Effects of a ration change from a silage and concentrate- to a pasture-based ration on the production, health and rumen physiology of dairy cows
University of Veterinary Medicine Hannover

Institute for Physiology

“Effects of a ration change from a silage and concentrate- to a pasture-based ration on the production, health and rumen physiology of dairy cows”

THESIS

Submitted in partial fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

(PhD)

awarded by the University of Veterinary Medicine Hannover

by

Melanie Schären

Basel

Hannover, Germany 2016
Supervisor: Prof. Dr. med. vet. Gerhard Breves

Prof. Dr. Dr. Sven Dänicke

Supervision Group: Prof. Dr. med. vet. Gerhard Breves

Prof. Dr. Dr. Sven Dänicke

Prof. Dr. Johannes Isselstein

Prof. Dr. med. vet. Jürgen Rehage

1st Evaluation: Prof. Dr. med. vet. Gerhard Breves (Department of Physiology, University of Veterinary Medicine Hannover, Germany)

Prof. Dr. Dr. Sven Dänicke (Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany)

Prof. Dr. Johannes Isselstein (Department of Crop Science, Grassland-Science, Georg-August University Göttingen, Germany)

Prof. Dr. Jürgen Rehage (Clinic for Cattle, University of Veterinary Medicine Hannover, Germany)

2nd Evaluation: Prof. Dr. Karl-Heinz Südekum (Institute of Animal Science, Animal Nutrition, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany)

Date of final exam: 04.04.2016
Parts of the thesis have been published previously in: Journal of Dairy Science

Schären et al. 2016, The Effects of a Ration Change from a Total Mixed Ration to Pasture on Health and Production of Dairy Cows.


Sponsorship: This thesis has been sponsored by the “Niedersächsisches Ministerium für Wissenschaft und Kultur” in the scope of the research project “Systemanalyse-Milch”.
To my family

„Man liebt das, wofür man sich müht, und man müht sich für das, was man liebt.“

Erich Fromm (1900 – 1980)
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BCS</td>
<td>Body Condition Score</td>
</tr>
<tr>
<td>BHBA</td>
<td>Beta-Hydroxybutyrate</td>
</tr>
<tr>
<td>CLA</td>
<td>Conjugated Linoleic Acid</td>
</tr>
<tr>
<td>CP</td>
<td>Dietary Crude Protein</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty Acid</td>
</tr>
<tr>
<td>fOM</td>
<td>fermentable Organic Matter</td>
</tr>
<tr>
<td>IOFC</td>
<td>Income Over Feed Cost</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolyaccharide</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral Detergent Fibre</td>
</tr>
<tr>
<td>NEB</td>
<td>Negative Energy Balance</td>
</tr>
<tr>
<td>NH₃</td>
<td>Ammonia</td>
</tr>
<tr>
<td>NPN</td>
<td>Non-Protein-Nitrogen</td>
</tr>
<tr>
<td>NUE Milk</td>
<td>The percentage of N intake secreted as milk N</td>
</tr>
<tr>
<td>OM</td>
<td>Organic Matter</td>
</tr>
<tr>
<td>PUFA</td>
<td>Poly Unsaturated Fatty Acid</td>
</tr>
<tr>
<td>RDP</td>
<td>Rumen Degradable Protein</td>
</tr>
<tr>
<td>RNB</td>
<td>Rumen Nitrogen Balance</td>
</tr>
<tr>
<td>RPM</td>
<td>Rising Plate Meter</td>
</tr>
<tr>
<td>SARA</td>
<td>Subacute Ruminal Acidosis</td>
</tr>
<tr>
<td>TMR</td>
<td>Total Mixed Ration</td>
</tr>
<tr>
<td>UDP</td>
<td>Rumen Undegradable Protein</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile Fatty Acid</td>
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<tr>
<td>WSC</td>
<td>Water Soluble Carbohydrate</td>
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Summary

Melanie Schären, “Effects of a ration change from a silage and concentrate- to a pasture-based ration on the production, health and rumen physiology of dairy cows”

In pasture-based dairy production systems, dairy cows often receive a silage- and concentrate-based ration (= TMR, total mixed ration) during wintertime and are then gradually introduced to fresh herbage in spring. The present study aimed to investigate how the transition to this new nutritional situation influenced different production and health indicators as well as rumen physiology. A 10-week trial (wk 1-10) was performed in spring 2014, which included 60 dairy cows of the German Holstein breed (166 ± 23 days in milk, 23.5 ± 3.7 kg milk/d; means ± SD) of which 10 were rumen- and duodenum-fistulated. The cows were divided into a pasture- and a confinement group (PG and CG, n = 29 and 31, each group contained 5 fistulated animals). The CG stayed on a TMR-based ration (35% corn silage, 35% grass silage, 30% concentrate; DM basis), while the PG was gradually transitioned from a TMR to a pasture-based ration (wk 1: TMR-only, wk 2: 3 h/d on pasture & TMR indoors, wk 3 & 4: 12 h/d on pasture & TMR indoors, wk 5-10: pasture-only). A continuous grazing system was implemented on a ryegrass dominated pasture and temperature humidity indices (THI) were assessed based on a continuous recording of temperature and humidity; indoors as well as outdoors.

Dry matter intake (DMI) indoors was measured using automatic weighing throughs. To estimate the DMI outdoors the n-alkane technique (in wk 7 and wk 9) and exclosure cages (in wk 5-6, wk 7-8 and wk 9-10) were used. Milk production and body weight (BW) were measured twice a day at milkings. Samples for milk components were collected twice a week and body condition score (BCS) was assessed in 14-d intervals. Urine samples were collected once a week and analyzed for creatinine and purine derivatives concentrations to measure alterations in nitrogen excretion and microbial protein production. To assess possible negative effects of this ration change on metabolic and liver health different clinical chemistry measures and complete blood counts were measured weekly. To observe changes in rumen fermentation patterns pH, volatile fatty acids (VFA), NH₃-N and lipopolysaccharide (LPS) concentrations were measured in rumen fluid samples collected medially and ventrally on a weekly basis. To evaluate a possible subacute ruminal acidosis (SARA) risk ruminal pH was additionally recorded weekly during 1-4 consecutive days using continuous ruminal pH measuring devices. In wk 1, wk 5 and wk 10 the total rumen content was weighed and the DM and non-DM content determined. Subsequently, rumen papillae were collected at three locations to determine the mean papillae area and for a histopathological evaluation. Additionally, a VFA absorption test (VFA-AT) was performed.
DMI from TMR, milk production, body weight (BW) and BCS decreased as soon as the PG had partial access to pasture. Milk production and BW decreased even further in the first week on a full grazing ration, but thereafter BW increased again and milk production stabilized. DMI estimation using the n-alkane method in wk 7 and wk 9 revealed an increase in DMI from pasture between the two time points and indicates an adaptation of grazing behavior and metabolism over several weeks. Increased serum β-hydroxybutyrate and nonesterified fatty acids concentrations at several time points as well as a continuous BCS decrease during the whole course of the trial indicate an energy deficit in the PG.

These alterations in production and metabolic parameters in the PG were also reflected in various rumen variables. Mean rumen pH and molar acetate proportions decreased, and molar butyrate proportions increased continuously over the course of the trial, which can most likely be ascribed to an increased intake of rapidly fermentable carbohydrates. During the first weeks on a full grazing ration (wk 5-7) variation of rumen pH decreased and in wk 5 a lower rumen content, papillae surface area and potential for VFA absorption was observed. In wk 8-10 variation of rumen pH and total VFA concentrations increased again, and acetate/propionate ratio decreased. In wk 10 rumen content, papillae area and VFA absorption characteristics similar to initial levels were observed. Although continuous rumen pH assessments and LPS concentrations did not reveal an increased risk for subacute rumen acidosis (SARA) during the adaptation period, histopathology of rumen papillae and VFA absorption potential indicate a possible risk for rumen health. An increased risk for SARA was observed in wk 9 and wk 10 in the PG, but rumen LPS concentrations and histopathology were not adversely affected.

As expected an increase in N excretion occurred in the PG, as indicated by an increase in serum and milk urea concentrations as well as an increase in the urine total N to creatinine ratio. However, no biological relevant changes were observed for serum albumin, total protein, cholesterol, aspartate transaminase, γ-glutamyltransferase and glutamate dehydrogenase concentrations as well as for white and red blood cell counts. It was therefore concluded that higher metabolic N concentrations did not have any negative transitional impact on the liver.

During wk 5 and wk 7-8 mild heat stress was observed in both groups and metabolic variables were consequently altered. Serum glucose concentrations decreased and urine total N/creatinine and purine derivatives/creatinine ratios increased. Concurrently rumen LPS concentrations increased in both groups.

Results of the present study suggest that during the transition from a TMR to a pasture-based ration an initial decrease in DMI occurred accompanied by possible negative effects on rumen
physiology. After a behavioral and metabolic adaption DMI and ruminal fermentation rate increased again with no adverse effects on rumen morphology and VFA absorption capacity, although rumen pH after adaptation to pasture indicated increased risk of SARA.
**Zusammenfassung**

Melanie Schären, “Einfluss eines Rationswechsels von einer Silage und Kraftfutter basierten auf eine Weide basierte Fütterung auf die Leistung, Gesundheit und Pansenphysiologie von Milchkühen”.

Weidewirtschaft betreibende Milchviehbetriebe füttern während der Wintermonate meistens eine Silage und Kraftfutter basierte Mischration (= TMR, totale Mischration). Im Frühjahr, nach Beginn der Vegetationsperiode, erfolgt in der Regel über mehrere Wochen ein schrittweiser Übergang von der Stall- auf die Weidefütterung. Das Ziel dieser Untersuchung war die Erforschung des Einflusses dieser Umstellung auf Leistungs- und Gesundheitsparameter sowie auf die Pansenphysiologie. Dazu wurde im Frühjahr 2014 ein zehnwöchiger Versuch (w1-10) mit 60 Milchkühen der Rasse Deutsche Holstein durchgeführt, wovon 10 Tiere pansen- und duodenumfistuliert waren. Die Tiere befanden sich zu Beginn des Versuches im Mittel 166 ± 23 Tage in Milch und wiesen eine Leistung von 23,5 ± 3,7 kg Milch pro Tag (Mittelwert ± Standardabweichung) auf. Die Tiere wurden in eine Weide- und eine Stallgruppe eingeteilt (WG und SG, n = 29 und 31, jede Gruppe beinhaltete fünf fistulierte Tiere). Die SG erhielt während des gesamten Versuchszeitraumes eine TMR (35 % Maissilage, 35 % Grassilage, 30 % Kraftfutter; auf Trockenmassebasis) während die WG kontinuierlich während mehrerer Wochen auf die Weidefütterung umgestellt wurde (w1: nur TMR, w2: 3 Std. pro Tag Zugang zur Weide plus TMR im Stall, w3 und 4: 12 Std. pro Tag Zugang zur Weide plus TMR im Stall, w5-10: nur Weidefütterung plus 1,75 kg Kraftfutter pro Tag). Als Weidesystem wurde eine Kurzrasenweide auf zwei Weidelgras betonten Flächen à 6 ha verwendet. Die Temperatur und Luftfeuchtigkeit wurden sowohl im Stall als auch im Bereich der Weideflächen kontinuierlich erfasst.

Die tägliche T-Aufnahme wurde im Stall mittels automatischer Wiegetröge erfasst. Auf der Weide wurden sowohl die n-Alkan Methode (w7 und w9) als auch Weidekörbe (in w5-6, w7-8 und w9-10) zur Schätzung der T-Aufnahme eingesetzt. Die Milchleistung und die Körpermaße (KM) wurden zweimal täglich nach dem Melken erfasst. Milchproben zur Bestimmung der Milchinhaltsstoffe wurden zweimal je Woche gesammelt und die Körperkondition (BCS) wurde in 14-tägigen Intervallen ermittelt. Um Veränderungen in der Stickstoffausscheidung und der mikrobiellen Proteinsynthese aufzuzeigen wurden wöchentlich Harnproben zur Bestimmung der totalen Stickstoff- (total-N), Kreatinin- und Purinderivatkonzentration gesammelt. Um mögliche negative Effekte auf die Tiergesundheit, insbesondere auf den Leberstoffwechsel, zu erfassen, wurden wöchentlich Blutproben genommen und sowohl verschiedene klinisch chemische Parameter als auch das weiße und rote Blutbild bestimmt. Um Veränderungen im


In w5 und w7-8 wurde leichter Hitzestress in beiden Gruppen beobachtet, was zu einer Veränderung verschiedener metaboler Parameter in diesem Zeitraum führte. Die Serumglukosekonzentrationen und die Urin-N/Kreatinin und Purinderivate/Kreatinin Verhältnisse wurden weiter. Zeitgleich erhöhten sich die LPS-Konzentrationen im Pansen.

In der vorliegenden Studie konnte gezeigt werden, dass bei einem Rationswechsel von einer TMR auf eine Weide basierte Fütterung die T-Aufnahme vorerst abgenommen hat und dies möglicherweise einen negativen Einfluss auf die Pansengesundheit hatte. Danach hat eine Anpassung des Tierverhaltens und des Metabolismus stattgefunden wodurch es über einen Zeitraum von mehreren Wochen zu einem erneuten Anstieg in der T-Aufnahme kam. Dies hatte eine erhöhte Pansenfermentationsrate zur Folge was zu einem erhöhtem SARA Risiko führte, ohne jedoch die Pansenzottenmorphologie oder Absorptionskapazität negativ zu beeinflussen.
1. Background

1.1. General introduction

Due to different reasons a dairy farmer may choose to let his cows graze during the whole day (unrestricted stocking), only part of the day (restricted stocking) or he chooses a zero-grazing strategy where the cows are either fed with fresh cut grass or ensiled feed [260, 300]. Depending on the geographical location, available facilities, farm size, meteorological conditions, size and quality of the farmland available and many other reasons, one option may be more suitable than another [261]. During the last century a notable shift towards the confinement housing of dairy cows in European countries and North America has taken place [245]. However, in a number of regions in the world, for example New Zealand [5, 115, 262, 316] and Ireland [136, 208, 243, 245], pasture based dairy farming still forms the main part of the dairy business.

In Germany a nationwide survey of the federal office of statistics in 2010 showed that 55% of the dairy farms at that point were implementing a non-grazing system, representing 58% of dairy cows in the country. In the 45% of dairy farms, that apply some sort of grazing strategy, the cows had daily access to pasture during an average of 13 hours per day and 24 weeks per year [26].

The causes for this trend towards a non-grazing approach for dairy production systems are not only the reasons mentioned above, but also the evolution of know-how regarding precision feeding and genetic advances. During the last three decades ensiling techniques, know-how of total mixed ration (TMR) feeding and balancing rations has evolved considerably. Due to this co-evolution of precision feeding and genetic potential, exceptionally high milk yields are possible [31, 124, 237, 256, 295, 321].

In grazing systems grass is a limiting factor for milk production. Constant changes in nutrients due to weather influences and growth, the high rumen degradable protein (RDP) and fast fermentable carbohydrate content of grass make balancing a pasture-based ration very difficult [15, 147, 150, 223, 286, 300]. Even under perfect management conditions, a grazing dairy cow, which does not receive any concentrate supplementation, can only produce up to 30 kg milk/day [13, 15, 79, 289]. Comparing this to productions of 40 kg milk/cow/day and more in a TMR-based confinement system, non-grazing operations seem more attractive [13]. Especially at times where prices for energy and concentrate feed were low, income over feed costs (IOFC) was high in high producing dairy cows [126, 293]. Then again, since a few years, not only feed
but also energy costs rise and milk prices are volatile [124]. Therefore, the cost effectiveness of grazing operations has received some attention again [19, 62, 63, 79].

But not only economical and management aspects are part of the considerations. The consumer within our society holds the idea of a dairy cow, grazing and wandering a green pasture. The trend towards non-grazing systems and greater animal welfare awareness during the past few years have led to debates if a cow needs to graze or not. Especially diseases related to confinement housing systems and the idea of natural behavior have drawn the public’s attention [6, 180, 306]. But also the fact that milk from pasture fed dairy cows is perceived as being healthier has raised the demand for pasture derived milk products [10, 62, 72, 106, 135].

On this account the state of Lower Saxony has launched and financed a research project called: “Produktion von Milch in Weide- oder Stallhaltung: Eine Systemanalyse”- “The production of milk in pasture- or confinement-based systems: a production system analysis”, in which the production of milk in confinement versus pasture based systems is being compared. The project consists of 8 different research modules, each investigating a different aspect of dairy farming and/or animal health within the two systems. The goal of the research project is to gather data concerning sustainability, animal welfare and economical aspects to form a valid information source to advise government institutions and the dairy industry. The data presented in this thesis has been generated within the scope of a research project being part of this large-scale project and focuses on the effects of pasture and ensiled feed on the health, production and rumen fermentation of dairy cows.

In grazing systems in temperate climate zones dairy cows are often fed with a silage- and concentrate-based diet (TMR) during winter and are gradually introduced to a pasture-based diet in spring. It is a general understanding that animal behavior and metabolism as well as the rumen microbiota need to adapt to this new nutritional situation, but only little is known on the impact and the length of this adaption period. The aim of the study presented here was therefore to investigate the effects of a ration change from a TMR to a pasture-based ration on dairy cow production, health and rumen physiology.

Since only little data exists on the impact of such a ration change, the comparative aspects of the two systems will be first illustrated in a general overview, followed by a chapter focusing on the possible impacts of a ration change.
1.2. Comparative aspects of pasture and confinement systems

1.2.1. Production and efficiency

In a confinement TMR-based system generally a highly balanced ration is fed and energy expenditure for maintenance of the cow is minimized by short walking distances to the milking parlor and feed bunk, an easily ingestible ration, and a maximized laying-time by the provision of comfortable bedding [93, 152]. In pasture-based systems however grass needs to be harvested by the cow itself. This causes a general lower dry matter intake (DMI) and higher energy expenditure due to grazing and walking [150, 220, 252]. Additionally, the nutrients in grass aren’t as well balanced as compared to a TMR. Pasture-based rations generally exhibit a much higher crude protein and lower metabolisable energy content causing a lower efficiency in nitrogen (N) usage and energy availability [150]. These three aspects (with the lower DMI being the most important one) form the major reasons why milk production in pasture-based systems is generally lower compared to TMR-based systems [13, 15, 63, 73, 147, 150, 289].

1.2.1.1. Dry matter intake

Factors influencing DMI

Kolver and Muller [147] reported that dairy cows fed a TMR consumed 4.5 kg DM/d more than cows on pasture and produced 44.1 kg, compared to 29.6 kg milk per day. Reason for this lower DMI are physical constraints (grazing and walking), rate of removal from the rumen through degradation and passage, and higher water consumption associated with pasture [289]. Whereas in a confinement system the TMR composition, bunk space, time management and climate conditions form the major and in comparison relatively easy controllable influencing factors on DMI, on pasture DMI is influenced by a range of additional and sometimes also more volatile parameters [63, 93, 115, 152, 166, 254, 306].

Environmental factors such as temperature and humidity do not only influence the cow’s metabolism [90, 166], but also the nutritive value of the plant resulting in daily, weekly and seasonal changes in chemical composition of the pasture [63, 115, 190, 223, 224, 268, 300]. For example, the water soluble carbohydrate (WSC) content of grass is subjected to diurnal variation, with lowest concentrations in the morning and highest in the afternoon [3, 268]. This explains why the highest grazing activity and intake can be observed at dusk [3, 95, 254, 288]. Furthermore, spring grass compared to a pasture in autumn is generally lower in fiber and higher in protein and WSC content rendering it much tastier, fast fermentable, and also nutritive more valuable [223, 224, 300]. This is the reason why generally in pasture-based systems a seasonal
calving pattern is favored, synchronizing the peak milk production with the highest protein and WSC content of the grass in spring [62, 115].

Aside from climate, also other factors such as soil quality and sward species influence the sward structure and thereby the DMI and productivity of dairy cows [63, 206, 270, 289]. In the Alps for example very short but species rich swards dominate, resulting in very flavorful milk but low milk production [74, 159, 179, 296]. Whereas in regions with mild climate and a lot of rainfall such as New Zealand and Ireland easy digestible and highly nutritive perennial ryegrass species can be cultivated almost all year round [62, 115, 270].

As in confinement also in pasture-based dairy systems management factors form the most important tool to maximize DMI and efficiency [63]. There are different grazing systems such as rotational and continuous stocking and their suitability and cost-effectiveness mainly depends on geographical and labor aspects [62, 63, 186]. Furthermore, sward height, herbage allowance, and stocking and grazing intensity are the main management depending factors to maximize DMI and milk production per hectare grassland [63, 86, 138, 155, 183, 186, 193, 228, 230, 300]. Another way to increase DMI and to better synchronize protein and energy availability in the rumen leading to a higher milk production and N efficiency is the additional feeding of carbohydrates (further discussed in the chapter 1.1.2) [15, 186, 300].

During the last centuries the Holstein-Friesian has established itself as the number one breed in high yielding confinement dairy systems due to its high milk production and efficiency per animal [294]. However, in grazing systems the cost-effectiveness is not so much dependent on the total milk production per animal but rather on the milk yield per hectare grassland [62, 186]. It is therefore that rather smaller animals with lower maintenance requirements and high DMI capacity from pasture are favored [115, 207]. There have been studies investigating whether certain strains or breeds are more suitable for and efficient in a grazing system than others [50, 100, 118, 149, 236, 240, 241, 291, 304]. For example Thomet et al. (2010) and Piccand et al. (2013) showed that the New Zealand Holstein Friesian breed has a higher fat and protein content at similar milk yield compared to Swiss Breeds (Swiss Holstein Friesian, Swiss Brown and Fleckvieh) [236, 291]. And Prendiville et al. (2009 and 2010) showed that Jerseys are more efficient than Holstein Friesian dairy cows at pasture [240, 241].

*Estimation of DMI in pasture-based systems*

DMI in confinement systems can be estimated (for research purposes) relatively easy using electronic identification and weighing systems [52, 265]. Estimating DMI in grazing animals is much more difficult. Studies have been conducted recording bite weight or diet selection using
oesophageally fistulated animals. But this technique is laborious, expensive, compromises animal welfare and may also result in abnormal animal behavior [254]. To estimate DMI over a short period weighing of turves and artificially constructed sward or the animal before and after grazing has been implemented. But both methods exhibit constraints concerning representativeness of normal foraging behavior [157, 226, 254]. Another technique to estimate DMI on pasture is the use of prediction equations that account for different factors such as season, liveweight, body condition, milk production, supplementation with concentrate, the pre-grazing herbage mass of the sward, daily herbage allowance, fiber content of the pasture, etc. This is a valid approach for estimating DMI especially under non-experimental conditions, since several studies showed that results are comparable with actual DMI [48, 214, 269]. Nevertheless, prediction equations are not an animal-based measurement and their use for research purposes should therefore be regarded as critical. A method that has been often used is DMI estimation by cutting and weighing of the pre- and post-grazing pasture mass, using either exclosure cages (Figure 1), a sward height or rising plate meter (RPM, Figure 2), or by just cutting and weighing representative areas before and after grazing activity [41, 113]. The method is easy, fast, does not require labor-intensive or expensive laboratory analyses and gives reliable results if grazing period is short and stocking rate is high [55, 178]. However, also this method is susceptible to bias since it does not account for selective grazing by the animal, animal influence on plant growth and senescence by urinary and dung restitution, and specific defoliation [55, 254]. Further, it cannot be used to obtain the individual intakes of animals in a group [171, 254, 269].

To estimate individual DMI over a period of time indigestible markers techniques have been developed [254]. Nowadays, the most commonly used and well established technique is the n-alkane method [41, 67-69, 76, 172, 178, 185, 244, 258, 269]. N-alkanes are plant wax compounds with the general chemical formula C_nH_{2n+2}. N-alkanes with an uneven chain length are frequent in legumes of temperate climate zones and are therefore ideal internal markers for digestibility estimation [69]. N-alkanes with an even chain length are synthesized and used as external markers to calculate the fecal output [69]. During an experimental period, the external marker is either administered twice a day under the form of a bolus [43, 189, 213, 241, 320], mixed into a portion of concentrate [269] or a rumen controlled-release device is used (single administration) [68, 199]. After seven days a steady-state in marker excretion is achieved and for seven consecutive days manure and pasture sample are collected twice a day. Thereafter, n-alkane concentration is determined in grass and manure samples by gas chromatography and extrapolated to the corresponding DMI. Mayes and Doves published an exact protocol for this
method in 2006 [69]. Even though, the n-alkane method is considered being the most precise method, measuring the DMI of grazing cows, it is still associated with sources of variation [269]. For example, the diurnal pattern of excretion, administration route of the marker, herbage sampling and sward composition, as well as analysis aspects such as drying of samples (heat vs. freeze drying) have been referenced as possible sources of error [269].

**Figure 1:** Harvest of herbage mass grown underneath an exclosure cage.

**Figure 2:** Measuring herbage height with a rising plate meter (RPM, source: Haus Riswick).
1.2.1.2. Nutrient composition of rations
Immediately after harvesting of grass, plant respiration still goes on and during this aerobic phase, sucrose and fructans are rapidly hydrolyzed to glucose and fructose. Also lipolysis and proteolysis take place in that stage, reducing the nutritional value of the feed. As soon as feed is ensiled and an anaerobic environment is created, a rapid fermentation of WSC by lactic acid bacteria takes place. The production of lactic acid decreases the pH, causing a conservation of the feed by inhibiting the growth of undesirable microorganisms such as Clostridia or Enterobacteria. Due to microbial activity the content of certain amino acids, especially arginine, decreases. Others on the other hand, such as alanine, increase [188]. The breakdown of amino acids causes a rise in non-protein-nitrogen (NPN), leading to a decreased quality of the N compounds available [87]. The fermentation of WSC causes a relative increase in slow fermentable carbohydrates. Consequently the Neutral Detergent Fibre (NDF) content of the ration rises and the NEL content decreases [188]. Comparing the nutrient composition (DLG Nutrient Composition Tables [66]) of perennial ryegrass (Lolium perenne), fresh and ensiled grass differ in following aspects: the NEL decreases from 7.12 to 6.88 MJ/kg and the dietary crude protein (CP) content drops from 19.7 to 17.7%. Crude fiber content increases from 19.7% to 21.4%. Due to microbial fermentation, the WSC content of grasses drops from >10% down to ~2% during ensiling [66].

During the last decades elaborate nutritional studies have made the development of dietary requirements, models and equations which simulate digestion and nutrient use of dairy cows very precisely, and optimal ration compilation possible [111, 112, 124]. For the most optimal digestion and nutrient use of feed in the rumen, sufficient protein and energy sources need to be available at the same time. This model of a balanced nutrient supply to rumen microorganisms is called rumen “synchronization theory” [78, 267, 300]. The changes in chemical composition that occur during ensiling of grass, therefore cause a faster fermentation of fresh compared to ensiled grass in the rumen [87, 286]. To counterbalance this increased imbalance in energy and protein availability grass silage is often fed not as a single component in confinement systems but as part of a TMR. A TMR mostly consists of a high quality protein source (such as grass or legume silage and soybean meal), which is combined with an energy source (such as corn silage and grains), sources of fiber (such as straw and hey), and smaller amounts of different products such as mineral feed and different additives [212, 234]. By combining protein and energy sources that allow an optimal fermentation rate and nutrient supply microbial synthesis can be maximized [78]. To further enhance nutrient efficiency ration components can be used that are rich in rumen undegradable protein (UDP), thereby enhancing the amount of protein that
is available at the small intestines for resorption [78]. With an optimal protein quality, proportion of RDP and UDP, and starch and fiber degradability in a ration nutrient supply is maximized and high milk yields are possible [78, 85, 212, 238].

High quality pastures generally exhibit a high protein content and the main carbohydrate sources are cell wall and WSC. Fresh grass is therefore fast fermentable, high in RDP and exhibits a relatively high ratio between rumen-available CP and rumen-available organic matter (OM) [150, 286, 300]. These nutritional characteristics and the lower DMI are the main reasons for a lower metabolisable energy availability and a maximal milk production of approximately 30 kg milk/day in pasture compared to confinement TMR-based systems [78, 147]. Therefore, to optimize rumen synchronization dairy cows on pasture are often supplemented with an energy source [13, 15, 112, 121, 144, 146, 147, 150, 163, 262-264, 308, 315]. In 2003 Bargo et al. reviewed the different studies conducted to evaluate the benefit of different supplements and supplementing methods of cows on pasture [15]. In addition, in the same year, Schroeder et al. reviewed the effects of fat supplementation on milk production and composition by dairy cows on pasture [262]. Different studies have also shown that higher milk yields are achieved by supplementing grazing dairy cows with UDP rich supplements [15, 194]. In summary it can be concluded that the amount and kind of supplement that should be used depends on the pasture quality, grazing system, milk production and supplement availability [15, 300]. Further, also the application of perennial ryegrass species high in WSC to increase metabolisable energy availability has been tested, but results on influence on animal production and N usage have been inconclusive so far [133, 164, 202, 286, 287, 290].

1.2.1.3. Nitrogen efficiency

The theory of rumen synchronization also implicates that whenever an effluent amount of N is available in the rumen (= positive rumen N balance (RNB)) it is converted into ammonia (NH₃) [162]. The NH₃ overflow then needs to be detoxified in the liver through conversion into urea and is either recycled in the rumino-hepatic cycle or excreted [158]. This occurs at an energy cost of 30 kJ ME/g N and needs to be considered when calculating rations [133, 150, 221]. There are several studies indicating that NH₃ in high metabolic concentrations has toxic effects and incriminates different organs in their function [133, 221, 232, 242].

But N overflow is not only a strain to the organism of the cow, but also represents an economical loss and an environmental risk. Nitrogen overflow from animal systems has notably effects on the nitrate content of water resources and on the emission of nitrogenous gases into the
atmosphere [221, 231, 239]. It is therefore of major interest to optimize N utilization in animal production [285].

N efficiency of dairy cows is expressed as the percentage of N intake secreted as milk N (NUE-milk) [133, 235, 239]. Several studies have shown that N efficiency of grazing dairy cows, solely receiving nutrients from grass, is much lower than from cows in confinement systems, where a well-balanced TMR is fed. The NUE-milk of grazing dairy cows ranges between 13-31%, compared to the NUE-milk of TMR fed dairy cows of 40-45% [58, 133, 239, 302].

Research has shown that the N efficiency of pasture based systems mainly depends on fertilization quantity, pasture management and adequate nutrient supplementation of grazing dairy cows [13, 15, 112, 132, 133, 286, 299, 300]. The CP content of grass is the most important factor influencing N efficiency of dairy cows, since a CP content >16% leads to higher urinary and milk N excretion [32, 78, 112, 122, 133, 286, 302]. But even with the most optimal management system known, N efficiency of grazing dairy cows stays significantly lower compared to TMR fed cows [133].

However, this topic cannot only be discussed on a single cow level but also needs to be evaluated on a farm or system level [217, 239]. In pasture-based systems the cow harvests feed and for a large part also performs grassland fertilization by herself (= land-based livestock system). Therefore, the only N leaving the system in the form of animal product (milk and meat) and direct environmental losses (NH₃ volatilization, denitrification, leaching and immobilization). The remaining N enters the cycle immediately again [8, 91, 217]. Further, as illustrated above, the main reason why N-efficiency is relatively low in pasture based systems is that the CP content of grass is generally > 16 % [286]. But by decreasing the N availability within the system plant growth is limited and pasture yield decreases [117, 302]. In confinement TMR systems, feed stuffs need to be harvested, processed, transported and stored, and are often partially or completely imported (sometimes even from other countries or continents, e.g. soy beans in Europe). The excreted N is thereafter mechanically distributed on farmland for fertilization or sold again (= (partial) land-less livestock systems) [8, 91, 217]. The cow herself might be extremely efficient, but the system itself is very labor-intensive and less efficient on different other levels [217]. On the other hand confinement systems exhibit the possibility of storing and spreading manure in a controlled way and at the most effective time points considering weather and other factors influencing N losses [217]. Oenema and Tamminga (2006) and Oenema (2006) reviewed the different aspects considering N-efficiency on a farm and global level,
illustrating that aside an improvement of N utilization on animal level other system optimizing aspects play a key role (such as manure, soil and pasture management) [216, 217].

1.2.1.4. Milk quality and components
Dairy products from cows either pastured or fed hay, grain or ensiled feed differ in organoleptic properties [130]. For example, cheese produced from the milk of grazing dairy cows, is much softer than when grain or hay rich diets are fed [47]. This is due to the differences in fatty acids (FA) and other components of the feed such as terpenes, tocopherols and carotenoids [130]. The FA content of pasture is highly unsaturated (average 70-90%), with a large amount of linoleic (C\textsubscript{18:2}) and linolenic acids (C\textsubscript{18:3}) [134, 262]. During ensiling extensive lipolysis takes place [70, 129]. Van Ranst et al. (2010) reported a lipolysis level of 90% for perennial ryegrass [298], explaining why ensiled grass has a much lower unsaturated FA content than fresh grass.

The first step in the ruminal lipid metabolism is the lipolysis of ester linkages, followed by biohydrogenation of unsaturated non-esterified FAs [70, 266]. The result of the biohydrogenation of these unsaturated FAs is mainly trans vaccenic acid (trans-11 C\textsubscript{18:1}), which is then converted into rumenic acid (C\textsubscript{18:2} cis-9, trans-11) by $\Delta^9$ desaturation in the mammary gland. Rumenic acid is the predominant conjugated linoleic acid (CLA) isomer in ruminant fats [75]. Therefore milk of grazing dairy cows contains larger amounts of unsaturated FAs and trans-FAs (CLA and vaccenic acid) compared to milk of cows receiving a silage- and concentrate-based diet [61, 129, 134].

CLAs are of growing interest since in the last decade several studies with experimental animals have shown that CLAs promote various beneficial health-related effects, such as anti-carcinogenic and anti-atherogenic effects. Nevertheless several people call for caution, saying these claims have to be made with care, since clinical evidences for health benefits in humans are very few [75, 296].

The FA composition of pastures, and thereby the amount of CLAs in milk, is dependent upon species, variety and growing conditions [47, 60, 74, 262, 281, 292]. In 2006 Van Dorland et al. reviewed the influence of species-rich swards of the alps on beneficial FA content in alpine dairy products [296]. Cows grazing in higher altitude do produce higher amounts and different CLA than cows grazing lowland pasture [179, 296].

It is a known phenomenon that supplementation with unsaturated FA leads to milk fat reduction [262]. This is due to an anti-lipogenic mechanism of specific biohydrogenation intermediates in the udder [16, 70, 262, 266]. The same effect can be observed with dairy cows on pasture [203,
The molecular pathways responsible for this phenomenon are being investigated and for several molecules a connection with unsaturated FA induced milk fat depression has been made. Examples are sterol response element-binding protein-1 (SREBP1) and thyroid hormone-responsive spot 14 (S14). These molecules are key mammary lipogenic factors and are downregulated during milk fat depression [16, 131].

1.2.2. Animal welfare and health

Due to elaborate vaccine and eradication programs, advances in housing, feeding strategies, and breeding programs efficiency of livestock production systems has increased substantially during the last decades [161]. Whereas 50 years ago a range of transmissible infectious diseases played an important role, nowadays dairy farmers and veterinarians are mainly confronted with management associated production diseases such as lameness, mastitis and metabolic diseases [51, 88, 128, 161, 204, 272]. Especially in high yielding systems the genetic potential for high milk production exposes the limitations of housing systems concerning cow comfort and feeding strategies [93, 205]. As mentioned before, these systems are extremely efficient on cow level, but are very labor-intensive [204, 217]. A high yielding dairy cow requires optimal housing, ration composition, and time budgeting and shortcomings will directly result in poor animal health and economic losses [93, 128, 204, 205, 272]. Grazing systems are often less labor-intensive, characterized by lower milk yields and a more “natural behavior” of cows compared to confinement systems, and are therefore often perceived as more beneficial regarding animal welfare [6, 21, 44, 62, 175]. However, as the following paragraph illustrates, several studies have tried to compare the different systems regarding animal welfare and disease incidence, but the results are inconclusive.

Several studies have shown that grazing high yielding dairy cows, receiving non or not enough supplementation, are more severely subjected to a negative energy balance (NEB), do loose more weight and exhibit higher serum beta-hydroxybutyrate (BHBA) and FA concentrations after calving [5, 13, 79, 112, 135, 137, 147, 192, 193, 262]. Ribeiro et al. (2013) have shown that also in seasonally calving grazing farms periparturient diseases are highly prevalent and different studies confirm that also in grazing systems liveweight changes are negatively associated with reproductive performance [4, 5, 193, 247]. In a trial with 46 Holstein-Friesian cows Olmos et al. (2009) confirmed that cows in a pasture-based system exhibit an inferior metabolic or nutritional status during the early post-calving period compared with cows in a TMR-based system [219]. However, this greater nutritional and metabolic stress did not appear to trigger an increased
incidence of health or reproductive problems. Interestingly, the cows in the grazing group had a better reproductive health and welfare. The authors therefore speculate that, due to a lower milk production, cows in pasture-based systems are able to match their energy requirements more closely with an adequate energy supply. In a study involving 401 Danish dairy herds Burow et al. (2011) have investigated the effect of grazing on cow mortality and described that in farms with a traditional milking system the mortality was 75% lower in grazing compared to zero-grazing herds [28]. In this context different studies indicate that pasture-based production systems are more beneficial regarding udder health [89, 311] and lameness incidence [103, 107, 218], but elaborate studies are lacking.

Due to the limitations of metabolisable energy supply and a high crude protein intake, the N efficiency of grazing dairy cows is generally lower [150]. Excess N is converted into urea by the liver and excreted mainly via milk and urine. Urea synthesis incurs a metabolic energy cost which imposes an additional metabolic effort on a system already limited by energy supply (elaborately discussed in chapter 1.2.1.2 and 1.2.1.3) [150]. Several studies in confinement systems indicate that NH$_3$ in high metabolic concentrations has a toxic effect, incriminates different organs in their function [133, 221, 242] and is associated with reduced fertility (summarized in Pfeffer and Hristov 2005 [232]). Moller et al. (1993) have confirmed this correlation in a study including several pasture-based dairy farms [200]. Contrary to this conclusion, Smith et al. (2001) could not find any relationship between milk urea N content and reproductive performance of pasture fed New Zealand dairy herds [271].

Studies that directly compare the health and metabolism of grazing versus non-grazing cattle are very scarce. In 2011 Kaufmann et al. published a study on the differences in hepatic and blood plasma parameters in cows that were either grazing or received freshly cut grass in a confinement system [131]. The cows on pasture had higher plasma concentrations of triiodothyronine, BHBA and total protein than cows housed in a free-stall. This was most likely due to a higher metabolic turnover and a difference in triglyceride intake (triiodothyronine is an important determinant of overall energy expenditure). Cut grass is always subjected to respiratory losses, such as lipolysis, proteolysis and a decrease in sugar content. The authors therefore suggest that the lower energy intakes in the confinement group has probably lead to a high CP/WSC ratio, causing higher urea levels and subsequently a higher GLDH activity (indicates damaging of liver cells) [131]. Few studies have investigated the influence of high altitude grazing on different metabolic traits [105, 154, 255]. Ruhland et al. (1999) have shown carry-over effects that indicate a long-term influence of alpage. The authors hypothesize that the
increased activity and metabolic turn-over during this period could be beneficial for later health and longevity of the animals [255].

In confinement as well as pasture-based systems different management aspects form challenges regarding cow performance and animal welfare. In confinement systems time budgeting, bedding comfort and ration composition form the most important elements which need to be controlled and adjusted on a daily basis [93]. Moreover, silage quality is a key element in animal health in confinement systems. Hazards to animal health associated with silage are for example infections with microorganisms (e.g. Listeria, Enterobacteria and Clostridia), intoxications (e.g. nitrate, NH$_3$, mycotoxins, phytoestrogens, biogenic amines and other plant toxins), and by decreasing the buffering capacity in the rumen through excess acidity [277, 319]. In pasture-based systems however a range of different aspects, such as pasture management, stocking density and supplementation play an important role in daily management directly influencing animal performance and health [63, 86, 186, 254, 300]. The major nutritional disorders relating to animal health of grazing dairy cows concern mineral and trace element nutrition (e.g., hypomagnesaemia), gastro-intestinal disorders (e.g., bloat) and parasitic diseases such as liverfluke (Fasciola hepatica), longworm (Dictyocaulus viviparus), parasitic gastroenteritis (Ostertagia, Trichostrongylus, etc.) and coccidiosis [18, 186]. To prevent economic losses and guarantee animal welfare, preventive measures, such as rotational grazing to interrupt parasitic reproduction cycles and increasing the magnesium intake against hypomagnesaemia, have to be part of the management strategy in pasture-based systems [186].

Furthermore, in pasture-based systems the chemical composition of the ration as well as the cows themselves are much more subjected to weather influences. Grazing dairy cows have to deal with not only seasonal but sometimes daily and weekly changes in protein and energy availability [3, 223, 268] and are often more exposed to certain weather conditions such as rain, wind and heat [166].

In this context some studies have investigated the preference of cows themselves for being indoors or on pasture. It was demonstrated that preferences vary throughout the day and that cows simply prefer to stay wherever climate, bedding and feed conditions are the most ideal (in these studies being indoors during the day and on pasture during nighttime) [35, 36, 166].

This short overview illustrates that a vast amount of variables play a role in both systems and that one can come across good as well as bad management practices in confinement as well as
pasture-based systems. This is also the reason why it is not possible to answer the question whether one system is superior to another regarding animal welfare and health.

1.2.3. Rumen physiology

The rumen is a huge fermentation vessel in which a vast range of bacteria, protozoa and fungi ferment plant material into substances that can be utilized by the cow. Carbohydrate sources are fermented into volatile fatty acids (VFA; mainly acetate, butyrate and propionate) and absorbed by the rumen wall. Plant protein and other rumen degradable N sources (RDP) are turned into microbial protein, which is then absorbed together with UDP in the small intestine [196, 256]. Ration composition and feed intake patterns are major influencing factors on this microbial ecosystem and therefore also on nutrient supply of the cow [256].

1.2.3.1. Rumen microbiota

Already centuries ago farmers discovered that they could alter the flavor and consistency of the milk of their cows produced by feeding different rations. It is for example commonly known that during spring and summertime butter produced from cows grazing grass is softer compared to butter produced during wintertime when predominantly hay and silage is fed [49, 129]. This is due to an increased concentration of polyunsaturated FA (PUFA) in fresh grass which are partly biohydrogenated by rumen microorganisms and incorporated into milk fat in the mammary gland (described in chapter 1.2.1.4). Furthermore, milk components can also be influenced by the concentrate-roughage ratio in a ration. Milk fat % for example is increased by feeding a roughage rich diet, and the reverse effect can be observed in concentrate rich diets [16, 23, 222, 312]. The reason for this lies in the alteration of the rumen microflora and fermentation pattern. In roughage rich diets fermentation occurs at a lower rate and acetate producing bacteria are favored [18, 297]. This leads to a higher rumen pH and increases the production of milk fat [18, 23, 222]. Whenever an increased amount of non-structural carbohydrates is fed, the rate of flow of carbohydrate carbon through the glycolytic pathway to pyruvate leads to a higher ratio of NADH relative to NAD⁺ and an increase in H ions. This causes a lower rumen pH and favors bacteria species that convert carbohydrates to propionate via the succinate pathway. Additionally, a carbohydrate rich ration increases the production of FA in the rumen that have a milk fat depressing effect most likely by directly influencing milk fat synthesis in the mammary gland (described in chapter 1.2.1.4). Rumen microbiota does therefore not only play a key role in dairy cow nutrition, but also influence product palatability and quality, and animal health.
Up to a few years ago microbiota analysis and research was mainly culture and staining based and very labor-intensive. It was often only possible to bring certain (culturable) bacteria species into picture or to remain on a rough classification level. It is only in recent years, with the development of microbiota fingerprinting and sequencing methods, that a better understanding of the population and dynamics of the rumen microbiota is created [25, 127, 256, 279, 309, 310]. It has for example been discovered that the bacterial community structure in the liquid phase of those attached to feed particles and those attached to the ruminal papillae is different [24, 37, 125, 168, 198, 229, 256, 257, 279, 280, 322]. There have been studies investigating the impact of a high concentrate diet [37, 97, 116, 119, 120, 165, 257, 284], ruminal acidosis [142, 143, 198, 229], the feeding cycle [165, 182, 314], ration changes [37, 97, 174, 201, 257, 284], supplementation on pasture [110], silage fermentation attributes and the use of silage inoculants [104, 197, 233], milk fat-depression conditions [312], environmental factors such as temperature [253], fumaric acid [246, 251], elevated CO₂ levels and drought stress during growth of maize plants [191] and many other factors on the ruminal microbial communities. Other studies focused on the role of the microbiota on the animal’s performance, such as Hernandez-Sanabria et al. who showed in 2010 that feed efficiency of steers can be correlated to the microbiota of the individual animal [98, 108, 109]. Another quite remarkable finding was made by Weimer et al. in 2010 by discovering, in a near-total exchange experiment of ruminal content, that the ruminal community seems to be host specific [313]. However, still a lot of questions remain and only slowly the dynamics and function between and of different bacterial, archaeal and protozoal species are revealed.

On the effects of a ration change from TMR to pasture on the rumen microbial community only two studies have been published so far. In 2011 de Menezes et al. showed that the rumen microbiota of cows, either pasture or TMR fed, differed significantly [54]. And Nakano et al. (2013) showed that rumen microbiota needs 3-4 weeks to adapt to a pasture-based ration when no adaption to the new nutritional situation is granted. Furthermore they observed an increase in *Butyrivibrio* sp. and *Provetella* sp. at 28 days after beginning of grazing. They therefore hypothesized that these two species form a key element in the design of a feeding and supplementation program before and after the beginning of grazing [209].

**1.2.3.2. Rumen fermentation**

As illustrated in the previous chapters a TMR and a pasture-based system do not only differ substantially in the ration composition, but also in the way feed is acquired. It can therefore be
expected that rumen fermentation patterns do not only exhibit differences in molar proportions of VFA and other parameters, but also exhibit a different evolution throughout a 24 h period.

Ruminal fermentation patterns throughout a 24 h period are mainly dependent on the feed intake pattern of the cow and ration composition. In confinement systems feed intake is strongly related to feeding times (mostly once or twice a day) and other management decisions (such as milking times and stocking density) [211]. Generally, an increase in VFA, NH$_3$-N and a decrease in pH can be observed after feed has been ingested. After a longer period in which no feed is ingested (mostly at night) VFA and NH$_3$-N concentrations decrease, and pH increases again [14, 211]. Similar observations have been made in grazing cows [29, 39, 144, 184, 274, 288, 299, 307]. Taweel et al. (2004) showed that cows under continuous stocking conditions exhibit three major grazing bouts: dawn, afternoon and dusk, with the largest being at dusk. They further observed that the fermentation variables VFA, NH$_3$-N and pH follow this pattern by increasing or decreasing respectively throughout the day and exhibiting a reciprocal development at night [288]. In two studies Abrahamse et al. (2008 and 2009) showed that total DMI, rumen fermentation pattern and milk production or composition can be altered by changing the allocation frequency and daily movement of cows to fresh grass (morning or afternoon) [2, 3].

Bargo et al. (2002a and 2002b) confirmed that daily fermentation patterns in cows either receiving a TMR or being on pasture differ due to different feed intake patterns. In a trial involving six cannulated Holstein cows they investigated the performance as well as the ruminal digestion and fermentation in three different feeding systems combining pasture and TMR. During this 21-wk experiment the pasture group received an average of 8.7 ± 0.1 kg DM/day of a corn-based concentrate and was compared with a group receiving either TMR and pasture or a TMR-only group. Only significant differences were observed for ruminal NH$_3$-N concentrations. Mean ruminal pH and individual VFA proportions were unaffected by treatment. However, a significant treatment*time interaction was present for pH and NH$_3$-N concentrations mainly due to the concentrate supplement feeding after milking and the movement to a new paddock [13, 14].

Other studies that have chosen for a single sample per day have come to different conclusions. Holden et al. (1994) for example observed a higher VFA and NH$_3$ concentration in grazing cows compared to cows receiving either hay or silage, but no effect on rumen pH [114]. Vibart et al. (2010) used an continuous culture system to investigate the effect of replacing a TMR with fresh grass and observed an increase in digestibility, total VFA concentration, and molar proportions
of butyrate and valerate, and a decrease in NH$_3$-$N$ concentrations and methane concentrations [303].

In 2013 Steinwidder et al. published a study illustrating the effects of a ration change from a partially mixed ration (PMR; consisting of hey, grass silage and individual concentrate allowance) to a pasture-based ration on rumen pH pattern. In their study they observed a decrease in daily average pH and an increase in min < pH 5.8/d during this transitional period. After two weeks on a full grazing ration no difference was observed compared to initial status. They concluded that this data shows that such a ration change has a substantial influence on rumen microbiota and fermentation and underlines the importance of a gradual transition from one ration to another [278].

Rumen digesta stratification and intraruminal differences in pH and VFA concentrations is depended on feed fiber content and particle length [280]. It has been shown that if diets with a higher fiber content and longer particle length are fed, ruminal stratification is more pronounced [280, 283]. Storm and Kristensen (2010) hypothesized whether feeding a low fiber diet could result in a more homogenous ruminal content and thereby increasing ventral VFA concentrations and increasing the risk of ruminal acidosis [280]. Since young lush pastures exhibit a low fiber content it could be hypothesized whether this could lead to a homogenization of the ruminal content. Unfortunately, currently studies investigating ruminal stratification in grazing cows are lacking.

1.2.3.3. Subacute ruminal acidosis (SARA)

Several studies have shown that the rumen bacterial community is very dynamic and responds to changes in diet and environmental conditions [54, 56, 57]. Transitioning cows from one diet to another leads to a shift in the microbiota community of the rumen [109]. Feeding high amounts of starch for example results in the overgrowth of starch-fermenting, lactate-producing bacteria, causing the pH to drop and inducing a ruminal acidosis [82, 142]. Acute ruminal acidosis is a phenomenon more known in the beef industry than in dairy cows, since rations in feed lot cattle are often very high in starch and contain only little amounts of fiber [38, 237]. In dairy cows the symptomatology caused by repeated periods of (moderately) depressed ruminal pH (= subacute ruminal acidosis (SARA)) due to improper feeding regimes and management (e.g. high concentrate-roughage ratio, wrong feed-bunk management, etc.) is more prevalent [145, 151, 237]. Subacute ruminal acidosis has been elaborately investigated during the last two centuries and has been associated with laminitis, lung and liver abscesses and decreased production [46, 145, 151, 237]. It is assumed that 10-20% of dairy cows in early and mid-lactation suffer from
this disease [142, 145]. Several studies have discussed a threshold for SARA, but consensus among the scientific community has not been achieved yet [17, 139, 153, 324]. A range that is often used to define an increased risk for SARA has been developed by Zebeli et al. (2008). After a meta-analysis of different studies, the authors suggest a threshold of 314 min/d < pH 5.8 and a daily average pH of 6.14 [324].

It has been discussed if high quality pastures with high concentrations of WSC and low concentrations of physical effective fiber could have an adverse effect on rumen health [148, 215]. Furthermore, grazing dairy cows are also often supplemented with concentrates that could possibly increase the risk for SARA. O’Grady et al. (2008) and Bramley et al. (2008) have shown that approximately 10 % of cows in pasture-based systems could be classified as being affected by SARA [22, 215]. But since most research investigating the connection of a low ruminal pH and adverse effects on health and production has been conducted in confinement TMR-based system [237], it is questionable if the developed cut-off values for SARA can be translated onto pasture-based systems. In 2001, Kolver and de Veth have stated that in several studies a lower ruminal pH in pasture-based rations was associated with higher concentrations of ruminal VFA, ruminally degradable OM, and OM intake, and with a lower milk fat percentage, forage NDF content, and particle length index, but since no starch is present in pasture-based diets a low ruminal pH is not associated with high ruminal concentrations of lactic acid. They therefore suggest that a low ruminal pH does not necessarily compromise cow performance on pasture.

In this context Khafipour et al. (2009a and 2009b) showed that the pathological consequences of SARA are possibly substrate dependent [140, 141]. Whenever SARA is induced by high amounts of grain a lactic acid accumulation in the rumen occurs. When SARA is provoked with high fermentable products, such as grass or legumes, accumulation of high amounts of VFA are responsible for the low pH [237]. By using either grain or alfalfa pellets to induce SARA, they showed that grain induced SARA was dominated by Streptococcus bovis and Escherichia coli. Whereas the mild grain-induced SARA was dominated by Megasphaera elsdenii and alfalfa pellet-induced SARA was dominated by Prevotella albanensis [142]. Streptococcus bovis has a fast replication cycle and is the main lactic acid producing bacterium in the rumen. M. elsdenii has a much slower replication cycle and is one of the main lactic acid eliminating bacteria. These results lead to the assumption that in grain induced SARA there is a built up in lactic acid due to high amounts and fast fermentation of starch by S. bovis and took 1o slow elimination by M.elsdenii. In milder forms of SARA mainly M. elsdenii was present, probably due to a higher degree of microbial balance in the rumen [142]. In both acute and subacute acidosis, there is an increase in lipopolysaccharides (LPS) in the rumen [71, 92, 169]. Interestingly, an immune
response in the peripheral blood has only been observed in grain induced SARA [140, 141]. Further research of Khafipour et al. in 2010 showed that population structure of E.Coli changes during grain induced SARA into a more pathogenic type and was highly associated with virulence factors such as curli fibers [143]. They concluded that low rumen pH and high osmolarity, although damaging to the rumen epithelium, are not the only decisive factors that trigger immune activation during SARA [143]. Calsamiglia et al. (2012) even go as far as renaming SARA into a “high-concentrate syndrome”, claiming that two events, namely a high proportion of concentrate in the diet and a low ruminal pH are confounded [30].

This ongoing discussion on the relevance, definition and consequence of SARA illustrates the need for further research on ration depended influences on ruminal fermentation and health, especially for pasture-based systems.

1.2.3.4. Rumen morphology and volatile fatty acid absorption

Ruminal pH and fermentation patterns are just one aspect of different rumen characteristics that are influenced by alterations in feeding regime. Also rumen papillae morphology and absorption capacity [7, 11, 12, 33, 181, 225, 227, 282], histology [12, 275] and gene expression [45, 64, 225, 276] are altered under the influence of different ration types and during SARA. Slowly increasing the concentrate intake for example leads to an increase in the size of the papillae and the number of epithelial cells [65, 170, 325]. But also the reverse phenomenon can be observed in food-deprived animals, where a decrease in available fermentable material leads to a decrease in absorptive capacity of the rumen wall [83].

Bannink et al. (2012) have investigated the influence of a faster versus a slower increment of a starch-rich-concentrate intake after calving on rumen papillae. An influence of treatment on epithelial thickness but no significant difference was observed for papillae size, histopathological variables and fractional absorption rate of VFA (only numerical differences). They therefore suggest that the results show that the adaptation of the morphological characteristics may be relevant, but grant that interpretation of results was clearly hampered by the heterogeneity and small number of cows [12].

As summarized above, rumen morphology plays a key role in nutrient absorption and adaptation to ration changes. However, effects of a ration change from TMR to a pasture-based ration or different supplementation and grazing strategies on rumen morphology have not been investigated yet.
1.3. Ration Change

1.3.1. General aspects

It is known that the transition from one ration type to another, depending on the extent of difference between the rations, causes a change in the rumen microbiota [99, 256] and rumen stratification [280], which leads to alterations in fermentation patterns [297] and thereby to physiological and structural adaptations of the rumen epithelium [84] (see chapter 1.2.3.). The alterations in the nutrient availability are then transduced to the cow’s metabolism and are possibly reflected in changes in performance [123].

During the last 60 years various studies have been conducted investigating the time needed for adaptation of different variables after a ration change [94]. It has been shown that eating behavior normally stabilized within 1-7 days and therefore behavior research has commonly assessed 7-14-day adaptation periods [6, 59, 94, 273, 305]. Since feed intake is strongly related to behavioral changes also for DMI 7-10 days are required for adaptation. However, when the new ration represents a major change from the previous diet or elicit neophobia the adaptation period should be extended to at least 2 weeks [9, 27, 34, 80, 81, 94, 96, 123, 160]. For rumination a very fast initial response (1 day) and 4-10 days for a complete adaptation has been described [94].

Adaptation of rumen fermentation is mainly depended on the time required for the rumen microbiota to stabilize, but does not necessarily coincidence [25, 99]. Generally, a preliminary increase in abundance of total and culturable bacteria is observed, followed by stabilization [97, 99, 176, 177, 310]. Depending on the group of bacteria and the ration change a complete stabilization can be observed within 24 h, but can still be incomplete after 3 weeks [1, 99, 176, 201, 284]. Further, only few studies have been conducted illustrating the time required for methanogens and fungi to stabilize [99]. More recent studies employing culture independent methods (fingerprinting and sequencing methods, qPCR) confirmed shifts in taxonomic composition of gastro-intestinal microbiota during dietary changes [53, 284]. But further research focusing on the change in microbial function (for example using metagenome sequencing) and more frequent sampling is needed to reveal at what exact point it stabilizes [24, 99, 187].

Since dietary digestibility closely relates to rumen microbiota and fermentation it is not surprising that also for this variable commonly an adaption period of 10-14 days can be observed [42, 94, 174, 210]. Also milk yield and components follow this trend [80]. Further, different studies have
shown that the time course of and recovery from milk fat depression lies between 3-5 days and 2-3 weeks respectively [101, 248-250].

Protein metabolism generally adapts very rapidly to alterations in the diet [167, 323]. Rumen NH₃ concentrations are elevated rapidly when protein levels are increased in a ration and blood and milk urea and urinary N excretion follow this pattern almost contemporaneously [20, 132, 167, 195, 221].

In summary it can be stated that the time course needed for adaptation to a new ration is affected by physical and chemical attributes of the diet (such as particle size, carbohydrate fermentability, and fat or protein content and characteristics) as well as by possible required behavioral adaptations [94]. Commonly 7-21 days are assessed for adaptation under experimental and practical circumstances, depending on the extent of change [94]. Recently however questions arose whether for research purposes these commonly assessed 7-21 days might need to be re-evaluated and adapted depending on factors such as the basal diet, the cows physiological state, parity, genetics, environment, etc. [102, 123, 323]. It has even been discussed whether in future we need to account for factors such as possible metabolic imprinting and epigenetics [102].

1.3.2. Transition from TMR to pasture

It is commonly known that a fast transition from the slower fermentable winter-diet to lush spring grass harbors a potential threat for rumen and animal health (e.g., bloat) [18]. Therefore, dairy cows in pasture-based systems are generally gradually transitioned from one diet to another at the start of the growing season. However, only little to no data exists on the effects of this ration change on different physiological variables, and its ideal length and feeding regime.

In different nutritional studies comparing TMR and pasture-based systems mostly 7-14 days were assessed in which the amount of TMR fed or the time indoors is gradually reduced, but only little attention was given to this transitional period itself [77, 147, 213]. In some studies, the animals were switched onto a full-grazing ration from one day to another, but sample collection mostly only started after 7-14 days [13, 54, 114]. Other studies following production parameters from the beginning of the lactation onwards switched the cows directly from a dry-cow ration to the either TMR or pasture ration after calving [73, 219], gradually transitioned the animals as soon as daily herbage growth was sufficient [301], or fed supplements as long as the daily herbage growth did not cover nutritional needs [79].
Being one of the few studies differentiating the transition period from the grazing period, Kolver and Muller (1998) described a gradual decrease in milk production, protein %, serum glucose, BW and body condition score (BCS), and an increase in serum FA and BHBA as soon as the animals had a partial access to pasture [147]. Unfortunately, the transitional as well as the grazing period of the trial only lasted two weeks and it is therefore not possible to determine whether the cows had already fully adapted to the new nutritional situation or to estimate how long this adaptation (would have) lasted. Also Ferris et al. (1999) have observed a decrease in body weight and milk yield in the first 10 days post-turnout, but unfortunately further data on changes in body condition, milk composition and DMI during the transitional period have not been illustrated [77].

Chilibroste et al. (2012) have shown that in primiparous cows grazing time increases with increasing days in milk. This indicates that the transition to a pasture-based ration requires substantial behavioral adaptation [40].

A study investigating the influence of the transition from a silage and concentrate-based to a pasture-based ration on rumen pH has been conducted by Steinwidder et al. in 2013 [278]. The authors described a decrease in average daily rumen pH from 6.09 to 5.84 and an increase in time pH < 5.8 from 6 to 85 min/d during the transitional period (7 h pasture per day). Thereafter the animals were on a full-grazing ration and an increase to initial levels within three weeks was observed. Unfortunately, no further data on production and rumen fermentation were published in this work.

Nakano et al. (2013) investigated the time needed for rumen microbiota to adapt after an abrupt change from TMR to pasture in Holstein steers (described in chapter 1.2.3.1) [209]. The authors suggest that due to a negative daily gain, high serum FA concentrations, low rumen VFA concentrations and a continuous increase in DMI over the course of the trial the animals only started covering their nutritional needs at day 21 after switching to the new ration. Furthermore, PCR-DGGE analysis indicated that at least 3-4 weeks were needed for the rumen microbiota diversity to fully adapt.
2. Aims of study

In temperate climate zones dairy farmers that implement a pasture-based system often feed a silage and concentrate-based ration (TMR) to bridge the cold season of the year. After the start of the following vegetation period the cows are then gradually introduced to fresh spring grass.

As illustrated in the previous chapter, several studies have been conducted investigating the impact of pasture and confinement dairy housing systems on production and health aspects of dairy cows, demonstrating that in both systems different management aspects form challenges regarding cow performance as well as welfare. However, to date there are no studies (known to the author) published that focus on the impact of the transition from a TMR to a pasture-based ration on dairy cow production and health.

It is a general understanding that animal behavior and metabolism as well as the rumen microbiota need to adapt to the new nutritional situation and generally it is advised to introduce cows to pasture gradually over several weeks. But data illustrating the impact of this nutritional change and duration of adaption are lacking.

Thus it is hypothesized that the change from a confinement to a pasture based system involves complex nutritional and metabolic adaptations with consequences on health and performance:

- The ration change from TMR to pasture increases energy expenditure and decreases DMI causing an energy deficit. This will influence body weight and condition, and milk composition.
- Nitrogen intake and excretion is increased during this ration change and liver health is incriminated due to a higher metabolic urea load on a pasture-based ration.
- Due to different energy expenditure, environmental conditions and nutritional situation, total blood cell count and clinical chemistry parameters are altered during this ration change.
- During this ration change rumen fermentation, VFA absorption and morphology are affected due to an altered ration composition, DMI and feed intake pattern.
- The increased intake of fast fermentable organic matter (fOM) on a pasture-based ration leads to an increased risk for SARA and negatively influences rumen health.
- Animal production and rumen physiology variables need between 7-21 days to adapt to this new nutritional situation.

For testing these hypotheses, a ten-week trial with two groups (TMR only vs. gradual transition from TMR to pasture) with repeated measurements was conducted.
3. The Effects of a Ration Change from a Total Mixed Ration to Pasture on Health and Production of Dairy Cows

Authors: M. Schären,* S. Jostmeier,* S. Ruesink,* L. Hüther,* J. Frahm,* M. Bulang,† U. Meyer,* J. Rehage,‡ J. Isselstein,§ G. Breves,# and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany

†Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Karl-Freiherr-von-Fritsch-Str. 4, 06120 Halle (Saale), Germany

‡Clinic for Cattle, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany

§Department of Crop Sciences, Grassland Science, Georg-August University Göttingen, Von-Siebold-Str. 8, 37077 Göttingen, Germany

#Department of Physiology, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany

State of publication: printed in February 2016 issue of Journal of Dairy Science

Contribution of authors:

- Head of organization and execution: MS, UM, SD, JI, GB
- Trial and project design: MS, UM, SD, JI, MB, GB, LH, JF
- Trial implementation and sample collection: MS, SR, SJ
- Sample analysis: LH, JF, MB, SR, SJ
- Data analysis and interpretation: MS, SR, SJ, SD, UM, GB, JR
- Writing of manuscript: MS
- Revision of manuscript: SD, UM, GB, JI, LH, JF, MB

Own contribution: 70 %
The Effects of a Ration Change from a Total Mixed Ration to Pasture on Health and Production of Dairy Cows

M. Schären,* S. Jostmeier,* S. Ruesink,* L. Hüther,* J. Frahm,* M. Bulang,† U. Meyer,* †J. Rehage,‡ J. Isselstein,§ G. Breves,# and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Karl-Freiherr-von-Fritsch-Str. 4, 06120 Halle (Saale), Germany
‡Clinic for Cattle, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany
§Department of Crop Sciences, Grassland Science, Georg-August University Göttingen, Von-Siebold-Str. 8, 37077 Göttingen, Germany
#Department of Physiology, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany

Published February 2016 in Journal of Dairy Science

Volume 99, Issue 2, Pages 1183–1200
http://dx.doi.org/10.3168/jds.2015-9873

Abstract

In pasture-based dairy production systems, dairy cows often receive a silage- and concentrate-based ration (= TMR, total mixed ration) during wintertime and are gradually introduced to fresh herbage in spring. The present study aimed to investigate how the transition to this new nutritional situation influenced different production and health indicators. A 10-week trial (wk 1-10) was performed in spring 2014, including 60 dairy cows of the German Holstein breed (166 ± 23 days in milk, 23.5 ± 3.7 kg milk/d; means ± SD). The cows were divided into a pasture- and a confinement group (PG and CG). The CG stayed on a TMR-based ration (35 % corn silage, 35 % grass silage, 30 % concentrate; DM basis), while the PG was gradually transitioned from a TMR to a pasture-based ration (wk 1: TMR-only, wk 2: 3 h/d on pasture, wk 3 & 4: 12 h/d on pasture, wk 5-10: pasture-only). A continuous grazing system was implemented on a ryegrass dominated pasture and temperature humidity indices (THI) were assessed based on continuous

1Corresponding author: Ulrich.Meyer@fli.bund.de
recording of temperature and humidity indoors as well as outdoors. Dry matter intake (DMI) from TMR, milk production, body weight (BW) and body condition score (BCS) decreased as soon as the PG had partial access to pasture. Milk production and BW decreased even further in the first week on a full grazing ration, but thereafter BW increased again and milk production stabilized. DMI estimation using the n-alkane method in wk 7 and wk 9 revealed an increase in DMI from pasture between the two time points and indicates an adaptation of grazing behavior and metabolism over several weeks. Increased serum β-hydroxybutyrate (BHBA) and fatty acids concentrations at several time points as well as a continuous BCS decrease during the whole course of the trial indicate an energy deficit in the PG. A significant correlation between serum glucose concentrations and the THI was observed. An increase in serum and milk urea concentrations as well as an increase in the urine total N to creatinine ratio occurred in the PG. To assess possible negative effects of the ration change on metabolic and liver health different clinical chemistry variables and complete blood counts were assessed. No biological relevant changes were observed for serum albumin, total protein, cholesterol, aspartate transaminase, γ-glutamyltransferase and glutamate dehydrogenase concentrations as well as for white and red blood cell counts.

**Key Words:** pasture, confinement, ration change, health

**Introduction**

In temperate climate zones dairy cows are often fed a TMR during winter time and are gradually transitioned to a pasture-based ration in spring. Especially for farms with a seasonal calving pattern milk production from pasture can be economically beneficial due to lower production costs. Also a larger demand for pasture-based milk products, higher feed costs and volatile milk prices have made grazing dairy systems more attractive in recent years (Dillon et al., 2005). Pasture-based rations generally exhibit a higher crude protein and lower metabolisable energy content. Due to this imbalance in available nutrients, a lower DMI and a higher energy demand, grazing dairy cows generally have a lower milk production when no dietary attempts are made for counterbalancing (Osuji, 1974; Kolver, 2003; Roca-Fernandez et al., 2013). Milk production can be supported by a pasture-based ration up to 25 to 30 kg milk/d whereas a TMR can support milk production of more than 40 kg milk/d (Kolver and Muller, 1998; Bargo et al., 2002).

Different studies have shown that in a grazing- compared to a TMR-based system especially cows of high yielding breeds with similar production potential are subjected to a more pronounced negative energy balance after calving, and as a consequence undergo a more
extensive loss of body weight (Washburn et al., 2002; Fontaneli et al., 2005; O'Neill et al., 2011) and exhibit higher serum BHBA and fatty acids concentrations postpartum (Kolver and Muller, 1998; Bargo et al., 2002). However, up to now there is no clear evidence that this inferior metabolic and nutritional status is related to an increased incidence of health or reproductive problems (Olmos et al., 2009b; Alawneh et al., 2012; Ribeiro et al., 2013).

Further, different studies suggest that pasture-based compared to confinement systems are more beneficial regarding different general health related traits such as mortality (Burow et al., 2011), udder health (Goldberg et al., 1992; Washburn et al., 2002) and lameness incidence (Haskell et al., 2006; Olmos et al., 2009a), but elaborate studies are lacking.

Due to the limitations of metabolisable energy supply and a high crude protein intake, the nitrogen (N) efficiency of grazing dairy cows is generally lower (Kolver, 2003). Excess N is converted into urea by the liver and excreted mainly via milk and urine. Urea synthesis incurs a metabolic energy cost which imposes an additional metabolic effort on a system already limited by energy supply (Kolver, 2003). Several studies indicate that NH₃ in high metabolic concentrations has a toxic effect and incriminates different organs in their function (Rajala-Schutz et al., 2001; Pacheco and Waghrorn, 2008; Keim and Anrique, 2011). It has been shown in confinement systems that high metabolic urea concentrations are associated with reduced fertility (summarized in Pfeffer and Hristov (2005)). Moller et al. (1993) confirmed this correlation in a study including several pasture-based dairy farms. Contrary to this conclusion, Smith et al. (2001) could not find any relationship between milk urea N content and reproductive performance of pasture fed New Zealand dairy herds.

In a pasture-based, in contrast to a confinement TMR-based system, the chemical composition of the ration as well as the cows themselves are much more subjected to weather influences. Grazing dairy cows have to deal with not only seasonal but sometimes also daily and weekly changes in protein and energy availability (Parker and Edwards, 1996; Smit et al., 2004; Abrahamse et al., 2009) and are often more exposed to certain weather conditions such as rain, wind and heat (Legrand et al., 2009).

This short summary illustrates that in pasture-based as well as confinement systems different management aspects form challenges regarding cow performance as well as welfare. However, up to now, there are no studies (known to the author) published that focus on the impact of the transition from a TMR to a pasture-based ration on dairy cow production and health. It is generally accepted that animal behavior and metabolism as well as the rumen microbiota need to adapt to the new nutritional situation and it is therefore advised to introduce cows to pasture
gradually over several weeks. But data illustrating the impact of this nutritional change and duration of adaption is lacking.

We thus hypothesized that the change from a confinement to a pasture based system involves complex nutritional and metabolic adaptations with consequences on health and performance. Therefore, the objective of this study was to investigate the influence of a ration change from TMR to pasture on DMI, body condition, milk production, N metabolism and health. A ten-week trial with two groups (TMR only vs. gradual transition from TMR to pasture) with repeated measurements was conducted to assess the impact and duration of the adaption period.

**Materials and Methods**
Experimental work was conducted from April until June 2014 at the experimental station of the Friedrich Loeffler Institute (FLI) in Brunswick, Germany. The experiment was carried out according to the German Animal Welfare Act approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, Germany).

**Experimental Design and Treatments**
Sixty primi- and pluriparous German Holstein cows (166 ± 23 days in milk; mean ± SD) were randomly assigned to either a pasture group (PG; n = 29) or a confinement group (CG; n = 31). Each group contained five rumen- and duodenum fistulated animals. Cows had been exposed to intensive grazing prior to their first calving and during dry periods in previous seasons. Treatments were balanced for milk production (23.5 ± 3.7 kg milk/cow per day), body weight (613 ± 48 kg), BCS (3.1 ± 0.6; five-point scale; Edmonson et al. (1989)) and mean number of lactations (1.9 ± 1.6). The experimental period lasted 10 weeks (wk 1-10) from April 21st until June 27th 2014. In the months preceding the trial a TMR (similar components as fed during the trial) and additional concentrate to match individual milk production (available at an automated feeding station) was fed. Two weeks prior to the trial all cows were switched to the trial TMR and individual concentrate feeding was ceased. The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR and 3 h pasture/d, wk 3 and 4: TMR and 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d. The TMR consisted of 35% corn silage, 35% grass silage and 30% concentrate. To prevent the risk of hypomagnesaemia Mg-oxide was added to the TMR and concentrate of the PG. The components and chemical composition of the different experimental diets are illustrated in Table 1 and 2. The cows were milked 2 times per day at 5:30 h and 15:00 h. The TMR was fed daily at approximately 11:00 h. In wk 5-10 0.88 kg DM concentrate was offered to the PG in troughs after morning and evening milking. Pastures were located directly
adjacent to the barn including the milking parlor, with a walking distance averaging less than 100 m.

**Pasture Management**

A continuous grazing system was implemented with two pastures, measuring 6 ha each (pasture 1 & 2). Depending on pasture height, weather and management decisions such as irrigation, one or the other pasture was used (stocking density: 2.5 animals/ha). The average pasture height was measured 3 times/week using an electronic rising plate meter (RPM F400, Farmworks Systems Ltd., Manawatu-Wanganui, New Zealand). Daily pasture allowances were estimated using exclosure cages (described in “animal measurements, DMI”). Plant species and their estimated pasture coverage were assessed in wk 1. The pastures were situated on sandy loamy soil and sufficiently supplied with plant available phosphorous (P), potassium (K) and magnesium (Mg) (soil sampling 6 months prior to the trial: pasture 1: pH = 5.5; P = 5.3 mg/100g; K = 11.6 mg/100g; Mg = 10 mg/100g; pasture 2: pH = 6.3; P = 9.6 mg/100g; K = 11.7 mg/100g; Mg = 10 mg/100g). Both pastures were fertilized with approx. 40 kg N/ha one month preceding the trial and every 4-6 weeks thereafter (total annual fertilization: 137 kg N/ha (pasture 1) and 107 kg N/ha (pasture 2) with either calcium ammonium nitrate, ammonium nitrate urea solution or liquid manure).

**Weather and Barn Climate Measurements**

Daily mean temperature and humidity as well as minima and maxima were recorded by a weather station of the “Deutscher Wetterdienst” adjacent to the pastures (station nr. 662; 52°17’29”N, 10°26’47”E). Barn climate (temperature and humidity) was recorded every 10 min via data loggers (Tinytag Plus 2, TGP-4500, Gemini Data Loggers, Chichester, UK). The climate conditions were characterized by the calculation of the daily temperature humidity indices (THI) described in Hahn (1999).

**Pasture and Feed Measurements**

Representative TMR and silage samples were collected daily and pooled over 4-week periods and concentrate samples were taken representatively during the manufacturing process. Pasture samples were collected 3 times/week in the morning along a defined pathway using an electric mower, selected and cut representatively according to observed grazing behavior (only where cows were currently grazing and only the upper half of the plant) and pooled per pasture and week. Feed samples were then dried at 60 °C for 72 h and ground to pass through a 1.0 mm-screen. The chemical composition was analyzed according to the methods of the VDLUFA (VDLUFA (2006); method numbers are given) for dry matter (DM, 3.1), ash (8.1), CP (Dumas
method, 4.1.2), starch (7.2.1), sugar (Luff-Schoorl method, 7.1.2), ether extract (EE, 5.1.1), crude fiber (CF, 6.1.1), neutral detergent fiber (NDFom; 6.5.1) and acid detergent fiber (ADFom; 6.5.2). NDF and ADF were expressed without residual ash and are therefore referred to as NDFom and ADFom. Utilizable crude protein (uCP), ruminal nitrogen balance (RNB) and netto energy lactation (NEL) of silage and pasture samples were determined by near infrared spectroscopy (NIRS, according to VDLUFA (2006)) and calculations according to GfE (2008). For calculations of uCP, RNB and NEL contents of concentrates tables values according to DLG (1997) and calculations according to GfE (2001) were used. A weekly average for the pasture chemical composition was calculated in consideration of the amount of days cows were on pasture 1 or 2 in the respective week.

**Animal Measurements**

**DMI.** In the confinement system individual water and TMR intake was continuously recorded using electronic weighing troughs and RFID (in wk 1-4 in the PG and throughout the whole trial in the CG; manufacturer: Insentec, B.V., Marknesse, The Netherlands). The concentrate which was fed in the PG in wk 5-10 was individually fed after milkings and if some concentrate remained it was weighed back (which was rarely the case and found negligible). In the PG daily pasture allowances and group DMI was estimated three times in wk 5-10 using 12 exclosure cages (each 2.75 m²). Every 10-14 days the grass grown underneath each exclosure cage and a reference area next to it was harvested, weighed and the DM content was determined. The average growth rate was determined by subtracting the amount of DM harvested from the reference area prior to the growth period from the amount of DM underneath the exclosure cage and extrapolating it to kg/ha/d. The estimated average growth rate was then used to assess the average pasture allowance in kg/cow/d. The average DMI was estimated by subtraction of the amount of DM harvested from the reference area from the amount DM underneath the exclosure cage and extrapolation to kg DM/cow/d. To estimate the individual pasture DMI the n-alkane method was used (Dove and Mayes, 2006). The C32 alkane (800 g; Th-Geyer, Renningen, Germany; VWR, Darmstadt, Germany; Sigma-Aldrich, Seelze, Germany) was mixed into 1,800 kg concentrate (3 batches à 600 kg) as described by Taweel et al. (2006). Concentrate samples were collected representatively during the manufacturing process (4 samples per batch) and analyzed thereafter to assess marker distribution prior to the trial. The marker was evenly distributed within batches but slightly different between batches (batch 1: 342 ± 8.5; batch 2: 363 ± 4.7; batch 3: 372 ± 9.2 mg/kg DM; means ± SD). Therefore, batches were mixed thoroughly before feeding and the average of the measured concentrations (359 mg C32/kg DM) was used for DMI calculation. During wk 6-9 of the trial 0.88 kg DM of this concentrate was fed twice daily.
after milking. In wk 7 and wk 9 grass and individual feces samples were collected twice daily after milking and stored at -20°C. At the end of each week the feces samples were pooled per animal. The pooled feces samples and each grass sample were freeze-dried and ground to pass a 1-mm screen. Samples were analyzed for n-alkanes as described in Elwert et al. (2004). The DMI was calculated using the n-alkane pairs C31:C32 and C33:C32 as follows (Dove and Mayes, 1996):

\[
\text{DMI (kg DM/d)} = \frac{F_i^*D_i}{F_j^*G_j}
\]

Where \( F_i \) = average concentration of n-alkane in feces (mg/kg DM), \( G_j \) = average concentration of n-alkane in grass-sample (mg/kg DM), \( D \) = daily dosage of n-alkane marker (mg), \( i \) = marker alkane (C32), \( j \) = natural herbage alkane (C31 or C33).

**Milk and BW.** Individual milk yields were recorded daily and BW was automatically recorded when leaving the milking parlor. Morning and evening milk samples were collected at 2 days/week (Monday evening & Tuesday morning; Thursday evening & Friday morning) and stored at 4°C until analysis. Milk samples were analyzed for fat, protein, lactose, urea and SCC concentrations using an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 5000; Foss Electric A/S, Hillerød, Denmark). BCS was recorded every 14 days using a 5-point scale according to Edmonson et al. (1989).

**Blood.** Blood samples were collected weekly from the *Vena caudalis mediana* in a 10 mL evacuated serum separating blood tube and a 10 mL blood tube containing EDTA to prevent coagulation. A complete blood count (electrical resistance detection) of each sample was performed within 2 h after sampling using an automated hematology analyzer (Celltac alpha MEK-6450, Nihon Kohden Corporation, Tokyo, Japan; including hemoglobin (surfactant/colometric method), hematocrit, white blood cell population, and red blood cell and platelet distribution width (based on histogram calculation)). The serum was separated (centrifuged at 2123 x g for 15 min at 15 °C) and stored at -80 °C before chemical analysis using an automatic clinical chemistry analyzer (Euroliser CCA180, Eurolab, Austria; analysis methods are indicated in brackets following variables. Serum glucose (enzymatic colorimetric, GOD-PAP), BHBA, fatty acids (both enzymatic colorimetric) and urea concentrations were determined weekly. Serum albumin (bromocresol green), total protein (biuret), cholesterol (CHOD-PAP, with ATCS), aspartate transaminase (AST; mod. IFCC with pyridoxal phosphate), γ-glutamyltransferase (γ-GT; IFCC), total bilirubin (DC-test), glutamate dehydrogenase (GLDH; DGKC) and triglyceride (GPO-PAP) concentrations were measured in wk 1, wk 6 and wk 10. All
metabolites were determined using commercial kits (Greiner Diagnostic, Bahlingen, Germany) according to the manufacturer’s instructions.

**Urine.** Mid-stream urine samples were collected weekly during voluntary urination of cows either in the barn or on pasture. Urine pH was measured immediately after collection using a glass electrode (digital pH measurement devise, pH 525, WTW, Weilheim, Germany). Samples for total N analysis were directly stored at -20 °C. Samples for creatinine, allantoin and uric acid analysis were directly cooled to 4 °C. For further preparation samples were brought to room temperature, homogenized, diluted 1:50 and stored at -20 °C at the same day. Weekly urinary creatinine, allantoin and uric acid concentrations were determined using reverse-phase chromatography (HPLC system, Shimadzu, Kyoto, Japan). After filtration (amcro filter, PVDF, 0.45 μm) 20 μl were injected into a HPLC system. Samples were run through a C18 column (Inertsil ODS, 150 × 3 mm, 5 μm particle size, 150 Å pore size), using a binary gradient system. Mobile phase A consisted of 0.5 % MeCN, 10 mM IPCC6 in ultrapure water at a pH of 2.3., and mobile phase B of 100 % acetonitrile. Quantification was performed by a multi wavelength detector at a wavelength of 218 nm (allantoin), 225 nm (creatinine) and 284 nm (uric acid) (described in detail in Winkler et al. (2014)). Nitrogen concentrations were determined in wk 1, wk 4, wk 7 and wk 10 using the Kjeldahl method.

**Statistical Analysis**

If variables were recorded more than once a week, means were calculated per cow and week prior subjecting to statistical evaluation. Repeated measurements were analyzed using PROC MIXED in SAS Enterprise Guide 6.1 (SAS Institute 2013, Cary, NC, USA) using a restricted maximum likelihood model (REML). Week and diet group and their interaction were defined as fixed factors. To account for the individual variation of the cows a “REPEATED” statement was included. Best fitting covariance structures were tested using the “Akaike information criterion” for a finite sample size (AICC). Significant weekly effects were further evaluated by multiple t-test (procedure “PDIFF”) with Tukey adjusted p-values. Multiple comparisons were presented within experimental groups to describe time effects within groups (indicated by different letters), while group differences were evaluated for corresponding time points only (indicated by symbols). Results are presented as least square means (LSMeans) and pooled standard error of means (PSEM). Correlation coefficients between different traits were estimated using STATISTICA 12.0 (StatSoft, Inc. 2014, Tulsa, Oklahoma, USA).
Results

Weather and Barn Climate
Outdoor daily average ambient temperature with minima and maxima, outdoor humidity and outdoor and indoor THI are illustrated in Figure 1. The THI was generally $5.1 \pm 0.8$ (mean ± SD) units higher indoors compared to outdoors. The average daily THI was $57.9 \pm 5.5$ outdoors and $62.7 \pm 5.1$ indoors (means ± SD). Periods of mild heat were measured in wk 5 and between wk 7 and wk 8 with average daily THI between 65 and 70 outdoors and 65 and 75 indoors.

Feed composition
The chemical composition of the TMR, concentrates and pasture are indicated in Table 2. Chemical composition of the pasture varied considerably during the experimental period, with an average DM content of $183 \pm 14$ g/kg (mean ± SD), CP content of $193 \pm 21$ g/kg DM, sugar content of $113 \pm 32$ g/kg DM and CF content of $216 \pm 8$ g/kg DM. In wk 7 and wk 10 the highest sugar (174 and 148 g/kg) and lowest CP contents (159 and 167 g/kg) were observed.

Pasture Measurements
The average pasture height was $6.6 \pm 0.7$ cm (mean ± SD) and daily pasture allowances were wk 5/wk 6: $24.2 \pm 2.2$; wk 7/wk 8: $23.8 \pm 4.3$; wk 9/wk 10: $16.5 \pm 4.5$ kg DM/cow/d (means ± SEM). Pasture 1 consisted of (estimated pasture coverage; mean ± SD) 80.2 ± 13.8 % perennial ryegrass (Lolium perenne), 7.8 ± 11.6 % shepherd's purse (Capsella bursa-pastoris), 6.6 ± 1.1 % timothy-grass (Phleum pratense), 2.0 ± 1.9 % meadow fescue (Festuca pratensis), 1.0 ± 1.3 % Kentucky bluegrass (Poa pratensis), 0.6 ± 0.4 % annual meadow grass (Poa annua), 0.5 ± 0.4 % common chickenweed (Stellaria media), 0.4 ± 0.8 % white clover (Trifolium repens), 0.2 ± 0.4 % red fescue (Festuca rubra), 0.2 ± 0.4 % common dandelion (Taraxacum officinale), 0.1 ± 0.1 % couch grass (Elymus repens), 0.1 ± 0.1 % purple deadnettle (Lamium purpureum), 0.1 ± 0.1 % cut-leaved Crane's-bill (Geranium dissectum). Pasture 2 consisted of 60.0 ± 5.3 % perennial ryegrass, 22.0 ± 10.3 % meadow fescue, 10.0 ± 5.4 % timothy-grass, 0.2 ± 0.1 % common chickenweed, 0.2 ± 0.0 % common dandelion.
**Animal Measurements**

DMI. The average DMI from TMR and pasture & concentrate as well as in barn water intake are illustrated in Figure 2. The average DMI from TMR of the CG was 18.1 ± 0.3 kg DM/cow/d and exhibited only small fluctuations throughout the trial. The average water intake of the CG was 61.2 ± 1.9 kg/cow/d and correlated significantly with THI (R = 0.90; P < 0.001). The DMI from TMR and in barn water intake in the PG gradually declined in the first 4 weeks due to partial pasture access during wk 2-wk 4. The estimated DMI using the n-alkane method in wk 7 and wk 9 showed a significant difference between groups and an increase between wk 7 (12.7 ± 0.3 DM/cow/d) and wk 9 (15.0 ± 0.3 DM/cow/d) within the PG. The estimated DMI using the exclosure cage method also indicated an increase in DMI between wk 5/wk 6 and wk 7/8 but accounted for a much higher intake compared to the n-alkane method: wk 5/wk 6: 16.8 ± 1.5, wk 7/wk 8: 21.5 ± 3.2 and wk 9/wk 10: 22.2 ± 6.1 kg DM/cow/d (means ± SEM).

**Milk Yield and Composition.** Milk yield, and protein and fat content are illustrated in Figure 3. For milk, protein, fat and lactose yield a group (G), time (T) and group*time (G×T) effect was observed (protein yield: P_G < 0.01, P_T < 0.001, P_G×T < 0.001, fat yield: P_G < 0.001, P_T < 0.001, P_G×T < 0.001; lactose yield: P_G < 0.01, P_T < 0.001; P_G×T < 0.001; data for protein, fat and lactose yield not shown). Milk production decreased in the PG in the first week on a full grazing ration and significant differences between the groups were present during wk 5-7. Due to a decrease in milk production in the CG no difference between groups was present in wk 8-10.

Milk fat content remained uninfluenced by treatment until wk 4. In wk 5 the PG exhibited a significantly lower fat content compared to the CG. Starting from wk 7 the milk fat content of the CG slightly increased while the opposite was observed for the PG resulting in significant interactions between week and group and significant group differences in week 9 and 10. Milk fat content exhibited a group as well as time effect, and there was a significant effect of the group by time interaction. A significantly lower milk fat content in the PG was observed in wk 5, wk 9 and wk 10 compared to the CG. Milk protein content exhibited a significant time and group*time effect in the PG and a tendency for lower milk protein content was observed in wk 7. Milk lactose content exhibited a time and a time*group effect (P_G = n.s.; P_T < 0.001; P_G×T < 0.001; data not shown). Milk lactose content decreased in the PG from wk 5 and in the CG from wk 6 on continuously. No significant differences were present between groups at corresponding time points. Milk urea content (ppm) was influenced by a time, group as well as a significant group and time interaction (P_G < 0.001; P_T < 0.001; P_G×T < 0.001; data not shown). Average milk urea content was 155 ± 17 ppm (means ± SD) in the CG and exhibited only small fluctuations across the trial. In the PG milk urea content started increasing in wk 3 (wk 1: 172, wk 2: 182; wk
A tendency was observed in wk 3 (P < 0.06) and a significant difference between groups was present in wk 4-6 (P < 0.001) and wk 8-10 (P < 0.001). A tendency for a correlation of milk urea content with pasture CP content was observed (R = 0.81; P = 0.09). No group, time or group*time effect was observed for SCC (data not shown).

**BW and BCS.** Body weight as well as BCS was influenced by group, time as well as a group*time interaction (Figure 4). The BW of the CG exhibited an increase of 15 ± 16 kg (means ± SD) between wk 1 and wk 8. The BW of the PG decreased by 48 ± 22 kg from wk 1 until wk 7 and thereafter increased again until wk 10 by 26 ± 10 kg (means ± SD). The PG lost on average 0.18 ± 0.02 BCS score units (mean ± SD) per 14 days from wk 3 on. The CG increased slightly in BCS in the first half but lost 0.3 ± 0.3 BCS score (mean ± SD) on average in the second half of the trial.

**Blood.** Results of complete blood counts are illustrated in Table 3. Total white blood cell, lymphocyte and granulocyte concentrations exhibited a significant group*time interaction due to a decrease in the CG in wk 9 and 10. No significant group, time or group*time interaction effects were observed for monocyte concentration. For the eosinophil concentration only a time, no group or group*time interaction effects were observed. Red blood cell count exhibited a divergence between groups in red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) from wk 5 on. In wk 5-10 RBC, HGB and HCT were numerically higher in the PG compared to the CG. A significant difference between groups was observed for HGB and RBC in wk 9, and for HCT in wk 5, 6, 7 and 9. In the CG a decrease in RBC, HGB and HCT was observed in wk 7-9. (RBC P_{GxT} < 0.001; HGB P_{GxT} < 0.10; HCT P_{GxT} = 0.01). The mean corpuscular volume (MCV) decreased continuously from wk 1-10 in the CG. In the PG the MCV decreased simultaneously from wk 1-7 and increased in wk 8-10 again (P_{GxT} < 0.001). No significant difference between groups was observed for mean corpuscular hemoglobin (MCH) at any time during the trial. In the CG MCH decreased in wk 8-10 contributing to a significant group*time interaction. Mean corpuscular hemoglobin concentrations (MCHC) were higher in the PG compared to the CG in wk 1 and wk 3. Between wk 4-wk 10 no significant changes occurred in the PG. In the CG an increase from wk 5 until wk 7, with a subsequent decrease until wk 9 was observed. In wk 7 a tendency for a higher MCHC was observed in the CG compared to the PG (P_{GxT} < 0.001). Red blood cell distribution width (RDW) did not exhibit any significant differences between groups at any time but a group*time interaction due to an increase in the PG in wk 7-10. No significant changes in RDW were observed in the CG. Platelet distribution width (PDW) showed similar
development in wk 1-wk 5 in the PG and CG. In wk 6 and wk 8 PDW was higher in the PG ($P_{GxT} < 0.001$).

Clinical chemistry variables are illustrated in Table 4 and Figure 5-7. The group*time interaction was significant for albumin concentration since the values measured for the PG decreased again from wk 6 to wk 10 after an initial increase while the corresponding concentrations in the CG increased steadily over time. For serum total protein concentrations, a time effect was observed due to a decrease from wk 1 to wk 6 in both groups. But no group or group*time interactions were observed. Serum BHBA concentrations exhibited a group, time as well as group*time effect and were significantly higher in wk 7, wk 9 and wk 10 (1.02 ± 0.04 mmol/l; means ± SD) in the PG compared to wk 1 (0.68 mmol/l ± 0.04; $P < 0.05$). A significant difference between groups was only observed in wk 9 (Figure 5). Serum glucose concentrations were influenced by a significant time as well as a group*time interaction. An increase in both groups from wk 1 to wk 4 (from 55.8 to 62.3 ± 1.5 mg/dl) a succeeding decrease until wk 8 (44.5 ± 1.5 mg/dl) followed by an increase in wk 9 and 10 (56.5 ± 1.5 mg/dl) ($P_{GxT} < 0.001$) was observed. Groups did not differ significantly at any time during the trial. Serum glucose correlated positively with THI ($R = 0.55$; $P = 0.01$) and serum BHBA concentrations ($R = 0.45$; $P < 0.05$). For serum fatty acids as well as triglyceride concentrations a group, time and group*time effect was observed (Figure 6). Serum fatty acids concentrations increased in the PG from 0.23 in wk 3 to 0.42 ± 0.02 mmol/l in wk 8. Thereafter, a decrease to 0.39 ± 0.02 mmol/l in wk 10 was observed. A significant difference between groups was observed in wk 4 and wk 6-9. Concurrent to increased serum fatty acids concentrations a significant increase in serum triglyceride concentrations was measured in the PG during the full grazing period (wk 6 and wk 10). In the CG the serum triglyceride concentrations increased as well, but not as high as in the PG causing the significant group*time interactions. A significant difference between groups was present in wk 6 and wk 10. Serum cholesterol concentrations exhibited a group, time and group*time effect. No significant alterations were observed within the CG. In the PG a decrease from wk 6 to wk 10 contributed to a significant difference between groups at that point in time. Serum AST concentrations were generally higher in the PG, therefore a significant group effect was observed. In wk 6 a tendency for lower serum AST concentrations were observed in the PG compared to wk 1 ($P < 0.10$). A significant increase in the PG as well as CG was observed from wk 6 to wk 10 causing a significant time effect. No group*time interaction was present. Serum γ-GT concentrations were not altered in the CG over the course of the trial. In the PG a decrease over the course of the trial was observed contributing to a significant group, time and group*time effect. In wk 6 a tendency for and in wk 10 significant lower serum γ-GT concentrations were observed in the PG.
No change was observed in serum bilirubin concentrations within the PG over the course of the trial. Within the CG an increase was observed in wk 6, leading to a significant difference between groups at that point in time and a significant time and group*time effect. Serum GLDH activities were not influenced at any time by group, time or group*time effects. Serum urea concentrations exhibited a group, time and group*time effect and are depicted together with urine total N to creatinine ratio in Figure 7. Serum urea concentrations increased continuously in the PG from wk 3 on (except for wk 5 and wk 7). In the CG plasma urea levels were elevated in wk 8-10. A significant difference between groups for serum urea concentrations were observed in wk 4, wk 6, wk 9 and wk 10. Serum urea concentrations did not correlate significantly with pasture CP content.

**Urine.** Urine creatinine concentrations and purine derivatives (PD, allantoine and uric acid) to creatinine ratios are summarized in Figure 8. Both variables exhibited a group, time as well as group*time effect. Creatinine concentration decreased in the PG as soon as the animals were on a full pasture ration. In the CG a decrease in creatinine concentrations in the urine was observed during wk 6-8. A significant difference between groups was observed in wk 5-6 and wk 8-10. Urine creatinine concentrations correlated positively with water intake ($R = 0.25; P < 0.001$) in the CG. A significantly higher purine derivative to creatinine ratio was observed in the PG in wk 9 and wk 10. Urine total N to creatinine ratio was influenced by a group and time effect, as well as a group*time interaction (illustrated in Figure 7) and indicates an increase in N elimination via urine in the PG compared to the CG. Significant differences between groups were observed in wk 4 and wk 10. In wk 7, in the CG as well as the PG, a more than/almost 2-fold increase in the urine total N to creatinine ratio and urine PD to creatinine ratio respectively was observed. Urine pH was influenced by a group, time and group*time effect ($P_G < 0.001; P_T < 0.001; P_{GxT} < 0.001$; data not shown). In the CG no significant changes in urine pH were observed $(8.01 \pm 0.06; \text{means} \pm \text{SD})$. Urine pH of the PG decreased continuously from wk 4-9 and a significant difference between groups was observed in wk 8-10 (wk 8: 7.7; wk 9: 7.39; wk 10: 7.6; $P < 0.001; \text{PSEM} = 0.04$).

**Discussion**
The main cow associated difference in a TMR compared to a pasture-based system lies in the possible DMI, the ration composition and the energy demand. Dry matter intake is limited to approx. 20 kg DM/d and pasture-based rations are lower in metabolisable energy content (Kolver, 2003). Further, the form of feed acquisition (grazing and walking) results in higher energy expenses compared to eating ready available feed such as a TMR (Osuji, 1974; Kolver,
It is therefore not surprising that during this trial substantial changes were observed in the PG regarding DMI, body condition, milk production and energy metabolites. Due to a partial access of the PG to pasture in wk 2-4 we observed a decrease in DMI from TMR over the first four weeks of the trial. A decrease in BW, BCS and milk production indicates that a lower TMR intake and a higher energy demand due to grazing activity were not compensated by an adapted intake from pasture. This proposition is supported by a study of Bargo et al. (2002) describing a lower milk production and DMI in a feeding system combining TMR and pasture, compared to TMR-only. When the PG switched completely onto a full grazing ration in wk 5 milk production and BW dropped significantly, indicating a further decrease in DMI. The continuous decrease in BCS, an increased milk fat content in wk 6 and decreased serum glucose concentrations, increased serum BHBA and fatty acids concentrations in wk 6-9 clearly suggest that during the first weeks on pasture an energy deficit was present and lipomobilisation occurred. This is supported by several studies that have observed a lower BCS (Washburn et al., 2002; Fontaneli et al., 2005) and higher blood BHBA and fatty acids concentrations (Kolver and Muller, 1998; Bargo et al., 2002) in cows in grazing compared to confinement systems. In grazing systems especially cows of high yielding breeds are often supplemented with concentrates to counterbalance an energy deficit (Mayne et al., 2000; Bargo et al., 2003). Additionally, other management approaches such as different grazing systems are implemented to maximize DMI (Mayne et al., 2000). Further research is needed to investigate whether an appropriate supplementation or different grazing system would have attenuated the observed energy deficit.

To estimate DMI the exclosure cage as well as the n-alkane method were employed. The exclosure cage method resulted in much higher DMI estimations compared to the n-alkane method. A difference in DMI of approximately 8 kg DM indicates that depending on the method used, enormous variation in DMI estimations are possible. Comparing milk yield, body condition and blood metabolite data in this trial with the estimated DMI from the different methods we conclude that the exclosure cages most likely overestimated the DMI from pasture. To estimate the validity of the n-alkane method, the DMI to cover maintenance and milk production was calculated according to GfE (2001) guidelines (Schären et al., unpublished data) and compared with the estimated DMI. A difference of -1.6 kg DM/d in wk 7 and 0.3 kg DM/d in wk 9 was calculated by subtracting the calculated DMI (GfE) from the estimated DMI (n-alkane). As illustrated in the previous section, other traits measured during the trial suggest an energy deficit in the PG during the full grazing period. Combining this observation with the fact that the difference between the calculated DMI (GfE) and estimated DMI (n-alkane) was negative in wk 7.
we conclude that the n-alkane methods seems to be a fairly accurate method to estimate DMI from pasture. This observation is supported by other studies who have compared different techniques to estimate herbage intake (Reeves et al. (1996); Smit et al. (2005); reviewed in Decruyenaere et al. (2009)).

As expected the estimated DMI using the n-alkane method revealed a lower DMI in the PG compared to the CG in wk 7 and wk 9. DMI estimates from both exclosure cages and n-alkane method show an increase in DMI from pasture from the first half (wk 5-7) to the second half of the full grazing period (wk 8-10). In wk 1, wk 5 and wk 10 we weighed the total rumen content of the rumen fistulated cows and observed a decrease of 15 kg rumen content between wk 1 and wk 5 and an increase of 20 kg in wk 10 compared to wk 5 in the PG (Schären et al., unpublished data). This observation additionally underlines that the sudden decrease and later increase of BW in the PG was apart from tissue mobilization due to a rapid decrease and later continuous increase of DMI. No further decrease in milk yield, a decrease in serum fatty acids concentrations and an increase in BW to almost initial state in wk 10 in the PG suggests that an adaption of grazing behavior has led to a higher DMI and a decrease in energy deficit in the PG during this period. The further decrease in BCS and increased serum BHBA concentrations > 1.00 mmol/l however indicate that during this period tissue mobilization was still ongoing. At present the functional interrelations between the variables serum BHBA, fatty acids and triglyceride concentrations, and BCS during that period in the PG cannot be finally elucidated. It might be concluded that the increased serum triglyceride and concurrently decreased serum fatty acids concentrations are a sign of an increased rate of triglyceride synthesis in the liver (Drackley et al., 2001). A recent study by McCarthy et al. (2015) has shown that serum BHBA and fatty acids concentrations correlate poorly, which might be another explanation for our observation. In addition, since we observed an increase of approx. 45 % in butyrate concentration in rumen fluid over the course of the trial (from 10.3 mmol/L in wk 1, to 12.3 mmol/L in wk 5-7, and to 14.9 mmol/l in wk 8-10, P < 0.01, n = 5, PSEM = 0.65, LSMeans; Schären et al., unpublished data) we suggest that the increased serum BHBA concentrations might be attributed to an increased ketogenesis in the rumen epithelium, rather than a subclinical ketosis (Baird, 1981; Baldwin and Jesse, 1996). However, we conclude that to further elucidate the length and extent of the energy deficit in the PG a prolongation of the experimental period would have been necessary.

Since the cows were in the second half of their lactation a continuous increase in body condition was expected in the CG. But instead, especially in wk 8-10, a decrease in BCS combined with a stronger decrease in milk yield than expected was observed. During the trial grass silage from
two different silos was fed. Feed analysis after the trial revealed a poor grass silage quality in both silos. The CP of the grass silage fed in wk 1-4 exhibited a CP content of 151 g/kg DM and the grass silage fed in wk 5-10 of 111 g/kg DM. Consequently, the CP content of the TMR was reduced and the ruminal N balance (RNB) was negative (-2.1 and -3.7 g/kg DM), especially in the second half of the trial. We assume that the low CP content and a poor silage quality have led to a decrease in body condition and milk yield in wk 8-10 in the CG. No significant increase in serum fatty acids concentrations indicates that no extensive lipomobilisation occurred. Nevertheless, serum BHBA concentrations were significantly elevated in that period, probably either due to a subclinical ketosis due to a nutritional misbalance, or increased silage butyrate concentrations (LeBlanc, 2010). A rather unexpected observation is the concurrent increase in serum urea concentration in wk 8-10. We presume that due to an inadequate fermentation AA degradation occurred, leading to an increased amount of N available under the form of ammonia instead of AA or peptides in the grass silage fed. As a consequence, efficiency of microbial synthesis was possibly hampered (Kim et al., 1999; Dijkstra et al., 2007) and excess ammonia and non-limiting AA were metabolized and excreted, leading to increased serum urea concentrations. Another possible explanation is an increased tissue mobilization and a concurrent decrease in milk protein yield leading to an increase in hepatic deamination of mobilized AA from peripheral tissue (Wheelock et al., 2010).

Several studies have illustrated the decreasing effect of fresh grass and legumes on milk fat synthesis in dairy cows (White et al., 2001; Morales-Almaraz et al., 2010; Wiking et al., 2010). This effect was also observed in this study as soon as the PG was on a full grazing ration (wk 5). We hypothesize that the milk fat concentration decreasing effect of the unsaturated fatty acids in fresh grass was partially compensated by the lipomobilising effect of the energy deficit also present at that time. This is supported by an increase in wk 6 and continuous decrease in milk fat concentration in wk 7-10.

We did not observe any effect of an increased CP intake in the PG on milk protein concentrations, but as expected the excess ingested CP led to a significant increase in milk and serum urea concentrations. This concept of an increased N excretion is further supported by an increased urine total N to creatinine ratio in the PG in wk 4 and wk 10 and is a known phenomenon in dairy cows on a pasture- compared to a TMR-based ration (Kebreab et al., 2002; Keim and Anrique, 2011).

We observed an increased urine total N to creatinine concentration in wk 7 in the PG as well the CG and no significant difference between groups was present due to high standard deviations at
that point in time. The same pattern was observed for the PD to creatinine ratio. Even though earlier studies have confirmed the urine spot sampling, even throughout the day, as a valid method to estimate the total creatinine and PD output (Chizzotti et al., 2008), several factors in the present study could have affected the creatinine metabolism and thereby influencing the interpretability of the results. For example, urine creatinine concentrations halve in the PG from wk 4 to wk 5, suggesting that the PG ingested more fluid due to a lower DM content of grass and a higher direct water intake. Another aspect that should be considered is that simultaneously the muscle activity of the PG increased, possibly influencing the creatinine metabolism in addition (Baxmann et al., 2008). In the CG the urine creatinine concentration decreases substantially in wk 6-8 as well, indicating a higher water intake due to increased ambient temperature. This was confirmed by a significant correlation of urine creatinine concentrations with water intake. Various studies have discussed the THI threshold for heat stress in dairy cows. The general agreement is that it is localized somewhere between 60-70 depending on the climate zone, housing system and dairy breed (Kellaway and Colditz, 1975; Zimbelman et al., 2009; Gorniak et al., 2014). In wk 7 the THI was on average 6.6 points higher compared to wk 1, wk 4 and wk 10 indoors and outdoors, indicating possible heat stress for the CG as well as PG in that period. Kellaway and Colditz (1975) and Wheelock et al. (2010) describe an increased muscle catabolism and decreased N retention in an experiment with Holstein Friesian heifers and cows under heat stress. We therefore suggest that the high standard deviation in the urine total N and purine derivates to creatinine ratio in wk 7 was caused by individual differences in the extent of catabolic processes triggered by heat stress. We further suspect that due to difference in sampling days of blood and urine samples, no concurrent evolution of blood and urine urea variables was observed (blood samples were taken at the beginning of each week, whereas urine samples were collected throughout the week). We also observed a strong influence of the weather conditions on serum glucose concentrations. Our observation of a reciprocal relationship between THI and glucose agrees with multiple heat stress models and has been described earlier in other studies (summarized in Wheelock et al. (2010)). It is assumed that glucose is the favored fuel for heat-stressed animals since adipose tissue mobilization probably causes an increase in metabolic heat production (Wheelock et al., 2010).

In our study we observed a decline in urine pH in the PG from 8.03 in wk 1 to as low as 7.39 in wk 9. Roche et al. (2000) described that throughout the year the urine pH of dairy cows on a pasture-based diet was generally between 8.0 and 8.5, and was only lowered by a very low dietary cation-anion difference (DCAD <+15 mequiv/100 g) at certain times in the year. Given that we did not quantify the DCAD of the TMR nor the pasture we can only speculate that the
DCAD of pasture must have been lower than that of the TMR. However, since a urine pH in that range is considered physiological we conclude that no adverse health effect of either ration can be deduced from urine pH measurements during the trial.

In agreement with our observations several studies have described a lipomobilisation and higher metabolic urea concentrations due to an energy deficit and an excess CP intake in pasture-based rations (Kolver and Muller, 1998; Bargo et al., 2002; Fontaneli et al., 2005). The aim of this study was to investigate if this increased metabolic load could imply a negative effect on liver health. No relevant increase was observed for γ-GT, GLDH, AST and bilirubin, demonstrating that the increased N conversion in the liver and consequently increased metabolic urea concentrations did not have any negative effects on liver cell vitality. In the PG a decrease in serum cholesterol and γ-GT concentrations were measured in wk 10. Dänicke et al. (2014) described a positive linear relationship between cholesterol and γ-GT in bovine serum under the influence of an increase in concentrate feed proportion. It was discussed that the increase in γ-GT might be a reflection of the increased cholesterol associated bile acid turnover. Since in the present trial the total daily lipid intake did not differ between groups a different explanation would be that an increase in PUFA intake due to the pasture-based ration caused serum cholesterol to decrease and consequently the γ-GT activity to decrease as well. Ruminants receiving a pasture-based generally exhibit a higher cis-9, trans-11 CLA concentration in milk (White et al., 2001) and adipose tissue (Fincham et al., 2009) due to higher PUFA concentrations in pasture compared to TMR-based diets. Reklewska et al. (2002) showed that when supplementing linseed (α-linolenic acid/polyunsaturated fatty acid source) in a TMR, the proportion of unsaturated fatty acids increases and the cholesterol level in the milk decreases. Unfortunately, studies illustrating the influence of an increased PUFA intake on blood metabolites, especially with a focus on pasture-based diets and cholesterol, are lacking.

To identify other possible influences of ration and housing system on the health status we also measured immunological variables such as serum protein and albumin concentrations, white blood cell and milk SCC. Neither significant group effects nor biologically significant changes were observed at any time.

Red blood cell count revealed higher RBC, HCT and HGB concentrations in the PG during the full grazing period compared to the CG. We assume that these differences are possibly caused by increased activity of the PG due to grazing and walking, as well as differences in climate conditions indoors and outdoors (Lee et al., 1976; Hays et al., 1978; Fisher et al., 1980). But since the differences between groups are relatively small, we do not ascribe these observations
a great biological significance. Changes in MCV, MCH, MCHC, RDW and PDW did not reveal any conclusive differences between groups.

**Conclusion**
We hypothesized that the change from a confinement to a pasture based system involves complex nutritional and metabolic adaptations with consequences on health and performance. This was confirmed by a lower DMI causing an energy deficit and lipomobilisation during the transition from a TMR- to a pasture-based ration. These alterations in energy metabolism resulted in a lower milk production and loss in BW and BCS. After an initial sudden decrease DMI, milk production and BW increased continuously again in the PG, indicating a metabolic as well as behavioral adaptation. Further research is needed to investigate whether an appropriate supplementation or a different grazing system would have attenuated the extent of the energy deficit observed.

Due to a higher CP intake in the PG an increase in metabolic urea concentrations as well an increase in N excretion was observed. Metabolic health indicators such as clinical chemistry and total blood cell counts revealed no biological significant changes. We can therefore conclude that neither an increased exposure to higher CP feed intakes, nor the observed lipomobilisation, nor any other attribute of the pasture-based system had any short term negative effect on liver health.

**Acknowledgements**
The authors thank the “Niedersächsisches Ministerium für Wissenschaft und Kultur” (Hannover, Germany) for financial support. Thanks are also due to the „Versuchsstation Haus Riswick“ (Kleve, Germany) for scientific and material assistance associated with the pasture management. We thank Dirk Albers of the “Landwirtschaftskammer Niedersachsen” for excellent consultancy and collaboration. We further thank Jan Dijkstra for fruitful scientific discussions and exchange. Many thanks go to Berit Greune for assisting us in the pasture species assessment. Furthermore, the assistance of the coworkers at the Institute of Animal Nutrition and the experimental station of the Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany, in caring for the experimental animals, assisting with experimental measurements as well as performing the analyses is gratefully acknowledged.
References


Tables and Illustrations

Table 1. Ingredients of concentrates

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentrate (in % DM)</th>
<th>TMR CG</th>
<th>TMR PG</th>
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\(^1\)CG = confinement group; PG = pasture group.

\(^2\)Per kilogram of mineral feed: 140 g of Ca; 120 g of Na; 70 g of P; 40 g of Mg; 6 g of Zn; 5.4 g of Mn; 1 g of Cu; 100 mg of I; 40 mg of Se; 25 mg of Co; 1,000,000 IU of vitamin A; 100,000 IU of vitamin D\(_3\); and 1,500 mg of vitamin E.
<table>
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1CG = confinement group; PG = pasture group; DM = dry matter (g/kg); CP = crude protein; uCP = utilisable crude protein; NEL = netto energy lactation; RNB = ruminal nitrogen balance; CF = crude fiber; NDF_{om} = neutral detergent fiber; ADF_{om} = acid detergent fiber; EE = ether extract. NDF and ADF were expressed without residual ash and are therefore referred to as NDF_{om} and ADF_{om}. All components are indicated in g/kg DM except NEL (MJ/kg DM).
Table 3. Effect of a ration change from TMR to pasture on blood count

<table>
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<tr>
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a,bDifferent letters indicate difference between weeks within particular groups (P ≤ 0.05); † P ≤ 0.10; * P ≤ 0.05; ** P ≤ 0.01.
1WBC = white blood cells (10³/µl); LY = lymphocytes (10³/µl); MO = monocytes (10³/µl); GR = granulocytes (10³/µl); EO = eosinophils (10³/µl); RBC = red blood cells (10⁹/µl); HGB = hemoglobin (g/dl); HCT = hematocrit (%); MCV = mean corpuscular volume (fl); MCH = mean corpuscular hemoglobin (pg); MCHC = mean corpuscular hemoglobin concentration (g/dl); RDW = red blood cell distribution width (%); PDW = platelet distribution width (%); CG = confinement group (n = 31); PG = pasture group (n = 29);
2The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
3PSEM = pooled standard error of the mean.
Table 4. Effect of a ration change from TMR to pasture on blood clinical chemistry variables

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Different letters indicate differences between weeks within particular groups, (P ≤ 0.05).

1γ-GT = γ-glutamyltransferase; GLDH = glutamate dehydrogenase (IU/l); CG = confinement group (n = 31); PG = pasture group (n = 29).

The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.

PSEM = pooled standard error of the mean;

† P ≤ 0.10; * P ≤ 0.05; ** P ≤ 0.01
Figure 1: Outdoor daily average ambient temperature (solid line) with minima and maxima (dotted lines), outdoor humidity (small dashed line), outdoor THI (dashed line) and indoor THI (dash-dot line). THI = temperature humidity indices; calculated according to Hahn (1999); THI = 0.8 td + RH*(td - 14.4) + 46.4, where td = dry bulb temperature (°C) and RH = relative humidity.
Figure 2. Effect of a ration change from TMR to pasture on DMI and water intake. Dashed line = DMI from TMR; ▲ = DMI from pasture (PG) estimated using the n-alkane method (PSEM = 0.3); solid line = water intake recorded in barn (PSEM = 1.9). ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: DMI: group (G): P < 0.001, time (T): P < 0.001, GxT: P < 0.001; water intake: group: P < 0.01, time: P < 0.001, GxT: P < 0.001; stars indicate significant differences between groups in particular week; ** P ≤ 0.01; different letters indicate significant differences between weeks within particular groups (P ≤ 0.05). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 3. Effect of a ration change from TMR to pasture on milk yield (small dashed line; PSEM = 0.6), fat % (dashed line; PSEM = 0.10) and protein % (solid line; PSEM = 0.03). ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: milk yield: group (G): $P < 0.05$, time (T): $P < 0.001$, GxT: $P < 0.001$; fat %: group: $P = 0.01$, time: $P < 0.001$, GxT: $P < 0.001$; protein %: group: n.s., time: $P < 0.001$, GxT: $P < 0.001$; different superscripts indicate significant differences between groups in particular week; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; different letters indicate significant differences between weeks within particular groups ($P \leq 0.05$). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 4. Effect of a ration change from TMR to pasture on BW (solid line; PSEM = 8) and BCS (dashed line; PSEM = 0.11). BW was assessed daily and a weekly mean was calculated per cow. BCS was assessed at the beginning of the week in 14-day intervals. ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: BCS: group (G): P < 0.05, time (T): P < 0.001, GxT: P < 0.001; BW: group: P < 0.01, time: P < 0.001, GxT: P < 0.001; different superscripts indicate significant differences between groups in particular week; † P ≤ 0.10; * P ≤ 0.05; ** P ≤ 0.01; different letters indicate significant differences between weeks within particular groups (P ≤ 0.05). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 5. Effect of a ration change from TMR to pasture on serum glucose (solid line; PSEM = 1.5) and BHBA (dashed line; PSEM = 0.04) concentrations. ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: BHBA: group (G): $P < 0.01$, time (T): $P < 0.001$, GxT: $P < 0.001$; glucose: group: $P < 0.10$, time: $P < 0.001$, GxT: $P < 0.01$; stars indicate significant differences between groups in particular week; ** $P \leq 0.01$; different letters indicate significant differences between weeks within particular groups ($P \leq 0.05$). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 6. Effect of a ration change from TMR to pasture on serum triglyceride (no line; PSEM = 0.6) and fatty acids (dashed line; PSEM = 0.02) concentrations. ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans.; Significance: triglyceride: group (G): $P < 0.001$, time (T): $P < 0.001$, GxT: $P < 0.001$; fatty acids: group: $P < 0.001$, time: $P < 0.001$, GxT: $P < 0.001$; stars indicate significant differences between groups in particular week; ** $P \leq 0.01$; different letters indicate significant differences between weeks within particular groups ($P \leq 0.05$). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 7. Effect of a ration change from TMR to pasture on serum urea concentration (dashed line; PSEM = 1.4) and urine N to creatinine ratio (no line; PSEM = 0.5). ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: serum urea: group (G): \( P < 0.001 \), time (T): \( P < 0.001 \), GxT: \( P < 0.001 \); urine N:creatinine: group: \( P < 0.001 \), time: \( P < 0.001 \), GxT: \( P < 0.001 \); stars indicate significant differences between groups in particular week; ** \( P \leq 0.01 \); different letters indicate significant differences between weeks within particular groups (\( P \leq 0.05 \)). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 8. Effect of a ration change from TMR to pasture on urine creatinine concentrations (dashed line; PSEM = 0.05) and urine purine derivatives (PD) to creatinine ratio (solid line; PSEM = 0.20). ● = confinement group (CG, n = 31); ■ = pasture group (PG, n = 29). LSMeans. Significance: urine creatinine: group (G): $P < 0.001$, time (T): $P < 0.001$, GxT: $P < 0.001$; urine PD:creatinine: group: $P < 0.05$, time: $P < 0.001$, GxT: $P < 0.001$; stars indicate significant differences between groups in particular week; ** $P \leq 0.01$; different letters indicate significant differences between weeks within particular groups ($P \leq 0.05$).

The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
4. The Effects of a Ration Change from a Total Mixed Ration to Pasture on Rumen Fermentation, Volatile Fatty Acid Absorption Characteristics and Morphology of Dairy Cows

Authors: M. Schären,* G. M. Seyfang,† H. Steingass,‡ K. Dieho,‡ J. Dijkstra,‡ L. Hüther,*, J. Frahm,*, A. Beineke,§ D. von Soosten,*, U. Meyer,*, G. Breves,# and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany

†Institute of Animal Science, University of Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany

‡Animal Nutrition Group, Wageningen University, De Elst 1, 6708WD Wageningen, The Netherlands

§Institute of Pathology, University of Veterinary Medicine Hanover, Bünteweg 17, 30559 Hannover, Germany

#Department of Physiology, University of Veterinary Medicine Hanover, Bischofsholer Damm 15, 30173 Hannover, Germany

State of publication: Accepted for publication at the Journal of Dairy Science on January 3rd 2016

Contribution of authors:

- Head of organization and execution: MS, UM, SD, GB
- Trial and project design: MS, UM, SD, GB, KD, JD, GS, HS, LH, JF
- Trial implementation and sample collection: MS, GS, DvS
- Sample analysis: LH, AB, GB, MS, JF
- Data analysis and interpretation: MS, GS, KD, JD, SD, UM, GB
- Writing of manuscript: MS
- Revision of manuscript: SD, KD, JD, GS, HS, UM, DvS, LH, AB, GB, JF

Own contribution: 80 %
The Effects of a Ration Change from a Total Mixed Ration to Pasture on Rumen Fermentation, Volatile Fatty Acid Absorption Characteristics and Morphology of Dairy Cows


*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Institute of Animal Science, University of Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany
‡Animal Nutrition Group, Wageningen University, De Elst 1, 6708WD Wageningen, The Netherlands
§Institute of Pathology, University of Veterinary Medicine Hanover, Bünteweg 17, 30559 Hannover, Germany
#Department of Physiology, University of Veterinary Medicine Hanover, Bischofsholer Damm 15, 30173 Hannover, Germany

Accepted on January 3rd 2016 and in press at the Journal of Dairy Science
http://dx.doi.org/10.3168/jds.2015-10450

Abstract
To investigate the effect of the change from a concentrate and silage- (TMR) to a pasture-based ration a 10-week trial (wk 1-10) was performed, including 10 rumen- and duodenum-fistulated German Holstein dairy cows (182 ± 24 DIM, 23.5 ± 3.5 kg milk/d; mean ± SD). The cows were divided in either a pasture group (PG, n = 5) or a confinement group (CG, n = 5). The CG stayed on a TMR-based ration (35 % corn silage, 35 % grass silage, 30 % concentrate; dry matter (DM) basis), while the PG was gradually transitioned from a TMR to a pasture-based ration (wk 1: TMR-only, wk 2: 3 h/d on pasture, wk 3 and 4: 12 h/d on pasture, wk 5-10: pasture-only). Ruminal pH, volatile fatty acids (VFA), NH₃-N and lipopolysaccharide (LPS) concentrations were measured in rumen fluid samples collected medially and ventrally on a weekly basis. Ruminal pH was continuously recorded during 1-4 consecutive days each week using ruminal pH measuring devices. In wk 1, wk 5 and wk 10 rumen contents were evacuated and weighed.

Corresponding author: Ulrich.Meyer@fli.bund.de
papillae were collected from three locations in the rumen, and subsequently a VFA absorption test was performed. In the PG mean rumen pH and molar acetate proportions decreased, and molar butyrate proportions increased continuously over the course of the trial, which can most likely be ascribed to an increased intake of rapidly fermentable carbohydrates. During the first weeks on a full grazing ration (wk 5-7) variation of rumen pH decreased and in wk 5 a lower rumen content, papillae surface area and potential for VFA absorption was observed. In wk 8-10 variation of rumen pH and total VFA concentrations increased again, and acetate/propionate ratio decreased. In wk 10 rumen content, papillae area and VFA absorption characteristics similar to initial levels were observed. Although continuous rumen pH assessments and LPS concentrations did not reveal an increased risk for subacute rumen acidosis (SARA) during the adaption period, histopathology of rumen papillae and potential for VFA absorption indicated a possible risk for rumen health. An increased risk for SARA was observed in wk 9 and wk 10 in the PG, but rumen LPS concentrations and histopathology were not adversely affected. Results of the present study suggest that after behavioral and metabolic adaptation to the transition from a TMR to a pasture-based ration, no adverse effects on rumen morphology and absorption capacity occurred, although rumen pH after adaptation to pasture indicated increased risk of SARA.

Key words: pasture, ration change, rumen papillae morphology, rumen VFA absorption characteristics

Introduction
Upon transition from a silage and concentrate- to a pasture-based diet, dairy cattle and their rumen microbiota need to adapt to this new nutritional situation (de Menezes et al., 2011; Nakano et al., 2013). Dry matter intake is generally lower in pasture-based systems due to physical constraints, and energy expenditure is higher due to grazing and walking activity (Osuji, 1974; Kolver, 2003). The ration composition differs considerably between the two systems (Kolver, 2003) with the pasture-based ration generally characterized by a higher CP and water soluble carbohydrate (WSC) content and lower starch content (Kolver and de Veth, 2002; Kolver, 2003). Additionally, protein and energy availability in pasture-based rations are subjected to seasonal, weekly and even daily variations caused by changes in plant maturity and weather, as well as management decisions (Parker and Edwards, 1996; Mayne et al., 2000; Smit et al., 2004).
Few studies have investigated the difference in rumen fermentation patterns comparing TMR and pasture fed dairy cows. Generally higher rumen ammonia concentrations were observed when a pasture-based ration was fed, but results are inconclusive regarding ruminal pH and VFA concentrations (Holden et al., 1994; Bargo et al., 2002a; Bargo et al., 2002b). Rumen digesta stratification and intraruminal differences in pH and VFA concentration are influenced by feed fiber content and particle length (Storm and Kristensen, 2010). Diets with a higher fiber content and longer particle length promote ruminal stratification (Tafaj et al., 2004; Storm and Kristensen, 2010). Storm and Kristensen (2010) hypothesized that feeding a low fiber diet could result in a more homogenous ruminal content and thereby increasing ventral VFA concentrations and increasing the risk of ruminal acidosis. Since high quality pastures are often low in physical effective fiber and high in concentrations of WSC, pasture-based rations may adversely affect rumen fermentation and pH (Kolver and de Veth, 2002; O’Grady et al., 2008). O’Grady et al. (2008) and Bramley et al. (2008) have shown that approximately 10% of cows in pasture-based systems could be classified as being affected by SARA. Most research investigating the relationship of a low ruminal pH and adverse effects on health and production has been conducted in confinement TMR-based systems (Plaizier et al., 2008), and it is unclear if the developed cut-off values for SARA can be translated onto pasture-based systems. Kolver and de Veth (2002) suggested that a low ruminal pH arising from high fermentable OM (fOM) intake and low physical effective fiber does not necessarily compromise cow performance on pasture. This is further supported by several recent studies showing that the consequences of SARA are possibly substrate dependent (Khafipour et al., 2009a; b; Calsamiglia et al., 2012).

Ruminal pH and fermentation patterns are just one aspect of different rumen characteristics that are possibly influenced by a ration change. Also, rumen papillae morphology and absorption capacity (Bannink et al., 2012; Martens et al., 2012; Dieho et al., 2016), histology (Steele et al., 2011; Bannink et al., 2012) and gene expression (Connor et al., 2010; Penner et al., 2011; Steele et al., 2012) is altered under the influence of different ration types and during SARA. For example, slowly increasing the concentrate intake leads to an increase in the size of the papillae and the number of epithelial cells (Dirksen et al., 1984; Liebich et al., 1987). In food-deprived animals, a decrease in fermentable substrate leads to a decrease in absorptive capacity of the rumen wall (Gäbel et al., 1993).

Generally, the transition from one ration type to another, causes changes in the rumen microbiota (Russell and Rychlik, 2001) and rumen stratification (Storm and Kristensen, 2010), which leads to alterations in fermentation patterns (Van Houtert, 1993) and to physiological and structural adaptations of the rumen epithelium (Gäbel et al., 2002). Up to now only little research
has focused on the impact of a transition from a TMR to a pasture-based ration on rumen fermentation, VFA absorption capacity and morphology as well as the length required for adaptation. Since these two systems do not only differ substantially in ration composition, but also in the way feed is acquired we hypothesize that the change from a confinement TMR to a pasture-based system involves complex physiological and structural adaptations of the rumen. We suggest that a pasture-based ration in a continuous grazing system with a relatively short herbage height could lead to smaller intraruminal differences with regard to stratification and fermentation due to its possible lower fiber content and particle length. Further, a high content of fast fermentable carbohydrates and low amount of physical effective fiber could increase the risk for SARA and have adverse effects on rumen epithelium. The aim of the present study was therefore to investigate the influence of the transition from a TMR to a pasture-based diet on several rumen variables including the total rumen content and rumen fermentation characteristics (pH, VFA, ammonia-N (NH₃-N) and lipopolysaccharide (LPS) concentrations), and on VFA absorption as well as on morphological variables including papillae surface area and pathohistological parameters.

**Material and Methods**

Experimental work was conducted at the experimental station of the Friedrich Loeffler Institute (FLI) in Brunswick, Germany. The experiment was carried out in accordance with the German Animal Welfare Act approved by the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Germany).

**Experimental Design and Treatments**

The experimental design, treatments, rations, climate data, animal performance, urine variables, clinical chemistry and total blood counts have been reported in Schären et al. (2016). In brief, the full trial included 60 German Holstein cows (166 ± 23 DIM and 23.5 ± 3.7 kg milk/d; parity: 1.9 ± 1.6; mean ± SD; at the beginning of the trial) which were randomly assigned to either a pasture group (PG; n = 29) or a confinement group (CG; n = 31). Each group contained five rumen- and duodenum-fistulated animals (182 ± 24 DIM, 23.5 ± 3.5 kg milk/d; parity: 4.5 ± 2.2; mean ± SD; at the beginning of the trial). The experimental work and data describing rumen variables in the present paper have been exclusively conducted and collected in these 10 animals. The experimental period lasted 10 weeks (wk 1-10) from April 21st until June 27th 2014. The CG stayed on a TMR-based ration (35 % corn silage, 35 % grass silage, 30 % concentrate; DM basis), while the PG was transitioned from a TMR to a pasture-based ration (wk 1: TMR-only, wk 2: TMR and 3 h/d on pasture, wk 3 and 4: TMR and 12 h/d on pasture, wk
5-10: pasture and 1.75 kg DM concentrate/d offered in 2 equal meals in troughs after morning and evening milking). A continuous grazing system was implemented on ryegrass dominated pasture with an average herbage height of 6.6 ± 0.7 cm (mean ± SD; measured 3 times/week using an electronic rising plate meter; manufacturer: RPM F400, Farmworks Systems Ltd., Manawatu-Wanganui, New Zealand) and daily pasture allowances were: wk 5 and wk 6: 24.2 ± 2.2; wk 7 and wk 8: 23.8 ± 4.3; wk 9 and wk 10: 16.5 ± 4.5 kg DM/cow/d (estimated from undisturbed average grass growth using exclosure cages compared with a reference area next to the cage; described in detail in Schären et al. (2016); adverse weather conditions in wk 7 and wk 8 caused low herbage allowance in wk 9 and 10). The cows were milked two times per day at 0530 h and 1500 h. The TMR was fed daily at approximately 1100 h and individual DMI of TMR was continuously recorded (electronic weighing troughs, manufacturer: Insentec, B.V., Markenesse, The Netherlands). In the PG, individual DMI of pasture was estimated in wk 7 and wk 9 using the n-alkane method (described in detail in Schären et al. (2016). Body weight was measured twice daily after milking.

**Rumen pH and Fluid Composition**

Rumen fluid samples were collected once per week after morning milking. To prevent substantial grazing activity prior to sampling cows of the PG were rounded up for milking just before sunrise (Taweel et al., 2004; Abrahamse et al., 2009). To collect rumen fluid from the medial site the rumen mat content from the first 10 cm below the aperture of the rumen fistula was collected and pressed through a cheesecloth. Rumen fluid from the ventral site of the rumen was collected using a manual pump. Immediately after collection, pH was measured using a glass electrode (model: pH 525; WTW, Weilheim, Germany) and samples were cooled to 4 °C until further processing approximately 1-2 h after sample collection. Volatile fatty acids were determined as described in Geissler et al. (1976) and NH₃-N was determined using steam distillation according to the Kjeldahl method (DIN38406-E5-2, Anonymous (1998)). For determination of LPS concentration, rumen fluid samples were centrifuged at 10,000 × g in pyrogen-free tubes for 30 min. Thereafter, supernatants were passed through a 0.22-µm filter, heated for 30 min at 100 °C, and stored at -20 °C pending further analysis. Prior to analysis supernatants were diluted with endotoxin-free water at approximately 1:32,000 v/v. Lipopolysaccharide concentrations were measured spectrophotometrically at 405 nm using the Limulus amebocyte lysate (LAL) assay (Kinetic-QCLTM, Lonza, Walkersville, MD, USA; following the manufacturer’s instructions), a microplate reader with incubation chamber (Infinite M200, Tecan Group Ltd., Männedorf, Switzerland) and evaluated using the MagellanTM Data Analysis Software (Tecan Group Ltd., Männedorf, Switzerland)(Gozho et al. 2005).
Rumen content pH was continuously measured in the ventral rumen sac using a continuous ruminal pH measuring device in wk 1-10 in the PG and in wk 3-10 in the CG (Lethbridge Research Centre Ruminal pH Measurement System, Dascor, Escondido, CA, USA; Penner et al. 2006). No continuous rumen pH data were collected in wk 1 and wk 2 in the CG due to technical issues at the time. Before and after each period the system was calibrated in buffer solutions (pH 4 and pH 7) at 39 °C. Ruminal content pH was recorded every minute and measured of each cow between 1-4 consecutive 24-h periods each week (2.68 ± 0.99; mean ± SD). For each 24 h interval a logistic curve was fitted (AlZahal et al. (2007) using PROC NLMIXED in SAS 9.3 (SAS Institute 2011, Cary, NC, USA) and the variables β0 (the slope of the logistic curve at the inflection point, illustrating the variation in rumen pH over the assessed 24 h interval), β1 (describing the inflection point of the curve, representing the average pH of the assessed 24 h period) and time pH < 5.6 and pH < 5.8 (min/d) were assessed as described in Colman et al. (2012). To evaluate a possible increased risk for SARA a threshold of 314 min at pH < 5.8/d and average pH lower than 6.16 was chosen (Zebeli et al., 2008). To allow a representative interpretation the SARA risk was evaluated on basis of LSMeans on group level and based on a scoring system on individual basis. The score per group and week was calculated as: score = [sum of (number of positive SARA observations per animal in week i/total number of observations per animal in week i)]/total number of animals assessed in week i. This approach was chosen since the amount of measurements and assessed animals differed between weeks. Animals were not exposed to SARA challenges prior to this trial.

**Rumen Content**
In wk 1, wk 5 and wk 10 the rumen of each cow was evacuated by hand and total rumen content was separated into fluid and solid content using a self-made sieve with 10 mm aperture (2-3 cows per day between 0730 h and 1430 h; all cows within 5 days within particular week). Samples were collected of the solid and liquid content and stored at -20 °C pending analyses. Of each sample the DM content was assessed to determine the total rumen DM and non-DM quantity. Both fractions were weighed separately, combined again thereafter, and kept in insulated barrels to prevent cooling.

**Rumen Papillae Collection**
After evacuation the rumen was washed twice with 10 L of water (39 °C) and remaining fluid was removed using an industrial vacuum cleaner. Thereafter, papillae were collected at three different sites in the rumen (saccus caecus caudodorsalis, saccus ventralis and saccus caecus caudoventralis; always approximately 5 cm adjacent to the pila coronaria dorsalis or pila coronaria ventralis, respectively, at the most ventral site of the respective location) using a
biopsy forceps (Lloyd-Davis biopsy forceps 35cm, Zepf Instruments, Tuttlingen, Germany). Papillae samples were immediately washed in 0.9 % NaCl and stored in 4 % formaldehyde. Per location 14.6 ± 4.5 (mean ± SD) intact papillae were collected. Subsequently the papillae were photographed and the surface area (one side) was determined using the CellProfiler® (Broad Institute, Cambridge, MA, USA) software package. Thereafter, the rumen papillae were histopathologically examined for the presence of inflammation. To evaluate the samples representatively we grouped the samples into either “absence of lesions” and “presence of lesions” for statistical analysis and graphical illustration.

VFA Absorption Test
Subsequent to the papillae collection a VFA absorption test (VFA-AT) was performed as described by Dijkstra et al. (1993). A total of 36.5 ± 0.4 L of a VFA buffer solution (pH 5.0 ± 0.1; 400 mOsm/L; 39 °C) was prepared based on McDoughall’s buffer (Dijkstra et al., 1993), containing additionally 170 mM VFA (60 % acetic, 25 % propionic, 15 % butyric acid) and a marker (Co-EDTA, 0.07 g/L). The rumen was washed with 5 L of the buffer solution and remaining fluid was removed using an industrial vacuum cleaner. Thereafter 31.5 L of the buffer solution was introduced and a buffer solution sample was collected. After 60 min of incubation another buffer solution sample was collected and the buffer solution completely recovered and weighed. The pH and liquid volume were measured and the samples were stored at -20 °C. During the VFA-AT the pH of the buffer solution was assessed manually every 15 min from a 100 ml sample, which was reintroduced into the rumen immediately after measuring. In wk 10 an indwelling and recording pH probe (inPro® 3100/120/Pt100 combination pH electrode, Mettler Toledo, Giessen, Germany; mobile pH recording device PCE-228, PCE Deutschland GmbH, Meschede, Germany) was used to measure the buffer solution pH continuously during the incubation period in 9 animals (n = 5 CG; n = 4 PG, data of one animal was lost due to technical issues). Finally, the rumen content was reintroduced. Buffer solution VFA concentrations were determined as described in Geissler et al. (1976). Buffer solution cobalt concentrations were measured using ICP-OES (inductively coupled plasma optical emission spectrometry; Quantima, GBC Scientific Equipment Pty Ltd, Victoria, Australia). The water inflow, fractional liquid passage rate (FLPR), and fractional absorption rates of acetic (FAR C2), propionic (FAR C3) and butyric acids (FAR C4) were calculated according to Dijkstra et al. (1993).

Statistical Analysis
If variables were recorded more than once a week, means were calculated per cow and week prior to statistical evaluation. To obtain a normal distribution, LPS concentrations were logarithmically transformed prior to statistical analysis. To analyze repeated measurements
PROC MIXED in SAS Enterprise Guide 6.1 (SAS Institute 2013, Cary, NC, USA) was implemented using the following model [173]:

\[ Y_{ijkl} = \mu + G_i + W_k + (G \times W)_{ik} + C_j + \epsilon_{ijk} \]

and in case of multiple sampling sites the model was extended to:

\[ Y_{ijkl} = \mu + G_i + W_k + S_l + (G \times W)_{ik} + (W \times S)_{kl} + (G \times W \times S)_{ikl} + C_j + W(C)_{jk} + \epsilon_{ijkl} \]

where \( G \) = treatment group (\( i = \text{PG, CG} \)), \( W \) = sampling week (\( k = 1, ..., 10 \)), \( S \) = sampling site (\( l = \text{medial, ventral for pH, VFA, NH}_3\text{-N, LPS; } l = \text{saccus caecus caudodorsalis, saccus ventralis, saccus caecus caudoventralis for biopsies} \), \( (G \times W)_{ik} \) = fixed interaction, \( C_j = \text{cow (} j = 1, ..., 10) \), \( W(C)_{jk} \) = random effects of sampling week within cows, \( \epsilon_{ijk} \) and \( \epsilon_{ijkl} \) = error.

A restricted maximum likelihood method (REML) with the cows as experimental units was used. Week, diet group, sampling site within the rumen (where applicable) and their interaction were defined as fixed factors. A “REPEATED” statement was included to account for individual variation of the cows. Best fitting covariance structures were tested using the Akaike information criterion for a finite sample size (AICC). For the performance data of the animals, first order autoregressive and for all other data compound symmetry covariance structure was chosen. In case of multiple sampling sites, the site was nested within the cow and a “RANDOM” statement was included for cow and cow × week interaction to account for pseudo-replication. Significant effects at different points in time were further evaluated by multiple \( t \)-test (procedure “PDIFF”) and results are presented as least square means (LSMeans) with pooled standard error of means (PSEM). Histopathological scores of rumen papillae were arranged in contingency tables and analyzed by using Fisher’s Exact Tests (PROC FREQ) in SAS Enterprise Guide 6.1. Correlation coefficients between different parameters were estimated using STATISTICA 12.0 (StatSoft, Inc. 2014, Tulsa, Oklahoma, USA). Results were considered significant at \( P < 0.05 \) and a trend declared at \( 0.05 < P < 0.10 \).

**Results**

**Ration Composition and Weather Data**

The chemical composition of the different rations and weather data are presented in detail in Schären et al. (2016). Briefly, the average chemical composition of the TMR of the CG and the PG was: DM content: 330 ± 12 g/kg, CP: 128 ± 4 g/kg DM, net energy lactation (NEL): 6.8 ± 0.1 MJ/kg DM, starch: 262 ± 3 g/kg DM, CF: 204 ± 4 g/kg DM, NDFom: 394 ± 9 g/kg DM and ADFom: 226 ± 5 g/kg DM (mean ± SD). Chemical composition of the pasture was assessed
weekly: DM content: 183 ± 14 g/kg, CP: 193 ± 21 g/kg DM, sugar: 113 ± 32 g/kg DM, CF: 216 ± 8 g/kg DM, NDFom: 525 ± 40 g/kg DM and ADFom: 260 ± 16 g/kg DM (mean ± SD). In wk 7 and wk 10 the highest sugar (174 and 148 g/kg, respectively) and lowest CP contents (159 and 167 g/kg, respectively) were observed. Additionally, pasture NEL contents were assessed in wk 7 (6.7 MJ/kg DM) and wk 9 (6.6 MJ/kg DM). The average daily temperature humidity index (THI) averaged 57.9 ± 5.5 outdoors and was indoors generally 5.1 ± 0.8 (mean ± SD) units higher. Periods of mild heat were measured in wk 5 and between wk 7 and wk 8 with average daily THI between 65 and 70 outdoors and 65 and 75 indoors.

Animal Performance

For the presentation of the results the terms “group”, “time” and “location” were chosen to describe the effect of diet group, week and sampling location within the rumen, respectively. Milk production and body weight changes in the fistulated cows (Table 1) were similar to those in the 60 cows described in Schären et al. (2016). For all variables, except body condition score (BCS), a group×time interaction was observed. Dry matter intake from TMR decreased in the PG as soon as animals had part time access to pasture. In the PG, DMI of pasture and concentrate differed significantly in wk 7, but not in wk 9, from that of TMR in the CG. However, this difference was significant in wk 9 in all 60 animals. Between wk 1 and wk 6, milk yield in PG decreased by 4.3 kg/d followed by stabilization until the end of the trial. In the CG, milk yield decreased more steadily over the course of the trial. A tendency for a higher milk yield in the CG was observed in wk 5. In the PG, milk protein content slightly decreased from wk 4 to wk 5 and wk 6 followed by an increase until wk 9 leading to a tendency for a higher milk protein content compared to the CG in wk 8 and wk 9. Within the CG milk protein content marginally increased between wk 1 and wk 4. In the PG, milk fat content decreased as soon as the cows were on a full grazing ration. Milk fat content in the CG compared with PG was higher in wk 5, wk 9 (tendency only) and wk 10. Milk urea concentration in PG increased from wk 4 onwards, whereas no considerable alterations were observed in the CG. Milk urea concentrations were higher in the PG in wk 4-10 (except for wk 7). Body weight decreased in the PG between wk 1 and wk 6 by 32 kg and continuously increased thereafter until wk 10 to initial status. In the CG, an initial increase of BW by 18 kg until wk 4 followed by a decrease until wk 10 to initial status was observed. A higher BW was observed in the CG compared to the PG between wk 4-7. The decrease of 0.8 BCS points in the PG over the course of, and 0.3 BCS points in the CG during the last weeks of the trial (in all 60 cows) was only numerically reflected in the fistulated cows (no significant group×time interaction). There was however a significant group effect for BCS.
within the fistulated animals, since the BCS in PG was lower than in CG already at the beginning of the trial.

**Rumen pH and Fluid Composition**

**pH – sensor data.** Four main variables (β₀, β₁ and time pH < 5.8 and 5.6 (min/d)) describing the development of rumen pH throughout the trial in the PG and CG are illustrated in Figure 1A. For β₁, representing the average pH of the assessed 24 h period, a group×time interaction was observed. The PG exhibited a decrease between wk 2 and wk 10 (from 6.2 to 6.1), whereas in the CG a continuous increase during the course of the trial was observed (from 6.1 in wk 3 until 6.3 in wk 10). In wk 10, β₁ was significantly lower in the PG compared with the CG. The variable β₀, illustrating the variation in rumen pH over the assessed 24 h interval (the greater, the more constant), exhibited a time as well as group×time effect. In the PG an increase was observed as soon as the animals were on a full-grazing ration in wk 5. Subsequently a decrease in wk 8-10 occurred. In the CG, no considerable variations were observed except for an increase in wk 5. In wk 6 and wk 7, β₀ was higher in PG than in the CG. No significant time or group effect nor a group×time interaction was observed for the variable time pH < 5.8 (min/d). Time pH < 5.6 (min/d) did not exhibit a significant group or group×time effect. However, a significant time effect was observed due to an increase between wk 3 and wk 4, succeeding decrease until wk 7 and increase again until wk 10 in both groups.

No increased risk for SARA was observed on group level at any time during the trial in both groups. On an individual basis, the average score was 0.11 ± 0.08 for the CG and 0.11 ± 0.15 for the PG in wk 3-10 (means ± SD; no wk 1 and wk 2 due to technical problems with measurements in CG at the time). The highest score for CG was 0.30 in wk 4 and lowest in wk 6 (zero). In the PG the highest scores were observed in wk 1 (0.40), wk 9 (0.20) and wk 10 (0.40) and the lowest in wk 2 and wk 6-8 (zero).

**pH – manual.** For the weekly manual pH measurements, a time and location effect, as well as a group×time, group×location, time×location and group×time×location interaction was observed (Figure 1B). pH of rumen fluid samples collected in the ventral part of the rumen was generally 0.52 ± 0.04 higher compared to those collected in the medial part. In wk 1 and wk 3 a higher medial pH was observed in PG than CG, whereas during the full grazing period medial pH was higher in CG than in PG in wk 6, wk 8 and wk 9. A correlation between β₁ and manually assessed pH in the medial and ventral part of the rumen was found (medial: r = 0.40, P < 0.001; ventral: r = 0.30; P = 0.004).
**VFA concentrations and molar proportions.** A group and time effect as well as a group×time interaction was observed for total VFA concentrations due to a significant increase between wk 7-10 in the PG from 95.2 to 100.2 mmol/L (P < 0.05) and concurrent decrease in the CG between the first and second half of the trial (Figure 1 C; from 101.2 ± 2.4 in wk 1-4 to 91.6 ± 3.4 mmol/L in wk 5-10; mean ± SD; P < 0.05). A significant difference between groups was present in wk 3 (CG higher than PG), and wk 6, 8, 9 and 10 (PG higher than CG). Medial and ventral total VFA concentrations correlated with corresponding manually assessed medial and ventral pH (medial: r = -0.78, P < 0.001; ventral: r = -0.65, P < 0.001) as well as β1 (medial: r = -0.60, P = 0.009; ventral: r = -0.41; P = 0.094).

The acetate/propionate (C2/C3) ratio and the molar proportions of VFA are illustrated in Table 2. The C2/C3 ratio decreased in the PG between wk 1 and wk 3 (from 3.90 to 3.23; P < 0.001), increased thereafter until wk 6 (to 3.54; P = 0.082), followed by a decrease until wk 9 (to 3.03; P = 0.005). In the CG, a slight increase was observed over the course of the trial (from 3.21 in wk 2 to 3.58 in wk 9; P = 0.037) contributing to a significant group×time effect. A difference between groups was observed in wk 1 and wk 9 (wk 1 higher in the PG, wk 9 higher in the CG).

Molar acetate proportions (C2%) exhibited a time and location effect as well as a group×time and group×time×location interaction. In the CG, at both locations an initial increase in the first half of the trial (until wk 6), followed by a decrease at the ventral location in the second half was observed (no alterations at medial site). Medial C2% were higher compared to ventral from wk 4 on in the CG (tendency for difference in wk 4 (P = 0.062), significant differences in wk 6-10 (P < 0.05)). In the PG C2% decreased continuously over the course of the trial.

Molar propionate proportions (C3%) only exhibited a location effect due to generally higher C3% at the ventral compared to the medial sampling site (20.0 vs. 19.7 %, P = 0.012).

Molar butyrate proportions (C4%) continuously increased in both groups over the course of the trial (from 12.5 to 13.7 % in the CG and from 11.3 to 13.5 % in the PG between wk 1 and wk 10, P < 0.05), except for a decrease and subsequent increase in wk 6 and wk 9 in the CG, leading to a group×time interaction. A difference between groups was observed in wk 1 and wk 3 (CG higher), and wk 9 (PG higher). Medial C4% were generally lower compared to ventral C4% (12.3 vs 13.0 %, P < 0.001).

Molar isovalerate proportions (iC5%) exhibited a group and time effect as well as a group×time interaction. In the CG, iC5% decreased over the course of the trial (from 1.8 % in wk 1 to 1.1 % in wk 10, P < 0.001). In the PG, an initial decrease until wk 7 (from 1.4 % in wk 1 to 0.5 %, P <
and subsequent increase until wk 10 (0.8 %, P = 0.016) was observed. The CG exhibited higher iC5% than the PG between wk 3-9.

Ventral molar valerate proportions (C5%) were not altered significantly over the course of the trial (0.40 %), whereas medial C5% exhibited a decrease until wk 6 (from 0.50 to 0.22 %, P = 0.002), followed by a subsequent increase until wk 9 (0.70 %, P < 0.001) causing a time and time×location effect.

**NH3-N concentrations.** NH3-N concentrations were influenced by a time effect and group×time, time×location and group×time×location interactions (Figure 1D). No relevant changes were observed in the CG. In the PG a 2-4 folds’ increase was observed in wk 4 and wk 9 medially as well as ventrally leading to significant differences between groups in these weeks.

**LPS.** Both groups showed a similar development of rumen LPS concentrations over the course of the trial as indicated by the significant time effect and absence of any significant interactions (Figure 1E). Between wk 1 and wk 3, a decrease was observed followed by an increase from wk 4 until wk 6. Thereafter LPS concentrations continuously decreased until wk 10. A tendency for a group×time interaction was observed due to a significant difference between groups in wk 5 (CG higher, P = 0.012) and wk 10 (PG higher, P = 0.023). The significant location effect indicated that on average the LPS concentrations were higher ventrally than medially. Medial and ventral LPS concentrations correlated positively with THI (medial, r = 0.45, P = 0.045; ventral, r = 0.52, P = 0.018).

Serum glucose concentrations exhibited a similar development in both groups (PGroup = 0.113, PTime < 0.001, PG×T = 0.427, data not shown; similar development in fistulated and non-fistulated animals; sample collection and analysis, and data described in Schären et al. (2016)) and correlated negatively with rumen LPS concentrations (medial: r = -0.59, P = 0.006, ventral: r = -0.68, P < 0.001).

**Rumen Content**
Total rumen content averaged 84.7 ± 12.8 kg (mean ± SD) with an average DM content of 134 ± 18 g/kg (mean ± SD) over the course of the trial and groups (Figure 2A). Rumen DM content was influenced by a time and group×time effect due to a lower DM content in the PG in wk 5 compared to wk 1 and wk 10 and a lower DM content in PG than CG in wk 5 (7.1 vs. 10.2 kg DM). Within the CG, time did not affect rumen DM content. Rumen non-DM content exhibited a group×time interaction due to a lower non-DM content in the PG in wk 5 compared to wk 1 and wk 10, whereas non-DM content in CG did not differ in time.
**Rumen Papillae Collection**

The average mean papilla surface area was $0.64 \pm 0.07$ cm$^2$ (one side) and exhibited a significant group×time×location interaction (Figure 2B). A lower papillae area was observed in wk 5 in the PG at the location *saccus caecus caudodorsalis* ($P < 0.05$) and *saccus ventralis* ($P = 0.10$) compared to wk 1. In the CG, the mean papillae area decreased over the course of the trial at the locations *saccus ventralis* and *saccus caecus caudoventralis*. Papillae surface area was significantly lower in wk 5 for PG compared with GC at the location *saccus caecus caudodorsalis*. Total rumen DM content and total mean papillae area correlated significantly ($r = 0.54$, $P < 0.001$).

Histopathological analysis of rumen papillae revealed either no visible lesions, or a minimal or moderate focal or multifocal purulent-pustular inflammation in the epithelium, and minimal or moderate focal or multifocal lymphoplasmacellular infiltrates, either with or without neutrophils, in the *lamina propria*. Examples of inflammatory lesions are depicted in Figure 3. A higher number of tissue samples with inflammatory lesions in the PG were observed in wk 5 compared to wk 1, and in wk 5 a higher number of samples with lesions were present in PG than in CG, at the location *saccus caecus caudodorsalis* (Figure 2C).

**VFA Absorption Test**

The pH of the buffer solution increased linearly during the 60 min incubation period and is described as follows: $\text{pH} = 5.01 \pm 0.03 + 0.031 \pm 0.001 \times t(\text{min})$ ($r = 0.96; P < 0.001$). The linearity of the pH increase over time was further confirmed by continuous pH assessment during the incubation period in wk 10 (data not shown). For end pH, time as well as group×time effects were significant (Figure 2D). In the PG, a lower end pH was observed in wk 5 compared to wk 1 and wk 10, and in wk 10 compared to wk 1. In wk 5 end pH was also lower in PG than in CG (6.53 vs. 6.91). In the CG, a decrease in end pH was observed over the course of the trial. Papillae area at the three locations as well as the mean papillae area correlated with the end pH of the buffer solution (*saccus caecus caudodorsalis*: $r = 0.52$, $P = 0.004$; *saccus ventralis*: $r = 0.62$, $P < 0.001$; *saccus caecus caudoventralis*: $r = 0.53$, $P = 0.003$; mean: $r = 0.66$, $P < 0.001$). Buffer solution VFA concentrations at the beginning of the incubation were: $100.2 \pm 5.7$ mmol/L C2, $41.1 \pm 2.6$ mmol/L C3 and $23.4 \pm 1.5$ mmol/L C4 (mean ± SD). For the end concentrations of C2, C3 and C4 a time effect as well as a group×time interaction was observed (Figure 2E). In the PG, higher C2 concentrations were observed in wk 5 compared to wk 1 and wk 10, and in wk 5 C2 concentrations were higher in PG than in CG (71.5 vs. 55.6 mmol/L). In the CG, an increase occurred over the course of the trial. Similar to C2, C3 concentrations were higher in the PG in wk 5 (21.9 vs. 19.2 mmol/L) and increased in the CG between wk 1 and wk 10. For C4
concentrations, no significant differences between groups were present in wk 5, and patterns similar to those of C2 and C3 within groups (increase and subsequent decrease in PG, overall increase in CG) were observed. Papillae area at the location *saccus caecus caudodorsalis* correlated with the end concentrations of C2 and C3 (C2: $r = -0.37$, $P = 0.045$; C3: $r = -0.34$, $P = 0.065$).

Changes in FARs reflected the alterations observed in buffer solution pH and VFA concentrations numerically, but no significant group, time or group×time effects were observed (Figure 2F). Papillae area at the location *saccus caecus caudodorsalis* correlated with the FAR of C2 ($r = 0.34$; $P = 0.065$).

No statistical significant group, time or group×time interaction was observed for water inflow into the rumen and FLPR (Figure 2G and H).

**Discussion**

Recently we reported the influence of a transition from a TMR to a pasture-based ration on production and health traits (n = 60; Schären et al. (2016)). In the current work we present rumen variables and performance data collected in 10 duodenum- and rumen-fistulated cows assessed during this trial. Even though the average parity of the fistulated animals (4.5 ± 2.2) was higher compared to the overall group (1.9 ± 1.6), in general similar alterations in animal performance were observed. Further, none of the animals have been subjected to SARA challenge studies prior to this trial. Data on SARA risk can therefore be regarded as unbiased. The CP content of the TMR was unusually low due to unexpected poor grass silage quality. Its influence on animal performance and health has been discussed in Schären et al. (2016), and its influence on rumen variables is discussed in the relevant section of the present paper. Due to this unexpected, low feed quality in the CG, we decided to place the emphasis of the discussion not only on the comparison between groups, but also on alterations within the PG over time (horizontal development).

In the 60 animals we observed a decrease in milk production, BCS and BW as well as an increase in milk fat content, and in serum BHBA and fatty acid concentrations in the PG during the first weeks on a full grazing ration, indicating an energy deficit. After a few weeks on a full grazing ration serum fatty acid concentrations and milk fat content decreased and BW increased again. We suggested that these alterations may be related to an initially decreased DMI followed by a metabolic and behavioral adaptation leading to an increase in DMI in the second half of the full-grazing period (wk 8-10). This was supported by the fact that the measured DMI on pasture
in wk 7 and wk 9 (using the n-alkane method) were lower compared to the CG and exhibited an increase between the points in time.

We hypothesized that this ration change from a TMR to a pasture-based ration involved complex physiological and structural adaptations of the rumen. This was confirmed by alterations observed in various rumen variables presented in the current work. The lower rumen content in PG compared with CG in wk 5, which did not occur in wk 1 and wk 10, is in line with the assumption that DMI first decreased substantially as soon as the cows did not have access to a TMR indoors anymore and increased thereafter again. Increased clearance rate from the rumen of grass compared with TMR may also have resulted in reduced rumen contents in wk 5. However, since total rumen content in wk 10 did not differ between PG and CG, it seems unlikely that the lower rumen fill in wk 5 can predominantly be ascribed to a faster fermentation and passage of grass compared to TMR in the rumen, and a reduced DMI in wk 5 is a more likely explanation. We assume that this change in DMI in the PG group and thereby intake of fOM led to alterations in fermentation patterns and VFA yield, which is reflected in the initial decrease of variation in rumen pH in wk 5-7 and later increase in wk 8-10. We further assume that due to the increased intake of grass (being a fast fermentable substrate) throughout the trial, rumen average daily pH decreased continuously between wk 2 and wk 10 in the PG. This is also mirrored in total VFA concentrations, which increased in the PG from wk 7 onwards, as well as in the decrease of C2% and C2/C3 and concurrent increase in C4%, indicating an increase in fermentation rate as well as the fermentation of WSC (Van Houtert, 1993; Lee et al., 2002; Storm and Kristensen, 2010).

Diets with a higher fiber content and longer particle length promote ruminal stratification (Tafaj et al., 2004; Storm and Kristensen, 2010). Storm and Kristensen (2010) stated that in low fiber rations the rumen stratification might be less pronounced resulting in a more homogenous ruminal content. We therefore hypothesized that a pasture-based ration in a continuous grazing system with a relatively short herbage height would lead to smaller intraruminal differences. However, none of the measured variables were indicative for a decrease in ruminal stratification. A possible reason could be the higher NDF content of the pasture compared to the TMR in our case, but further research such as the measuring of average particle length at different sites within the rumen is needed to elucidate possible interrelations.

The concurrent evolution of total rumen content and mean rumen papillae area illustrates the influence of fermentation processes on rumen papillae morphology. Several studies have shown that an increased VFA load in the rumen is a strong stimulus for papillae growth (Dirksen et al.,
1984; Liebich et al., 1987; Bannink et al., 2012; Dieho et al., 2016). We therefore suggest that due to a decrease in fOM intake during the first days on a full grazing ration rumen papillae area decreased. Thereafter, due to an increase in ingestion of fOM in wk 8-10 epithelial cell proliferation was stimulated and therefore no significant difference between groups was observed in wk 10. A possible explanation for the fact that this alteration in papillae area mainly occurred at the location *saccus caecus caudodorsalis* could be that due to the lower rumen fill these papillae were less in contact with rumen content and thereby VFA.

Other studies have described a concurrent evolution of papillae area and VFA FAR in feed deprived animals as well as when dietary energy intake was increased over time (Dirksen et al., 1984; Gäbel et al., 1993; Martens et al., 2012). Although ration did not significantly affect FAR, it is likely that the higher VFA concentrations and lower pH in the buffer solution at the end of incubation, which we observed in the PG in wk 5, indicate reduced VFA clearance related to reduced rumen papillae surface area. This is also supported by significant correlations between papillae area and buffer solution end pH, VFA concentrations of C2 and C3 and FAR of C2. It could also be presumed that these alterations in buffer solution VFA concentrations and pH were caused by a decreased influx of water into the rumen and FLPR. But since for these variables no significant alterations over the course of the trial were observed this hypothesis seems to be unlikely.

As soon as the PG had access to pasture, milk and serum urea concentrations as well as the urine total N to creatinine ratio increased (details in Schären et al. (2016)) due to the high CP content of the ration. We therefore also expected a concurrent increase in ruminal NH$_3$-N concentrations, also in line with higher rumen NH$_3$-N concentrations in cows receiving a pasture- compared to a TMR (Bargo et al., 2002b) or grass silage- (Holden et al., 1994) based diet. However, no significant alterations and difference in medial and ventral ruminal NH$_3$-N concentrations between PG and CG were observed, except for peaks in wk 4 and wk 9 in the PG which cannot be correlated to any other measured variable (such as pasture CP content) or event. Further, the NH$_3$-N concentration measured were low in comparison with other studies describing rumen fermentation variables in grazing cows (Holden et al., 1994; Bargo et al., 2002b; Taweel et al., 2004; Abrahamse et al., 2008). A possible explanation for this observation could lie in the chosen sampling time. During our trial rumen samples were collected in the morning after milking and substantial grazing activity was prevented by rounding up the PG for milking at sunrise. Khalili and Sairanen (2000) and Soriano et al. (2000) reported similar rumen NH$_3$-N concentrations in the morning followed by a continuous increase as soon as grazing activity started (with a 2-3-fold increase 6-9 h later).
With advancing stage of lactation, a modest reduction in milk production during the trial was expected. In the CG, however, we observed a more pronounced decrease in milk yield and BCS in particular in wk 8-10 of the trial, probably due to poor quality of grass silage fed in wk 5-10 of the trial (elaborately described in Schären et al. (2016)). We suggest that several alterations observed in the CG concerning rumen fermentation, morphology and VFA-AT can be ascribed to the decreased amount of CP and consequential low rumen nitrogen balance (RNB) of the TMR as well. This change most certainly caused an alteration in rumen fermentation pattern and probably a decrease in fermentation rate. This is mirrored in following observations: low average NH$_3$-N concentrations, average rumen pH increased, papillae area decreased, total VFA concentrations decreased, medial C2% and C2/C3 increased (except wk 10), VFA-AT buffer solution pH and VFA concentrations after incubation decreased and increased, respectively, and VFA FAR decreased numerically.

To evaluate possible risk for SARA during a ration change from TMR to pasture we assessed ruminal pH using continuous rumen pH measuring devices, and collected ruminal fluid samples to determine LPS concentrations and rumen papillae for histopathological analysis at different time points. During the transition from TMR to a pasture-based ration (wk 2-8) no increased risk for SARA was observed on either group or individual level. Rumen LPS concentrations developed similarly between groups over the course of the trial indicating an influence by a common factor such as climate condition rather than ration type. However, a higher amount of animals with samples exhibiting lesions was observed in the PG compared with the CG in wk 5. Since this elevation in positive samples only occurred in the samples collected dorsally in the rumen and since this was also the site where the most pronounced decrease in papillae area was observed, a causal relationship with increased inflammation is not clear. Nevertheless, the concurrent observed lower ruminal VFA absorption potential suggests that the transition from a TMR to a pasture-based ration temporarily adversely affects rumen physiology. Further, a gradual decrease of rumen pH over the course of the trial, a numerical increase in time pH < 5.8, and individual scores based on rumen pH measurements in wk 9 and wk 10 in the PG are indicative for an increased risk for SARA on a full-grazing ration. This is in line with other studies that observed a low rumen pH in dairy cows on a pasture-based ration (Bramley et al., 2008; O’Grady et al., 2008). However, rumen LPS concentrations and papillae histopathology were not negatively influenced in that period. The absence of such negative effects support the assumption of other authors that a low ruminal pH arising from high fOM intake from pasture does not necessarily compromise cow performance (Kolver and de Veth, 2002).
An increase in rumen LPS concentration due to an increased concentrate proportion has been elaborately described, but only little data can be found on other factors that influence rumen LPS concentrations (Zebeli and Ametaj, 2009; Plaizier et al., 2012; Dänicke et al., 2014). In the previous publication we described possible heat stress during wk 5 and between wk 7-8 in both groups (Schären et al., 2016). We proposed that alterations in urine creatinine and total N excretion as well as serum glucose concentrations observed in both groups during that time were caused by heat stress. We also observed a positive correlation between THI and rumen LPS concentrations. Therefore, we suggest that alterations in fermentation conditions in the rumen occurred in these weeks due to increased THI. Baumgard et al. (2013) and (2014) suggested that the hyperinsulinemia causing a decrease in serum glucose concentrations during heat stress might be initiated by increased levels of LPS in circulation, since studies in other mammals have shown that heat-stressed animals exhibit an increased gut leakage. During our trial we observed a negative correlation between serum glucose and rumen LPS concentrations, which supports this hypothesis.

These correlations between THI and various variables illustrate the strong influence of climate conditions on dairy cow physiology. In pasture-based systems, in contrast to a confinement TMR-based system, the chemical composition of the ration (Parker and Edwards, 1996; Smit et al., 2004; Abrahamse et al., 2009) and the animals themselves (Legrand et al., 2009) are much more subject to weather and seasonal influences. We observed an interrelation between THI, pasture CP content and metabolic urea concentrations (serum and milk urea, and urine total N concentrations; described in Schären et al. (2016)). Further, also other factors influencing DMI and cow physiology, partly depend on weather conditions as well as management (such as herbage allowance, botanical composition of pasture, pre-grazing pasture mass, supplementation strategy, grazing system, etc.) (Mayne et al., 2000; Gibb, 2006; Van Vuuren and Van den Pol - Van Dasselaar, 2006). To exclude reasons other than a lower DMI for the alterations observed during this ration change, and to test whether this could be anticipated by an appropriate supplementation or grazing-system, further research is needed.

**Conclusion**

Rumen fermentation variables indicated a decreased fermentation activity during transition from a TMR to a pasture-based ration. Concurrently a lower rumen content, rumen papillae surface area and potential for VFA absorption was observed. After a few weeks on a full grazing ration fermentation activity increased and rumen content, papillae surface area and potential for VFA absorption were similar to initial levels again. Further, a continuous decrease in mean rumen pH
and molar acetate proportions, and an increase in molar butyrate proportions over the course of the trial was observed, which can most likely be ascribed to an increased intake of rapidly fermentable carbohydrates. Continuous rumen pH assessments and LPS concentrations did not reveal an increased risk for SARA during the transition period, but histopathological analysis of rumen papillae and a decreased potential for VFA absorption indicate adverse effects in initial phase of transition. Results of the present study suggest that after behavioral and metabolic adaptation to the transition from a TMR to a pasture-based ration, no adverse effects on rumen morphology and absorption capacity occurred, although rumen pH after adaptation to pasture indicated increased risk of SARA. Further studies are needed to confirm these results and exclude reasons other than the ration change as cause for the alterations observed.

Acknowledgements
The authors thank the “Niedersächsisches Ministerium für Wissenschaft und Kultur” (Hannover, Germany) for financial support. Special thanks go to Marion Burmester of the Institute for Physiology (University of Veterinary Medicine, Hanover, Germany) for the VFA analysis and Dirk Albers of the “Landwirtschaftskammer Niedersachsen” (Ovelgönne, Germany) for excellent consultancy and collaboration. Many thanks go to the coworkers at the Institute of Animal Nutrition and the experimental station of the Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany, for caring for the experimental animals, assisting with experimental measurements as well as performing the analyses.

References


### Table 1. Effect of a ration change from TMR to pasture on animal performance

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<td>3.03cde</td>
<td>3.14abc</td>
<td>3.02cde</td>
<td>3.05cde</td>
<td>3.10abc</td>
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<td><strong>BCS (scale 1-5)</strong></td>
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<td>3.5</td>
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<td>3.2</td>
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<td>2.2</td>
<td>1.9</td>
<td></td>
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</tr>
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</table>

1 Fistulated animals only; materials and methods described in Schären et al. (2016).

2 CG = confinement group (n = 5); PG = pasture group (n = 5); the CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.

3 Dry matter intake (DMI): CG (wk 1-10) and PG (wk 1-4) DMI from TMR only, PG in wk 7 and 9 DMI from pasture (n-alkane method) plus concentrate (1.75 kg DM/d); body condition score (BCS) was assessed at the beginning of each week in 14-d intervals and at the last day of wk 10; values presented as LSMeans.

4 PSEM = pooled standard error of the means; G = group, T = time.

Values within ration group in a row with different superscript letters differ (*P < 0.05*).

† P < 0.10; * P < 0.05; ** P < 0.01; symbols indicate difference between groups within a particular week.
Table 2. Effect of ration change from TMR to pasture on acetate/propionate ratio and VFA molar proportions in medial and ventral part of the rumen.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Week</th>
<th>PSEM</th>
<th>P-Values</th>
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<tr>
<td></td>
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<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>C2/C3</td>
<td>CG</td>
<td>3.39</td>
<td>3.28</td>
<td>3.31</td>
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<td>PG</td>
<td>3.93</td>
<td>3.49</td>
<td>3.23</td>
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<tr>
<td>C2/C3</td>
<td>CG</td>
<td>3.39</td>
<td>3.15</td>
<td>3.19</td>
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<td>CG</td>
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</tr>
<tr>
<td>C4 %</td>
<td>CG</td>
<td>12.9</td>
<td>12.6</td>
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</tr>
<tr>
<td></td>
<td>PG</td>
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<tr>
<td>IC5 %</td>
<td>CG</td>
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<td>1.70</td>
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<tr>
<td>C5 %</td>
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<td>0.57</td>
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<tr>
<td></td>
<td>PG</td>
<td>0.27</td>
<td>0.45</td>
<td>0.40</td>
</tr>
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</table>

1 Molar proportions in % of total VFA; C2 = acetic acid; C3 = propionic acid; C4 = butyric acid; IC5 = iso-valeric acid; C5 = valeric acid.

2CG = confinement group; PG = pasture group; the CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.

3PSEM = pooled standard error of the means; G = group, T = time; L = location.

4Values within ration group and site in a row with different superscript letters differ (P < 0.05).

† P < 0.10; * P < 0.05; ** P < 0.01; symbols indicate difference between groups in particular week (within particular location if applicable).
Figure 1. Effect of ration change from TMR to pasture on rumen fermentation variables measured weekly during the trial. ■ = confinement group (CG); ● = pasture group (PG). A. pH - sensor. Light dashed line = $\beta_0$, the slope of the logistic curve at the inflection point, illustrating the variation in rumen pH over the assessed 24 h interval (the greater, the more constant; PSEM = 0.7); long dashed line = $\beta_1$, inflection point of the logistic curve, representing the average pH of the assessed 24 h period (PSEM = 0.07); solid line = time pH < 5.8 (min/d) (PSEM = 75); short dashed line = time pH < 5.6 (min/d) (PSEM = 22). Significance: $\beta_0$: Group (G): $P = 0.242$, Time (T): $P < 0.001$, GxT: $P = 0.027$; $\beta_1$: Group: $P = 0.537$, Time: $P < 0.634$, GxT: $P = 0.008$; time pH < 5.8: Group: $P = 0.756$, Time: $P = 0.258$, GxT: $P = 0.208$; time pH < 5.6: Group: $P = 0.760$, Time: $P = 0.012$, GxT: $P = 0.595$. Logger data in wk 1 and wk 2 in CG is missing due to technical issues at the time. B. pH - manual. Dashed line = ventral part of the rumen; solid line = medial part of the rumen (PSEM = 0.12). Significance: Group: 0.818, Time: $P < 0.001$, Location (L): $P < 0.001$, GxT: $P < 0.001$; GxL: $P = 0.037$; TxL: $P = 0.010$, GxTxL: $P = 0.030$. C. Total VFA concentration. Dashed line = ventral part of the rumen; solid line = medial part of the rumen (PSEM = 5). Significance: Group: $P = 0.023$, Time: $P = 0.002$, Location: $P = 0.474$, GxT: $P < 0.001$; GxL: $P = 0.147$; TxL: $P = 0.050$, GxTxL: $P = 0.156$. D. NH$_3$-N concentration. Dashed line = ventral; solid line = medial (PSEM = 1.9). Significance: Group: $P = 0.272$, Time: $P = 0.003$, Location: $P = 0.303$, GxT: $P < 0.001$; GxL: $P = 0.124$; TxL: $P = 0.011$, GxTxL: $P = 0.002$. E. LPS concentration. Dashed line = ventral; solid line = medial (PSEM = 0.14). Significance: Group: $P = 0.700$, Time: $P < 0.001$, Location: $P < 0.001$, GxT: $P = 0.062$; GxL: $P = 0.973$; TxL: $P = 0.722$, GxTxL: $P = 0.414$. LSMeans; n = 5. Different superscripts indicate significant differences between groups in particular week (within particular location if applicable; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$). Letters indicate significant differences between weeks (within particular group and location if applicable; $P < 0.05$). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 2. Effect of ration change from TMR to pasture on different rumen variables measured in week 1, 5 and 10 (wk 1, wk 5 and wk 10) of the trial. Confinement group (CG); pasture group (PG). Numbers in graph B and C indicate different locations within the rumen: 1 = saccus caecus caudodorsalis, 2 = saccus ventralis, 3 = saccus caecus caudoventralis. A. Rumen content. Dry matter (DM) content: PSEM = 0.7, significance: Group (G): P = 0.339, Time (T): P = 0.001, GxT: P = 0.006; Non DM content: PSEM = 5.0, significance: Group: P = 0.703, Time: P = 0.084, GxT: P = 0.008. B. Mean papillae area. PSEM = 0.07. Significance: Group: 0.417, Time: P = 0.116, Location (L): P = 0.069, GxT: P = 0.077; GxL: P = 0.330; TxL: P = 0.456, GxTxL: P = 0.023. C. Histopathological analysis of papillae. Illustrated as fraction of samples with lesions (n = 5 per time, group and location; statistical analysis: Fisher’s Exact Tests) D. pH
of VFA-Absorption Test (VFA-AT) buffer solution after incubation. PSEM = 0.09, significance: Group: \( P = 0.310 \), Time: \( P < 0.001 \), GxT: \( P = 0.003 \). E. VFA concentrations in VFA-AT buffer solution after incubation. Acetic acid (C2): PSEM = 2.8, significance: Group: \( P = 0.058 \), Time: \( P = 0.001 \), GxT: \( P = 0.005 \); propionic acid (C3): PSEM = 0.8, significance: Group: \( P = 0.492 \), Time: \( P < 0.001 \), GxT: \( P = 0.003 \); butyric acid (C4): PSEM = 0.4, significance: Group: \( P = 0.793 \), Time: \( P = 0.003 \), GxT: \( P = 0.014 \). F. Fractional absorption rate of VFA during VFA-AT. C2: PSEM = 0.07, significance: Group: \( P < 0.478 \), Time: \( P = 0.085 \), GxT: \( P = 0.198 \); C3: PSEM = 0.07, significance: Group: \( P = 0.654 \), Time: \( P = 0.064 \), GxT: \( P = 0.245 \); C4: PSEM = 0.08, significance: Group: \( P = 0.548 \), Time: \( P = 0.137 \), GxT: \( P = 0.205 \). G. Influx of water into the rumen during VFA-AT. PSEM = 2.0, significance: Group: \( P = 0.482 \), Time: \( P = 0.608 \), GxT: \( P = 0.820 \). H. Fractional liquid passage rate during VFA-AT. PSEM = 0.07, significance: Group: \( P = 0.247 \), Time: \( P = 0.860 \), GxT: \( P = 0.471 \); LSMeans; \( n = 5 \). Different superscripts indicate significant differences between groups in particular week (and location in case of papilla area and histopathological analysis of papillae; † \( P < 0.10 \); * \( P < 0.05 \); ** \( P < 0.01 \)). Letters indicate significant differences between weeks within particular group (and location in case of papilla area and histopathological analysis of papillae; \( P < 0.05 \)). The CG stayed on a TMR based ration during the entire trial while the PG was slowly introduced to a pasture-based ration: wk 1: TMR, wk 2: TMR & 3 h pasture/d, wk 3&4: TMR & 12 h pasture/d, wk 5-10: pasture and 1.75 kg DM concentrate/d.
Figure 3: Examples of haematoxylin and eosinophil stained sections of rumen papillae. **A.** No lesions. **B.** Lymphoplasmacytic (bottom arrow) and neutrophilic granulocyte (top arrow) infiltration in lamina propria. **C.** Lymphoplasmacytic (arrow) infiltration in lamina propria. **D.** Pustular inflammation (containing neutrophilic granulocytes, arrow) of the rumen epithelium.
5. Discussion
In temperate climate zones dairy cows need to be fed with conserved feed during winter time and are then often gradually transitioned to a pasture-based ration in spring. The differences in production level, disease incidence and rumen fermentation between confinement- and pasture-based systems have been described in different studies and are relatively well known (summarized in chapter 1.2). Various studies have shown that physiological variables need between 1-21 days to adapt to a new nutritional situation whenever a ration change occurs. However, up to now studies investigating the effects of a ration change have mainly focused on the change between different conserved feeds and/or the increase in the concentrate proportion within a ration. Only little can be found on the changes occurring in a cow’s physiology during the transition from a TMR to pasture-based ration and the time needed to adapt to this new nutritional situation (summarized in chapter 1.3).

We hypothesized that the change from a confinement to a pasture based system involves complex nutritional and metabolic adaptations with consequences on health and performance, and therefore conducted a 10-week trial involving 60 primi- and pluriparous German Holstein cows in which the effects of a change from a confinement TMR to a pasture-based ration on dairy cow production, health and rumen physiology were investigated.

In summary, the main alterations observed in production, health and rumen variables were most likely caused by a decrease of DMI during the initial phase of transition, followed by a continuous increase in the weeks thereafter (data summarized and discussed in chapter 3 and 4). Production and rumen fermentation data suggest that after approximately three weeks on a full-grazing ration metabolic and behavioral adaptations were so far completed that the increase in fOM lead to an increase in rumen fermentation activity and a decrease in energy deficit occurred over the following weeks. This observation is additionally supported by a stabilization of rumen protozoal counts in wk 7 of the trial (master thesis, Künzel et al. 2015, [156]). However, a further decrease in average rumen pH and molar acetate proportion, BCS and a concurrent increase in BW, serum BHBA and urea concentrations, rumen total VFA and time < pH 5.8 (min/d) show that adaption to pasture was most likely still not terminated by the end of the trial. This is in line with other studies that have shown that whenever a ration change compromises a large change regarding feed composition, palatability and animal behavior the adaption period is extended (summarized in chapter 1.3).

During this trial rumen samples for microbial analysis were collected at three points in time (wk 1: TMR-only, wk 5: 4-6 days on full grazing ration, and wk 10: 6 weeks on a full-grazing ration).
Using a DNA-fingerprinting method (SSCP) the change in similarity over time within the population of the liquid-, fiber and epithelium-associated rumen bacteria was investigated. Results showed that at all three locations the average similarity decreased in the PG between wk 1, wk 5 and wk 10 (81.3 ± 4.1 in wk 5 and 71.0 ± 10.2 % similarity in wk 10, compared to wk 1), whereas in the CG only minor alterations were observed (93.7 ± 2.5 in wk 5 and 91.0 ± 2.4 % similarity in wk 10, compared to wk 1 [259]). These results illustrate that the rumen bacterial microbiota did not stabilize within a week after complete transition to a full-grazing ration and are in line with other variables showing that the adaptation to a pasture-based ration required several weeks. To further investigate the differences in bacterial populations sequencing of the 16S DNA samples is needed and will provide a more accurate and broader insight into rumen bacteria dynamics under the influence of a ration change from TMR to pasture. Additionally, future studies should include more frequent samplings to observe the time required for the rumen bacterial population to stabilize upon this nutritional change.

Since an abrupt decrease in DMI and therefore in animal performance during a changeover from one ration to another are highly undesired, research is needed to develop management strategies that allow a more gradual transition from one system to another. The grazing system that has been implemented in the current trial is characterized by infrequent allocation (continuous grazing) and a low concentrate supplementation. Different studies have shown that DMI can be increased in grazing systems by different management strategies, such as increasing the daily herbage allowance, increasing the frequency of pasture allocation (e.g., rotational grazing) and by supplementation of higher amounts of concentrate [15, 63, 86, 183, 186, 254, 300]. Therefore, future studies should include the comparison of different grazing and supplementation strategies and their influence on effects of a ration change on animal physiology. Further, the monitoring of behavioral variables such as activity and rumination could provide valuable data on energy expenditure and behavioral adaptation.

One of the central issues of this study was to investigate whether the change from a TMR to a pasture-based system could have a negative impact on rumen physiology and therefore animal health. We concluded that the negative alteration in histopathological analysis of rumen papillae and potential for VFA absorption during the initial phase of transition indicated a possible risk for rumen health. But since neither an increased risk for SARA nor negative alterations in blood chemistry variables, blood cell counts and rumen LPS concentrations were observed concurrently, we suggest that these alterations can be mainly ascribed to a lower intake of fOM and not to an increase in inflammatory processes in the rumen epithelium. Simultaneously with the increase in fermentation activity in wk 9 and wk 10 of the trial an increase in risk for SARA
was observed. Also in this case no concurrent alterations in blood variables and rumen LPS concentrations were observed, and rumen papillae histopathology revealed no increase in inflammatory processes in the rumen epithelium. However, since these observations were made only shortly before the trial ended, a prolongation of the experimental period should be considered in future trials. This would also allow the investigation of the long-term effects of an excessive N intake on organ health and effects of increased energy expenditure due to grazing and walking on animal physiology and health. Additionally, the measuring of serum acute phase proteins, serum LPS concentrations and continuous body temperature recording in future trials could deliver valuable additional data to evaluate the risk for and consequences of SARA.
6. References

(account for chapters 1. Background, 2. Aims of Study, and 5. Discussion)


316. White, J., **Chapter 1: The farmlands of New Zealand,** in New Zealand Pasture and Crop Science, J. White and J. Hodgson, Editors. 2000, Oxford University Press.


7. Affidavit

I herewith declare that I autonomously carried out the PhD-thesis entitled “Effects of a ration change from a silage and concentrate- to a pasture-based ration on production, health and rumen physiology of dairy cows”.

No third party assistance has been used.

I did not receive any assistance in return for payment by consulting agencies or any other person. No one received any kind of payment for direct or indirect assistance in correlation to the content of the submitted thesis.

I conducted the project at the following institution(s):

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany

Department of Physiology, University of Veterinary Medicine Hannover, Germany

The thesis has not been submitted elsewhere for an exam, as thesis or for evaluation in a similar context.

I hereby affirm the above statements to be complete and true to the best of my knowledge.

Date: ___________ Signature: _____________________
8. Acknowledgements

I thank my supervisors at the Institute for Animal Nutrition (FLI), Prof. Dänicke and Dr. Meyer for their support, guidance and giving me a great scope for development. I further thank Prof. Breves for his professional insights and support, especially during the time I resided at his institute. I also thank Prof. Isselstein and Prof. Rehage for their scientific input and advice.

The thesis would not have been possible without the funding of the “Ministerium für Wissenschaft und Kultur, Niedersachen” and the “Systemanalyse-Milch” project. The initiating and the decisive role of Dirk Albers in this project are greatly acknowledged. I as well would like to thank Mr. Albers for fruitful scientific exchange and practical insights.

I further would like to show my gratitude to the co-workers at the Institute of Animal Nutrition, namely the members of the immune-nutrition group (Jana Frahm, Nicola Mickenautsch, Lara Lindner and Elenia Scholz), basic-analytics group (Liane Hüther, Kathrin Hillendahl, Hans Eckardt, Axel Jagow, Johanna Sturm and Susanne Stock), the experimental station (Florian Mühlhaus, Jonas Knäpple, Dirk Wolters, Annerose Junghans, Katrin Kuhrmeier, Karin Rothe, Andreas Thies), administrative staff (Sabine Hartinger, Gudrun Mittelbach, Vanessa Tabatt and Sigrid Herweg) and others (Dirk von Soosten and Andreas Berk). I further would like to thank the members of the Institute of Physiology (TiHo Hannover) for the analysis of the VFA samples and support and guidance during my time at their laboratory (Kerstin Kiri, Marion Burmester, Karin Hustedt and Susanne Riede).

I am indebted to my many of my fellow doctoral students at the Institute of Animal Nutrition (Caroline Drong, Reka Tienken, Kirsten Schulz, Mohammad Ebrahem, Wendy Liermann, Katrin Steltler, Marleen Paulick and Janine Winkler) as well as Institute of Physiology (Kristin Elfers, Melanie Eger, Patrick Lange and Lisa Marholt) to support me during the trial as well as during my time in the laboratory. I especially would like to thank Marc-Alexander Lieboldt for creating a great work atmosphere and the “cow-chicken think tank”.

It is with immense gratitude that I acknowledge the support and help of Gero Seyfang and the masterstudents Stefanie Ruesink, Sandra Jostmeier and Susanne Künzel without whomes commitment the realization of the elaborate trial and sampling procedures would not have been possible.

It gives me great pleasure in acknowledging the support of our collaborating partners at Haus Riswick (Anne Verhoeven, Ingo Dünnebacke and Clara Berendonk), University of Halle (Michael Bulang), University of Bonn (Patrick Steuer, Daniela Martin, Prof. Südekum), University of
Hohenheim (Dr. Steingass and Prof. Rodehutscord) and the TiHo Hannover (Prof. Beineke). Special thanks go to Berit Greune for assisting us in the pasture species assessment.

It was an honor for me to be able to exchange thoughts with Jan Dijkstra (University of Wageningen) and I am very grateful for his support and critical revision of my manuscripts. Special thanks also go to his doctoral student Kasper Dieho for openly sharing his know-how with me and for critical scientific discussions.

I cannot find the words to express my gratitude to my parents and family for always supporting me in all my life decisions and showing me that the secret to success in life lies in hard work, stamina and empathy. My mother and grandmother have always been role models for me and I want to thank them for always being there for me. I also want to thank my father and his family (Sandra, Alessandro and Sienna) for supporting us in creating our own little family and bringing a lot of joy to our life.

I owe my deepest gratitude to Erik, my partner, best friend and father of our child.

Last but not least I want to thank all my friends (especially Renske, Nina, Jenny and Andries) for all the fun times and support!