fed ML showed a significantly higher proportion of eating (37.5%) compared to the control diet (31.9 %) and a lower proportion of rumination (62.7% vs. 68.1 %) of total daily chewing time (Chapter 4). On the one hand this effect is attributed to the tendentially higher feed intake and on the other hand an influence of lucerne silage can be assumed. When cows were fed GL, the proportion of eating time of the total chewing time tended also to be increased at similar feed intake compared to cows fed a TMR based on grass and maize silage ($p<0.1$). Kornfelt (2012), from her studies, concluded that the structural particles from legumes increased the time spent comminuting during eating compared to grass and this can lead to faster passage of the legume particles out of the rumen. The faster particle breakdown of lucerne during eating (Waghorn *et al.*, 1989, Flachowsky *et al.*, 1994) requires less rumination activity to reduce the feed particles to a size able to pass the rumen. Except the allocation of eating and rumination of total chewing time no effect on rumination characteristics was seen when diets contained lucerne silage or grass and maize silage. The higher proportion of eating and lower proportion of rumination on total chewing time for ML and as a trend for GL compared to grass as well as the lower digestibility of lucerne at higher feed intake point to a faster rumen clearance and passage rate, although this was not evaluated in this study.

The concentration and digestibility of nutrients account for the energy density of feed. As shown in Chapter 3 the energy value of lucerne silage (4.6 MJ NEL/kg DM (second cut) and 5.4 MJ NEL/kg DM (first cut)) was much lower compared to maize silage (6.6 to 6.9 MJ NEL/kg DM) or grass silage (6.5. to 6.6 MJ NEL/kg DM). Differences between the energy content of the silages are reflected in the energy concentrations of the TMR. Lucerne silage diluted the energy content of the TMR. The lower milk protein content of cows consuming lucerne diets (GL, ML, GML) compared to the control diet (CON) can also be regarded as a consequence of their lower energy content. This relationship is well described (Sutton and Morant, 1989, Yang and Beauchemin, 2007) and Huhtanen and Hristov (2010) emphasize the importance of energy in protein utilisation. The dilution effect of lucerne on energy content was not compensated despite higher feed intake of diets containing lucerne silage and, finally, resulted in a lower milk yield (Chapter 3). Lower digestibility followed by lower energy content, higher feed intake and lower milk yield (Bulang *et al.*, 2006) or the same milk yield (Ettle *et al.*, 2011) for dairy cows consuming rations containing lucerne silage was also seen in other German studies.

Thereby the feed quality of forages plays in important role. During maturation of the lucerne plant the stem fraction increases whereas the leaf fraction decreases. The content and digestibility of nutrients in the leaf changes just a few during maturation, in contrast all these variables in stems decrease radically. The main content of stems is cell wall material that becomes more lignified and therefore less digestible with age, because lignin is almost indigestible (Nordkvist and Åman, 1986, Südekum *et al.*, 1995a). In a companion study (unpublished) with different lucerne varieties (Oslava, Verko, Franken Neu and Orca) we determined the variation of chemical composition of the third cut 2011 depending on time of harvest. The lucerne varieties were cultivated as single-variety plots of 12 m^2 with four replications in the southwest of Germany (49°58'20.9"N 7°58'48.7"E, 290 above sea level, 690 mm rainfall, 8.8 °C average annual temperature, slightly sandy loam). Each sample for the analysis comprised the same number of plants from the four replications. The results are presented in Table 1. Obviously, a decline in the CP and energy content and an increase of the concentration of DM and fibre fractions occurred. Similar changes in chemical composition at different growth stages were reported by Balde *et al.* (1993) and Homolka *et al.* (2008). Taken together, this illustrates that the maturity stage at harvest plays an important role in the evaluation of forages and finally the feeding of animals.

Table 1 Chemical composition and energy value of four varieties of artificially dried lucerne harvested at five stages of maturity (estimated from near infrared spectroscopy (NIRS) data)

	Lucerne varieties				
Maturity[a]	Late bud	Late bud - early flower	Early flower	Early to late flower	Late flower
Cutting date in 2011	22 July	29 July	3 August	8 August	16 August
Height at harvest	35-40 cm	45-55 cm	55-65 cm	65-80 cm	65-85 cm
Franken Neu					
g DM/kg	178	191	184	197	222
g CP/kg DM	278	267	238	241	238
g CF/kg DM	206	212	214	213	237
g ADFom/kg DM	258	273	268	262	294
g aNDFom/kg DM	401	414	399	386	407
MJ NEL/kg DM	6.13	6.06	5.99	5.98	5.79
Oslava					
g DM/kg	166	180	176	205	217
g CP/kg DM	291	276	244	228	204
g CF/kg DM	189	199	217	221	270
g ADFom/kg DM	246	264	271	268	330
g aNDFom/kg DM	395	401	406	394	436
MJ NEL/kg DM	6.23	6.22	5.96	5.98	5.47
Verko					
g DM/kg	195	204	182	221	220
g CP/kg DM	277	244	253	247	232
g CF/kg DM	206	226	205	217	248
g ADFom/kg DM	259	273	266	267	302
g aNDFom/kg DM	404	438	410	403	419
MJ NEL/kg DM	6.05	6.01	6.04	6.07	5.81
Orca					
g DM/kg	166	183	188	207	217
g CP/kg DM	293	274	252	240	219
g CF/kg DM	181	203	208	214	245
g ADFom/kg DM	235	265	263	268	309
g aNDFom/kg DM	375	409	396	389	415
MJ NEL/kg DM	6.53	6.19	6.07	6.08	5.73

[a] Stage of maturity described according to Kalu and Fick (1983).

DM = dry matter, CP = crude protein, CF = crude fibre, ADFom = acid detergent fibre expressed exclusive residual ash, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, MJ NEL = mega joule for Net Energy Lactation

This data corroborates previous studies that, with an earlier harvest of the lucerne, a higher CP and energy content as well as a higher digestibility could have been obtained. Especially the second cut harvested between early and late flower was harvested too late. It is speculative but possible that earlier harvest would have allowed a higher milk yield. Furthermore the rate of particle breakdown for lucerne stem (higher proportion of lignin and lower proportion of hemicelluloses compared with grasses) and whole-crop ryegrass are similar (Waghorn *et al.*, 1989), whereas fermentation of lucerne leaves is faster compared to grass silage leaves (Bulang *et al.*, 2004). A decrease of the passage rate with advanced maturity stage of lucerne und therefore a less distinct increase of feed intake compared to grass silage is, therefore, plausible.

The milk fat percentage is an important variable that also reflects animal well-being and performance (Mertens, 1997). The milk fat content is strongly dependent on fibre and starch content of the rations (Sutton and Morant, 1989). Zebeli *et al.* (2008) described a positive correlation of the peNDF content of the diet with the milk fat concentration. Diet GL showed the highest peNDF content (31.1% of DM) and lowest starch content (20.4% of DM) and this was reflected in a significantly greater milk fat concentration (4.31%) compared with CON (3.92%) (Chapter 3). The peNDF is the fraction of feed that stimulates chewing activity and the fermentation of carbohydrates lead to an high amount of acid production (Allen, 1997). The peNDF$_{>1.18}$ content (DM basis) in our study varied between 26.3% (CON + ML) and 31.1% (GL). We did not find any differences among diets, neither in total chewing time per unit of DMI nor in rumination time per unit of DMI. The most probable explanation for this observation is that the structural properties affecting chewing activity were sufficient in all treatments, i.e. well above the minimum, such that no influence on chewing activity could be expected. Tafaj *et al.* (2005) determined only a reduced chewing activity if the peNDF content was as low as 19% peNDF of DM. The recommended peNDF content of 31.2% of DM (Zebeli *et al.* 2012) is not based on chewing activity alone, because more variables were incorporated into the model and only a poorly, although positive correlation between peNDF$_{>1.18}$ content in the diet and daily chewing and rumination was detected (Zebeli *et al.*, 2006). The peNDF$_{>8}$ concept better predict chewing and rumination activity than peNDF$_{>1.18}$ (GfE, 2014). For dairy cows, responses of chewing activity and milk fat content are not sensitive enough to estimate the structure supply. The ruminal pH is a crucial variable for normal and stable processes and a diet provides enough structure only when ruminal pH is maintained in 'normal ranges'. Therefore GfE (2014) recommends peNDF values to reach a daily average pH of 6.2 depending on dry matter intake and degradable starch (peNDF$_{>1.18}$) and total starch content in the diet (peNDF$_{>8}$), respectively. The recommended peNDF$_{>8}$ content was not achieved in all diets except diet GL. The higher peNDF$_{>8}$ as well as lower starch content in die GL lead to a sufficient structural supply after GfE (2014) (Chapter 4).

Mertens (1997) as well as Zebeli *et al.* (2006) pointed out the relationship between chewing activity and milk fat concentration. These authors determined a mean chewing time of 744 min/day or 36.1 min/kg of DM and 797 min/day or 36.5 min/kg of DM, respectively, to achieve a milk fat content of 3.6%. In our study, the chewing time per day ranged between 620 and 748 min and the time needed for 1 kg DM ranged between 25.6 and 36.9 min and, thus, was slightly slower than expected (Chapter 4). But we have to take into account the slight underestimation of the eating time that was due to the method of quantification of eating activities. We aggregated the time spent feeding at the trough and did not use the eating time recorded by the chewing halters. Aggregated trough data underestimate the eating time, because jaw movements which occurred during cows changing the feed bins within a meal were not recorded, in contrast eating data from the chewing halter overestimates the eating time, because other activities such as licking, grooming and drinking are often included (Beauchemin *et al.*, 1989). Overall the milk fat content of the selected group of cows as well as the whole herd was higher than 3.6% (up to 3.8%). The underestimation of the eating time can also, in part, explain that the recommended 150 min chewing activity per kg peNDF by Mertens (1997) was not achieved and ranged only between 93 and 140 min. However rumination activity required to maintain healthy ruminal conditions (360 min; Beauchemin *et al.* 1994) was achieved by cows on all diets (395 to 529 min).

Only few differences in chewing activity characteristics between diets were observed (Chapter 4), which can certainly be partly due to the small sample size. Also it seems reasonable that the supply of fibre and rumen-fermentable carbohydrates were suitable to maintain stable ruminal conditions in all diets. This suggests also the milk fat content of greater than 3.8% (Chapter 3). However ruminal pH data as direct indicator for 'normal rumen function' are not available.

CHAPTER 6

CONCLUSION

Lucerne silage in combination with grass and/or maize silage in diets for dairy cows enhance DMI. The highest feed intake was determined with cows fed a combination of maize and lucerne silage. Despite higher feed intake the daily milk yield and ECM yield, respectively as well as milk protein content was lower when cows were fed diets with lucerne silage. One reason for both could be the lower energy content of lucerne. The digestibility of lucerne silage compared with grass silage was lower for OM, ADFom, aNDFom as well as energy. Compared with maize silage, the digestibility of lucerne silage was lower for OM and energy, but higher for CP and (valid only for first-cut lucerne) the fibre fractions ADFom and aNDFom. The delayed harvest especially for the second cut is co-responsible for the decreasing digestibility of nutrients and lead to a lower energy concentration in the silage and lower energy density in the diet. Cows were only able to compensate for the lower energy density of the lucerne diets when they were fed the combination of maize and lucerne silages. The higher energy content in the maize silage (compared to grass silage) and the greater DMI of the ML diet relative to the CON diet allowed them to achieve the same net energy intake per day. Energy efficiency was, however, lower for the ML diet and resulted into lower milk yield compared to CON. It is possible that the lower CP and uCP content of the ML diet induced a slight protein (amino acid) deficiency relative to milk production.

Beside sufficient energy and protein supply, dairy cows need adequate structural fibre in their diet to maintain normal rumen function as measured e.g. by chewing activity, milk fat content or ruminal pH (GfE, 2014). Lucerne silage provides a higher amount of fibre (aNDFom, ADFom) compared to grass or maize silage. Total chewing, eating and rumination time as well as eating activity were not affected by forage combinations. However, cows fed lucerne and maize silages had the highest DMI and spent 37.3% of total chewing time on eating and 62.7% on rumination and therefore spent more time in eating and less time on rumination compared to cows fed grass and maize silages. Despite rations differed with regard to energy, fibre and starch concentrations as well as peNDF, overall effect on feeding behaviour and chewing activity was small and the investigated values are in line with recommended values in the literature (Beauchemin *et al.*, 1994).

This allows the conclusion, that fibre supply in all diets was sufficient. However current recommendation for peNDF content in the diet as indicator for rumen function depending on DMI and total starch content in the diet shows a deficit of structural fibre in all diets except GL (GfE, 2014). It is not possible to provide a conclusive assessment of structure supply for the diets, because data of the ruminal pH values are not available. And a uniform and repeatable guideline for the evaluation of eating behavior and chewing activity is to be striven for.

Lucerne has the potential to improve the protein and fibre supply from domestic farmland to dairy cows if harvested at the optimal stage of maturity. Depending on harvest condition and post-harvest treatment lucerne silage is recommended as component for dairy rations. Maize silage is able to compensate for the lower energy content of lucerne silage and is thus well suited as a further ration component.

REFERENCES (CHAPTER 5 AND 6)

Allen MS 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. Journal of Dairy Science 80, 1447-1462.

Balde AT, Vandersall JH, Erdman RA, Reeves JB and Glenn BP 1993. Effect of stage of maturity of alfalfa and orchardgrass on in-situ dry-matter and crude protein degradability and amino-acid-composition. Animal Feed Science and Technology 44, 29-43.

Beauchemin KA and Iwaasa AD 1993. Eating and ruminating activities of cattle fed alfalfa or orchard-grass harvested at 2 stages of maturity. Canadian Journal of Animal Science 73, 79-88.

Beauchemin KA, Zelin S, Genner D and Buchanan-Smith JG 1989. An automatic system for quantification of eating and ruminating activities of dairy cattle housed in stalls. Journal of Dairy Science 72, 2746-2759.

Beauchemin KA, Farr BI, Rode LM and Schaalje GB 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. Journal of Dairy Science 77, 1326-1339.

Bulang M, Boghun J, Engelhard T, Kluth H and Rodehutscord M 2004. Studies with alfalfa silage as a forage source for high yielding dairy cows. In Proceedings of the Society of Nutrition Physiology 13, p. 242.

Bulang M, Kluth H, Engelhard T, Spilke J and Rodehutscord M 2006. Zum Einsatz von Luzernesilage bei Kühen mit hoher Milchleistung. Journal of Animal Physiology and Animal Nutrition 90, 89-102.

Deswysen AG, Ellis WC and Pond KR 1987. Interrelationships among voluntary intake, eating and ruminating behavior and ruminal motility of heifers fed corn-silage. Journal of Animal Science 64, 835-841.

Deswysen AG, Dutilleul P, Godfrin JP and Ellis WC 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. Journal of Animal Science 71, 2739-2747.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ 2003. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. Journal of Dairy Science 86, 2612-2621.

El Khidir OA and Thomsen KV 1983. Effect of intake on digestibility of plant cell walls and cell contents of complete diets for ruminants. Animal Feed Science and Technology 9, 197-204.

Ettle T, Obermaier A, Weinfurtner S and Spiekers H 2011. Luzernesilage im Austausch gegen Grassilage bei der Milchkuh. In VDLUFA-Schriftreihe, Speyer, pp. 588-594.

Flachowsky G, Grün M, Polzin S and Kronemann H 1994. In sacco dry matter degradability and Ca, Mg and P disappearance from italian ryegrass, alfalfa hay and wheat straw in sheep and goats. Journal of Animal Physiology and Animal Nutrition 71, 57-64.

GfE 2014. (Ausschuss der Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Communications of the committee for requirement standards of the society of nutrition physiology: Evaluation of structural effectiveness of mixed rations for dairy cows – status and perspectives. Proc. Soc. Nutr. Physiol. 23, 165-179.

Gonzalez LA, Manteca X, Calsamiglia S, Schwartzkopf-Genswein KS and Ferret A 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Animal Feed Science and Technology 172, 66-79.

Hoffman PC, Combs DK and Casler MD 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. Journal of Dairy Science 81, 162-168.

Homolka P, Koukolova V, Nemec Z, Mudrik Z, Hucko B and Sales J 2008. Amino acid contents and intestinal digestibility of lucerne in ruminants as influenced by growth stage. Czech Journal of Animal Science 53, 499-505.

Huhtanen P and Hristov AN 2010. Effects of energy and protein supply on milk protein yield responses in dairy cows In Energy and Protein Metabolism and Nutrition: EAAP publication no. 127, Parma, pp. 287-298.

Kalu BA and Fick GW 1983. Morphological stage of development as a predictor of alfalfa herbage quality. Crop Science 23, 1167-1172.

Kornfelt LF 2012. Physical degradation of forage particles and digestion of structural fibre in alfalfa, red and white clover crops fed to cattle. PhD. Univ. of Copenhagen.

Mertens DR 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science 80, 1463-1481.

Nordkvist E and Åman P 1986. Changes during growth in anatomical and chemical composition and in-vitro degradability of lucerne. Journal of the Science of Food and Agriculture 37, 1-7.

Südekum KH, Oestmann A and Stangassinger M 1995a. Zur Rolle von Lignin und phenolischen Monomeren in Futtermitteln für Wiederkäuer II. Einfluss auf die Verdauung pflanzlicher Gerüstsubstanzen. Übersichten zur Tierernährung 23, 229-260.

Südekum KH, Roh H, Brandt M, Rave G and Stangassinger M 1995b. Comparative digestion in cattle and sheep fed wheat silage diets at low and high intakes. Journal of Dairy Science 78, 1498-1511.

Sutton JD and Morant SV 1989. A review of the potential of nutrition to modify milk fat and protein. Livestock Production Science 23, 219-237.

Tafaj M, Maulbetsch A, Zebeli Q, Steingass H and Drochner W 2005. Effects of physically effective fibre concentration of diets consisting of hay and slowly degradable concentrate on chewing activity in mid lactation dairy cows under constant intake level. Archives of Animal Nutrition 59, 313-324.

Waghorn GC, Shelton ID and Thomas VJ 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne L.*) and lucerne (*Medicago sativa L.*) fed to cows during a restricted feeding period. British Journal of Nutrition 61, 409-423.

Yang WZ and Beauchemin KA 2007. Altering physically effective fiber intake through forage proportion and particle length: Digestion and milk production. Journal of Dairy Science 90, 3410-3421.

Zebeli Q, Tafaj M, Steingass H, Metzler B and Drochner W 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of Dairy Science 89, 651-668.

Zebeli Q, Dijkstra J, Tafaj M, Steingass H, Ametaj BN and Drochner W 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. Journal of Dairy Science 91, 2046-2066.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN and Drochner W 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science 95, 1041-1056.

DANKSAGUNG

Mein besonderer Dank gilt Prof. Dr. Georg Dusel, der mein Interesse für die Wissenschaft geweckt hat, mich zur Promotion ermutigt und die Rahmenbedingungen dafür geschaffen hat, mich immer wieder aufgemuntert und stets an mich geglaubt hat. Ohne Dich Georg wäre diese Arbeit nicht entstanden.

Professor Dr. Karl-Heinz Südekum danke ich herzlichst für das Engagement, meine Promotion an der Universität Bonn trotz meines Fachhochschulhintergrundes durchzusetzen und danke für Dein Vertrauen in mich. Vielen Dank für diese Chance der externen/kooperativen Promotion, für Deine fachlichen Unterstützung und für den herzlichen meist telefonischen Austausch.

Vielen Dank Prof. Dr. Büscher für die Übernahme des Zweitgutachtens.

Dem Dienstleistungszentrum ländlicher Raum Westpfalz gilt mein Dank für die finanzielle Unterstützung bei der Versuchsdurchführung sowie Herrn Dr. Romberg für die Unterstützung bei der Datenauswertung.

Der Fütterungsversuch fand an der Lehr- und Versuchsanstalt für Viehhaltung, Hofgut Neumühle statt. Vielen Dank an Herrn Dr. Landfried, an die Mitarbeiter der Lehrwerkstätte Milchviehhaltung für die praktische Versuchsdurchführung und an Dr. Christian Koch für Dein stets offenes Ohr.

Vielen Dank an Herrn Dr. Steingaß für die Unterstützung v.a. beim Thema Kauaktivität.

Ein großes Dankeschön an meine Freunde und Kollegen der Technischen Hochschule Bingen für viele schöne gemeinsame Jahre und an Katja, Elmar, Freddy, Benjamin, Moni, Harald und an die Mitarbeiter des St. Wendelinhofes für eure tatkräftige Unterstützung bei den Anbauversuchen, Stoffwechselstudien und dem Fütterungsversuch.

Ganz besonders möchte ich meiner Familie danken, meinem Vater, der mich meinen eigenen Weg gehen lässt, mich immer unterstützt hat und immer an mich glaubt, meinem Mann und meinem Sohn, deren Liebe meine Motivation ist -ich liebe euch so sehr- und meinen Schwiegereltern, die bei jedem Engpass stets da sind und uns unterstützen.

Praktika und berufliche Laufbahn

2005	Landwirtschaftliches Praktikum bei der Lehr- und Versuchsanstalt für Viehhaltung Hofgut Neumühle; zugleich Betreuung der im Fütterungsversuch eingestallten Kälber im Rahmen der Diplomarbeit; 2 Monate
2005	Vertretung als landwirtschaftliche Fachkraft an der Lehr- und Versuchsanstalt für Viehhaltung Hofgut Neumühle, 1 Monat
Seit 2006	Assistentin/wissenschaftliche Mitarbeiterin im Bereich Tierernährung bei Prof. Dr. Georg Dusel an der Technischen Hochschule Bingen; angestellt am Institut für Innovation, Transfer und Beratung gGmbH, Bingen (An-Institut)
2007	Mutterschaftsvertretung (befristete Teilzeitanstellung) am Dienstleistungszentrum Ländlicher Raum (DLR) in Bitburg als Beraterin für Schweinehalter, 9 Monate
2008	Projektbetreuung (Rinderbeweidungsstudie) im Rahmen des Masterstudiums an der Forschungsstation Embrapa Amazônia Oriental in Belém, Brasilien (Nordost-Amazonien), 1 Monat
2012 - 2013	Lehrbeauftragte an der Fachhochschule Bingen Studiengang Agrarwirtschaft in Vertretung für Prof. Dr. Georg Dusel, für die Fächer Tierernährung, Grundlagen der Tierproduktion (Bereich Tierernährung), Tierhygiene und Tiergesundheit, Qualität tierischer Produkte und Grundlagen landwirtschaftlicher Tierhaltung, 2 Semester
2014	1 Jahr Elternzeit

CONFERENCES AND PUBLICATIONS

124. VDLUFA-Kongress, 2012

J. Trautwein, K.-H. Südekum, H. Steingaß, G. Dusel, C. Koch, F.-J. Romberg, K. Landfried

Vergleichende Bewertung von Milchviehrationen auf Basis von Luzerne-, Gras- und Maissilagen: Futteraufnahme, Milchleistung und Milchinhaltsstoffe (oral presentation)

Tagungsband Forum angewandte Forschung 2013, 82-85

J. Trautwein, G. Dusel, C. Koch und K.-H. Südekum

Futterrelevante Inhaltsstoffe von Luzernesorten in Abhängigkeit von Standort und Schnittzeitpunkt (poster presentation)

125. VDLUFA-Kongress, 2013

B. Losand, H.-J. Alert, Y. Arrigo. T. Ettle, H. Kluth, C. Koch, A. Menke, A. Priepke, O. Steinhöfel, J. Trautwein

Energiebestimmung von Raufuttermitteln aus kleinkörnigen Leguminosen

125. VDLUFA-Kongress, 2013

C. Böttger, K. Kolodzeyzik, J. Trautwein, K.-H. Südekum

In vitro-Untersuchungen zur ruminalen Abbaukinetik von Luzerne in Abhängigkeit von Sorte, Standort und Schnitt

GfE-Tagung 2017, Proc. Soc. Nutr. Physiol., volume 26, 129

J. Trautwein, K.-H. Südekum, G. Dusel, H. Steingaß, F.-J. Romberg, C. Koch, K. Landfried

Comparative evaluation of lucerne, grass and maize silage-based rations for lactating dairy cows: Feed intake, milk yield and milk composition (poster presentation)

GfE-Tagung 2017, Proc. Soc. Nutr. Physiol., volume 26, 194-202

Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Substanz von Grobfutterleguminosen für Wiederkäuer

Tagungsband Forum angewandte Forschung 2017, 50-53

J. Trautwein, K.-H. Südekum, G. Dusel, H. Steingaß, F.-J. Romberg, C. Koch

Vergleichende Bewertung von Luzerne-, Gras- und Maissilage basierten Rationen für Milchkühe: Futteraufnahme, Milchleistung und Milchzusammensetzung (oral presentation)