Grazing behavior of two Holstein dairy cow strains under organic farming conditions in Switzerland

Carolin Heublein

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

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2017

1st Edition 2017

Printed in Germany

édition linguistique
VVB LAUFERSWEILER VERLAG

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Institut für Tierwissenschaften

Rheinische Friedrich-Wilhelms-Universität Bonn

Grazing behavior of two Holstein dairy cow strains under organic farming conditions in Switzerland

Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität

Bonn

Vorgelegt im Juli 2016

von

Carolin Heublein

aus Köln

Referent:	**Prof. Dr. Karl-Heinz Südekum**
Korreferent:	**Prof. Dr. Michael Kreuzer**
Tag der mündlichen Prüfung:	**28.11.2016**
Erscheinungsjahr:	**2017**

Die vorliegende Arbeit wurde gefördert durch die AGFF (Zürich, Schweiz) und die Fondation Sur-la-Croix (Basel, Schweiz).

Meiner Familie

Grazing behavior of two Holstein dairy cow strains under organic farming conditions in Switzerland

Summary

The aim of the thesis was to test if concentrate supplementation is required in an organic, pasture-based feeding system and if concentrate supplementation influences grazing behavior. The study consisted of two trials, both with a crossover design performed on an organic farm in Switzerland with 12 Swiss Holstein cows and 12 Holstein cows of New Zealand origin. In the first trial the focus was on the impact of concentrate supplementation on milk yield and composition, grazing and rumination behavior, physical activity, and blood metabolites and the differences between the two cow strains. In the second trial the focus laid on the estimation of plant species selection by dairy cows with plant wax markers and whether differences exist between concentrates supplemented and non-supplemented cows in selection behavior.

Concentrate supplementation had an impact on milk yield and composition, the time animals spent grazing, herbage dry matter intake and physical activity, but no on rumination behavior. Supplemented cows had a more stable energy status, but no indices for strong negative energy balance were recorded for non-supplemented cows, for both cow strains. In the second trial the main focus was on the estimation of herbage composition of grazing dairy cows with plant wax markers, namely alkanes, long-chain fatty acids and long-chain alcohols (LCOH). Concentrate samples, feces samples from each cow and samples from each paddock were taken and plant species were manually separated. All plant species, concentrate and feces samples were analyzed for their marker contents. Corrections of fecal recovery were calculated in relation to dosed ytterbium. The estimations of diet composition were performed with the software “EatWhat” based on non-negative least squares. Results were compared to the botanical composition with the Aitchison distance. The most accurate diet composition estimation was achieved with alkanes, LCOH and a correction of fecal recovery. No differences in selected plant composition between cow strains were recorded, but supplemented cows selected more *Trifolium repens* compared to non-supplemented cows. However, further studies are required to confirm the feasibility of the approach and validate the calculation of fecal recovery. Understanding the grazing behavior and the consequences of concentrate supplementation may lead to management measures that increase production efficiency and ensure animal welfare. Minor differences between cow strains indicated that both are suitable for pasture-based feeding systems. However, short-term trials cannot give a conclusion for the whole lactation, and fertility and health traits should be included.

Untersuchungen zum Fressverhalten von zwei Holstein-Kuhtypen unter Bedingungen des Organischen Landbaus in der Schweiz

Zusammenfassung

In der vorliegenden Arbeit wurde geprüft, ob eine Kraftfutterergänzung in einem biologischen, weidebasierten Fütterungssystem notwendig ist und ob Kraftfutter einen Einfluss auf das Fressverhalten von zwei Holstein Kuhtypen hat. Die Arbeit besteht aus zwei Versuchen, die beide ein Crossover Design waren und auf einen Biobetrieb in der Schweiz durchgeführt wurden. Es wurden jeweils 12 Schweizer Holsteinkühe und 12 Holsteinkühe mit neuseeländischer Herkunft eingesetzt. Im ersten Versuch lag der Schwerpunkt auf den Unterschieden zwischen den Kuhtypen und dem Einfluss von Kraftfutterfütterung von 6 kg/Tag auf Milchleistung und -zusammensetzung, Fress- und Wiederkauverhalten, physische Aktivität und Blutmetaboliten. Im zweiten Versuch wurde untersucht, ob mit Hilfe von Markern bestimmt werden kann, welche Pflanzen und zu welchem Anteil von Milchkühen aufgenommen wurden, und ob es Unterschiede im Selektionsverhalten zwischen Kühen mit und ohne Kraftfutterergänzung gibt.

Die Kraftfutterergänzung hatte einen Einfluss auf Milchleistung und Milchinhaltsstoffe, sowie auf die Fresszeit auf der Weide, die Grünfutteraufnahme und die physische Aktivität, jedoch nicht auf das Wiederkauverhalten. Die mit Kraftfutter ergänzten Tiere hatten einen stabileren Energiestatus, jedoch gab es keine Anzeichen einer ausgeprägten negativen Energiebilanz bei beiden Kuhtypen ohne Kraftfutterergänzung. Zwischen den Kuhtypen gab es Unterschiede in den Milchinhaltstoffen und im Wiederkauverhalten. Die Neuseeländischen Holsteinkühe hatten höhere Fett- und Proteingehalte in der Milch und kauten längere Zeit wieder im Vergleich zu den Schweizer Holsteinkühen. Im zweiten Versuch wurde mittels Alkanen, langkettige Fettsäuren und langkettige Alkohole (LCOH) geschätzt, welche Pflanzen und zu welchem Anteil von grasenden Milchkühen aufgenommen wurden. Dazu wurden Kraftfutterproben, Kotproben von jeder Kuh und Grünfutterproben von der Weide genommen und nach Pflanzengruppen manuell aussortiert. In allen Pflanzengruppen, Kraftfutter- und Kotproben wurden die Marker-Konzentrationen analysiert. Außerdem wurden Korrekturen für die Wiederfindung im Kot in Bezug auf verabreichtes Ytterbium berechnet. Die Schätzungen der Pflanzenzusammensetzung erfolgten mit dem Programm „EatWhat“, welches auf „non-negative least sqares“ basiert. Die Ergebnisse der geschätzten Pflanzenzusammensetzung wurden mit der botanischen Zusammensetzung mittels ‚Aitchison Distanzmaß‘ verglichen. Die genaueste Schätzung der Pflanzenzusammensetzung wurde mit Alkanen, LCOH und einer Korrektur der Wiederfindung erreicht. Es gab keine Unterschiede in der Pflanzenauswahl zwischen den Kuhtypen, aber die mit Kraftfutter ergänzten Tiere fraßen mehr *Trifolium repens* im Vergleich zu Kühen ohne Kraftfutterergänzung. Weitere Studien sind notwendig, um die Methode zur Bestimmung der Futterauswahl zu optimieren und die Berechnung der Wiederfindungsrate zu validieren. Das Verständnis des Fressverhaltens und die Auswirkungen von Kraftfutterergänzung können Managementmaßnahmen beeinflussen, um die Produktionseffizienz zu steigern und den Tierschutz zu gewährleisten. Kleine Unterschiede zwischen den Kuhtypen zeigen, dass beide an ein weidebasiertes Produktionssystem angepasst sind. Jedoch können Versuche über einen kurzen Zeitraum keine Rückschlüsse auf eine ganze Laktation geben und Fruchtbarkeits- und Gesundheitsmerkmale sollten mit einbezogen werden.

TABLE OF CONTENTS

FIGURES

Chapter 4

TABLES

Chapter 3

Chapter 4

ABBREVIATIONS

ADF	Acid detergent fiber
ALAT	Alanine aminotransferase
alkanes	*n*-Alkanes
AP	Alkaline phosphatase
APDE	Absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen
ASAT	Aspartate aminotransferase
BCS	Body condition score
BHBA	*beta*-Hydroxybutyric acid
BW	Body weight
CCL	Carbon chain length
CF	Crude fiber
CK	Creatine kinase
Conc0	Cows without concentrate supplementation
Conc6	Cows with concentrate supplementation
CP	Crude protein
d	Day(s)
EE	Ether extract
ESC	Ethanol soluble carbohydrates
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk yield
FR	Fecal recovery
GC	Gas chromatograph
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase

HCH	Swiss Holstein cows
HC28	Octocosane, $C_{28}H_{58}$
HC32	Dotriacontane, $C_{32}H_{66}$
HC33	Tritriacontane, $C_{33}H_{68}$
HF	Holstein-Friesian
HNZ	New Zealand Holstein cows
IGF-1	Insulin-like growth factor 1
LCFA	Long-chain fatty acids
LCOH	Long-chain alcohols
NDF	Neutral detergent fiber
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NEL/NE_L	Netto energy lactation
OM	Organic matter
SD	Standard derivation
T3	3,5,3´-Trijodthyronine
T4	Thyroxin
VFA	Volatile fatty acids
wk	Week
WSC	Water soluble carbohydrates
Yb	Ytterbium

Chapter 1: General introduction

1.1. Organic dairy farming in Switzerland

The international interest in organic milk products is increasing and the number of farmers that have changed from conventional to organic milk production systems is increasing (Rosati and Aumaitre, 2004). In Switzerland 10% of dairy farms produce under organic conditions (BLW, 2015) and the number of organic farms has already doubled between 1996 and 2007 (BFS, 2009). Compared to other countries in Europe, Switzerland has one of the highest proportions of organic farmland (11%; BFS, 2009). With 70% pasture of all agricultural areas (BFS, 2009), Switzerland has reasonable conditions for pasture-based feeding systems. In alpine regions the portion of organic farms is higher than in regions in the valley (BFS, 2009). Intensive farming and crop production is not efficient in alpine regions because of soil quality and surface structure, and using these areas as pasture for dairy cows offers an opportunity for organic milk production, especially as organic milk obtains higher milk prices. Furthermore, pasture utilization in general conserves this kind of ecosystem and supports higher biodiversity, otherwise forest will establish there (Knaus, 2016).

For organic milk production several regulations have to be considered by farmers. The International Federation of Organic Agriculture Movements formulated four principles for organic farming (IFOAM, 2015). Among other factors, they claim that animals should be provided with conditions to display natural behavior. For ruminants, this means ensuring access to pasture to exhibit their natural grazing, social and explorative behavior and feeding them according to their physiological needs. Furthermore, guidelines for organic farming prohibit the use of mineral fertilizer which results in lower nutrient input (N, K, P) in soil in organic systems compared to conventional systems (Maeder et al., 2002). As a result herbage yield and content of protein in plants is declining (Spann et al., 2007), but plant species diversity is increasing (Gabriel et al., 2006). A different botanical composition on pasture and lower herbage yield in organic dairy farms might be a greater challenge for cows to match their requirements. Generally, cows with high genetic merit for milk yield may not match their energy requirements in early lactation with a pasture-only diet and supplementation of concentrate is required. Organic farming should be adapted to local conditions to ensure a balance between input and output. Inputs should be reduced by reuse, recycling and efficient management of materials to ensure a close cycle of production (IFOAM, 2015). Therefore importing supplements, which are not produced on the same farm and disturb a production cycle, are questionable. Guidelines exist to limit the use of

concentrate supplementation, but they differ between countries and organizations. The US regulations for organic milk production prescribe a daily dry matter intake (DMI) from grazing of no less than 30% throughout the grazing season and thus 70% daily DMI remains, which could be covered by concentrates or other feedstuff produced according to organic guidelines (GPO, 2015). A maximum of 40% of concentrate per day is allowed in the EU and even 50% in early lactation (Council Regulation No 889/2008). In Switzerland, it is more restricted as the organic guidelines allow 10% concentrate based on the annual ration (BioSuisse, 2014). As ruminants are able to digest fiber efficiently and assuming that competition for feed versus food use would intensify in the future, cereals and other field crops should primarily be destined to cover the needs of humans and successively of monogastric animals (Bocquier and Gonzalez-Garcia, 2010). According to Cassidy et al. (2013), 89% of crop-produced calories are lost when fed to animals and are not available for humans in the form of animal products. However, animal products are needed in human nutrition primarily to provide proteins and amino acids. Milk production is the most efficient livestock production system that converts potentially human-edible feed into animal product (Wilkinson, 2011). A dairy cow with a daily milk production of 10 kg produces 323 g edible protein per day, which is high compared to other animal species (Flachowsky and Kamphues, 2012). This protein can come from pasture or other by-products which are useless for human consumption. Pasture is the cheapest source of nutrients for dairy cows and provides the basis of sustainable farming systems. It preserves the rural landscape and promotes a clean, animal-welfare-friendly image of dairy production (Dillon, 2006).

1.2. Pasture-based feeding systems

Comparisons made at the world level indicate that an increase of herbage in the annual ration of cows decreases the total costs of milk production (Dillon et al., 2008). However, conserved-forage based systems, especially forage maize, are the most common feeding systems in most European countries (Dillon, 2006) and the numbers of farms that manage a pasture-based feeding system for dairy cows is decreasing in North-West Europe, expecting that in 2025 <5% of cows have access to pasture in the North-West of Germany (Reijs et al., 2013). As the number of cows per farm is growing, sufficient pasture available nearby the farm for grazing is hardly given. Pasture-based feeding systems are neglected in regions where it is more cost-effective to increase production through concentrate supplementation (Knaus, 2016). The trend to raise the amount of concentrate in dairy cow rations started in the 1960s, as the cost per unit of net energy for corn grain was less than forage (van Soest, 1994). With a favorable price of milk in relation to grain supplements it is most economical to add supplements to the diet rather than feeding only pasture to maximize feed intake and milk performance (Knaus, 2016). Keeping dairy cows on a pasture-only diet is challenging,

as quality and quantity of available feed is changing during the season, as well as the nutritive and energy requirements of the cows and may lead to a nutrient deficit or a waste of pasture feed. Energy is mostly the first limiting nutrient, especially for high producing dairy cows (Kolver and Muller, 1998; Hills et al., 2015). Therefore, cows may suffer from a lack of energy with a pasture-only diet and cows with a high genetic merit for milk production might reach a strong negative energy balance (NEB) at the beginning of lactation. However, concentrate supplementation influences grazing behavior on pasture (McCarthy et al., 2007; Bargo et al., 2003) and lowers cows´ motivation for grazing, the so called feeding drive, which means how effective the cow harvests the pasture (Baudracco et al., 2010). High feeding drive is important in pasture-based feeding systems as it ensures high pasture DMI throughout the grazing season with varying pasture quality. Understanding the behavior of dairy cows on pasture helps to optimize efficiency and support productivity, animal health and welfare.

In countries such as New Zealand, where over 95% of agriculturally used area is grassland (New Zealand Official Yearbook, 2012), the majority of the dairy cattle is kept on pasture. The principle of this system is based on seasonal calving with utilizing the rapid herbage growth in spring time to meet requirements of the cows and drying them off at the end of grazing season, so that reduced feed demand is in accordance with reduced pasture growth in winter. Therefore, high pasture utilization is a key factor, and the amount of milk produced per ha instead of milk yield per cow is important. Stocking rate (cows per ha) is an important management tool, because with higher stocking rate, milk yield per cow is lower, but milk production per ha is greater (Baudracco et al., 2010; McCarthy et al., 2011). Furthermore, lower grazing residuals on pasture improve pasture quality (Macdonald et al., 2008), as close defoliation of herbage plants is necessary to avoid high portions of dead plant material. The key for an efficient pasture-based feeding system is to ensure pasture in sufficient quantity and quality throughout the season by converting as much as possible of the green leaf mass for production.

1.3. Different Holstein cow strains

In New Zealand, pasture-based feeding systems are common as favorable climatic conditions allow a year-round pasture growth and utilization. This system assumes a seasonal calving interval close to 12 months (Harris and Kolver, 2001). Hence, New Zealand Holstein cows are bred for efficient pasture use with very little concentrate supplementation, high fertility and longevity (Harris and Kolver, 2001). They differ in body weight, body condition score (BCS) and milk yield compared to other Holstein cow strains (Piccand et al., 2013) and have a lower milk production response to concentrate supplementation compared

to high genetic merit Holstein cow strains (Horan et al., 2006). Studies suggest that high producing Holstein cows are less adapted for grazing systems, particularly under higher stocking rates and seasonal calving systems (Kolver and Muller, 1998; Baudracco et al., 2010). Without concentrate supplementation they may not meet their requirements and exhaust their potential for milk production with a pasture-only diet (Peyraud and Delagarde, 2013). Cows that reached a strong NEB at the beginning of the lactation have a greater post-calving BCS loss, which influence fertility, health, and welfare (Roche et al., 2006). New Zealand Holstein cows may be able to use herbage from pasture more efficiently for milk production (Macdonald et al., 2008) and therefore may be better suited for organic milk production systems. Nevertheless, it is still unclear which characteristics indicate a well-suited dairy cow and which characteristics a cow should have for the efficient use of pasture. Cows should also be able to deal with fluctuating conditions on pasture (Dillon, 2006) and as they are able to adapt to certain environmental and management factors (such as increasing the proportion of time spent grazing if access to pasture is restricted (Kennedy et al., 2009)), examining grazing behavior may provide some evidence about well-suited dairy cows.

References

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1-42.

Baudracco, J., N. Lopez-Villalobos, C. W. Holmes, and K. A. Macdonald. 2010. Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: a review. N. Z. J. Agric. Res. 53:109–133.

BFS. 2009. Schweizer Landwirtschaft – Taschenstatistik 2009. Eidgenössisches Departement des Inneren EDI, Bundesamt für Statistik. Neuchâtel, Switzerland.

BLW. 2015. Auswertung der Daten über die Milchproduktion. Eidgenössisches Department für Wirtschaft, Bildung und Forschung WBF. Bundesamt für Landwirtschaft BLW. Bern, Switzerland.

BioSuisse. 2014. Richtlinien für die Erzeugung, Verarbeitung und den Handel von Knospe-Produkten. BioSuisse, Basel, Switzerland.

Bocquier, F. and E. Gonzalez-Garcia. 2010. Sustainability of ruminant agriculture in the new context: feeding strategies and features of animal adaptability into the necessary holistic approach. Animal 4:1258-1273.

Cassidy, E. S., P. C. West, J. S. Gerber, and J. A. Foley. 2013. Redefining agricultural yields: from tonnes to people nourished per hectare. Environ. Res. Lett. 8:34015–34023.

Council Regulation No 889/2008. 2015. Accessed Apr. 03, 2016. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:250:0001:0084:EN:PDF

Dillon, P., D. P. Berry, R. D. Evans, F. Buckley, and B. Horan. 2006. Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production. Livest. Sci. 99:141-158.

Dillon, P. A. T., T. Hennessy, L. Shalloo, F. Thorne, and B. Horan. 2008. Future outlook for the Irish dairy industry: a study of international competitiveness, influence of international trade reform and requirement for change. Int. J. Dairy Technol. 61:16-29.

Flachowsky, G. and J. Kamphues. 2012. Carbon footprints for food of animal origin: what are the most preferable criteria to measure animal yields? Animals 2:108.

Gabriel, D., I. Roschewitz, T. Tscharntke, and C. Thies. 2006. Beta diversity at different spatial scales: plant communities in organic and conventional agriculture. Ecol. Appl. 16:2011-2021.

GPO. 2015. U.S. Government printing office. Accessed Apr. 03, 2016. http://www.ecfr.gov/cgi-bin/text-idx?SID=2d3d62fe28e906859e3c13bb0636dbb3&node=7:3.1.1.9.32&rgn=div5#7:3.1.1.9.32.3.354.11.

Harris, B. L. and E. S. Kolver. 2001. Review of holsteinization on intensive pastoral dairy farming in New Zealand. J. Dairy Sci. 8:E56-E61.

Hills, J. L., W. J. Wales, F. R. Dunshea, S. C. Garcia, and J. R. Roche. 2015. Invited review: an evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. J. Dairy Sci. 98:1363-1401.

Horan, B., P. Faverdin, L. Delaby, M. Rath, and P. Dillon. 2006. The effect of strain of Holstein-Friesian dairy cow and pasture-based system on grass intake and milk production. J. Anim. Sci. 82:435-444.

IFOAM. 2015. International federation of organic agriculture movements. Accessed Apr. 03, 2016. http://www.ifoam.org/en/organic-landmarks/principles-organic-agriculture

Kennedy, E., M. McEvoy, J. P. Murphy, and M. O´Donovan. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. J. Dairy Sci. 92:168-176.

Knaus, W. 2016. Perspectives on pasture versus indoor feeding of dairy cows. J. Sci. Food Agric. 96:9-17.

Kolver, E. S. and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403-1411.

Macdonald, K. A., G. A. Verkerk, B. S. Thorrold, J. E. Pryce, J. W. Penno, L. R. McNaughton, L. J. Burton, J. A. S. Lancaster, J. H. Williamson, and C. W. Holmes. 2008. A comparison of three strains of Holstein-Friesian grazed on pasture and managed under different feed allowances. J. Dairy Sci. 91:1693-1707.

Maeder, P., A. Fliessbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil fertility and biodiversity in organic farming. Science 296:1694-1697.

McCarthy, B., L. Delaby, K. M. Pierce, F. Journot, and B. Horan. 2011. Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. Animal 5:784-794.

McCarthy, S., B. Horan, M. Rath, M. Linnane, P. O'Connor, and P. Dillon. 2007. The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. Grass Forage Sci. 62:13-26.

New Zealand Official Yearbook. 2012. 2012 Yearbook agricultural tables. Accessed Mar. 11, 2016. http://www.stats.govt.nz/browse_for_stats/snapshots-of-nz/yearbook/environment/agriculture/yrbk-agri.aspx

Peyraud, J. L. and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply under grazing. Animal 7:57-67.

Piccand, V., E. Cutullic, S. Meier, F. Schori, P. L. Kunz, J. R. Roche, and P. Thomet. 2013. Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system. J. Dairy Sci. 96:5352-5363.

Reijs, J.W., C.H.G. Daatselaar, J.F.M. Helming, J. Jager, and A.C.G. Beldman. 2013. Grazing dairy cows in north-west Europe – Economic farm performance and future developments with emphasis on the Dutch situation. Pages 59-71 in LEI Report 2013-001, Wageningen, Netherlands.

Roche, J. R., D. P. Berry, and E. S. Kolver. 2006. Holstein-Friesian strain and feed effects on milk production, body weight, and body condition score profiles in grazing dairy cows. J. Dairy Sci. 89:3532-3543.

Rosati, A. and A. Aumaitre. 2004. Organic dairy farming in Europe. Pages 41-51 in Livestock Production Science Trends and Development in Organic Livestock Farming Systems. Vol. 90.

Spann, B., M. Diepolder, E. M. Schmidtlein, S. Tusch, A. Hermüheim, and D. Spengel. 2007. Umstellung zur ökologischen Milchviehhaltung - Vergleich verschiedener Leistungsparameter. Pages 65-77 in Angewandte Forschung und Beratung für den ökologischen Landbau in Bayern. Vol. 3. K. Wiesinger, ed. Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft, Freising, Germany.

van Soest, P. J. 1994. Ruminants in the world. Pages 1-6 in Nutritional ecology of the ruminant. Vol 2. Cornell University Press, New York, USA.

Wilkinson, J. M. 2011. Re-defining efficiency of feed use by livestock. Animal 5:1014-1022.

Chapter 2: Scope of the thesis

Organic dairy farming becomes more important nowadays and further research is necessary to optimize farming under organic conditions. Regulations for organic dairy farming claim that cows should have access to pasture and therefore, pasture during the grazing season is the main component in dairy cows rations, at least in Switzerland. The aim of present thesis was to examine if concentrate supplementation is required in a pasture-based feeding system and how concentrate supplementation influences grazing behavior. During two cross-over trials performed on an organic Swiss farm, the differences between Swiss and New Zealand Holstein cows and the impact of concentrate supplementation on grazing behavior was examined. During the first trial the focus was on milk yield, milk ingredients, grazing and rumination behavior, physical activity, and energy metabolism. It was examined how concentrate supplementation changes behavior and internal state of the animals and if there are differences between cow strains that indicate a better suitability to pasture-based feeding systems. The main focus on the second trial was the estimation of plant species selection on grazing dairy cows. It was tested if estimating diet composition on pasture is possible with plant wax markers, such as alkanes, long-chain fatty acids, and long-chain alcohols. Differences between cow strains in selection behavior were investigated and if concentrate supplementation has an impact on plant species selection on pasture. Studies with plant wax marker and free-ranging dairy cows are rare and results might give advises how to adapt pasture management to selection behavior.

The third and fourth chapters, as the main parts of this cumulative thesis, are manuscripts which are formatted according to the instructions of the journal chosen for submission.

Chapter 3

Impact of cow strain and concentrate supplementation on grazing behaviour, milk yield and metabolic state of dairy cows in an organic pasture-based feeding system

C. Heublein [1,2], F. Dohme-Meier [1], K.-H. Südekum [2], R. M. Bruckmaier [3], S. Thanner [1] and F. Schori [1]

[1] *Agroscope, Institute for Livestock Sciences, ILS, Tioleyre 4, 1725 Posieux, Switzerland*
[2] *Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany*
[3] *Veterinary Physiology, Vetsuisse Faculty, University of Berne, Bremgartenstr. 109a, 3001 Berne, Switzerland*

Heublein, C., Dohme-Meier, F., Südekum, K.-H. Bruckmaier, R.M., Thanner, S., Schori, F.
Impact of cow strain and concentrate supplementation on grazing behaviour, milk yield and metabolic state of dairy cows in an organic pasture-based feeding system.
Animal (in press: doi:10.1017/S1751731116002639).

Abstract

As ruminants are able to digest fibre efficiently and assuming that competition for feed *v.* food use would intensify in the future, cereals and other field crops should primarily be destined to cover the dietary needs of humans and monogastric animals such as poultry and pigs. Farming systems with a reduced or absent concentrate supplementation, as postulated by organic agriculture associations, require adapted dairy cows. The aim of this experiment was to examine the impact of concentrate supplementation on milk production, grazing and rumination behaviour, feed intake, physical activity and blood traits with two Holstein-Friesian cow strains and to conclude the consequences for sustainable and organic farming. The experiment was a cross-over study and took place on an organic farm in Switzerland. In all, 12 Swiss Holstein-Friesian (HCH) cows and 12 New Zealand Holstein-Friesian (HNZ) cows, which were paired according to lactation number, days in milk and age for primiparous cows, were used. All cows grazed full time and were supplemented either with 6 kg/day of a commercial, organic cereal-grain mix or received no supplement. After an adaptation period of 21 days, a measurement period of 7 days followed, where milk yield and composition, pasture dry matter intake estimated with the *n*-alkane double-indicator technique, physical activity based on pedometer measurements, grazing behaviour recorded by automatic jaw movement recorder and blood samples were investigated. Non-supplemented cows had a lower milk yield and supplemented HCH cows produced more milk than supplemented HNZ cows. Grazing time and physical activity were greater for non-supplemented cows. Supplementation had no effect on rumination behaviour, but HNZ cows spent longer ruminating compared with HCH cows. Pasture dry matter intake decreased with the concentrate supplementation. Results of blood analysis did not indicate a strong negative energy balance for either non-supplemented or supplemented cows. Minor differences between cow strains in this short-term study indicated that both cow strains are equally suited for an organic pasture-based production system with no concentrate supplementation. Many factors such as milk yield potential, animal welfare and health, efficiency, grazing behaviour and social aspects influence the decision to supplement grazing dairy cows with concentrates.

Keywords: concentrate supplementation, organic farming, dairy cow, Holstein, pasture

Implications

In the future, competition between feed and food will increase. Ruminants like dairy cows are able to digest forage fibre efficiently. As concentrate supplementation is limited in organic dairy farming, restrictions may cause health problems as energy requirements for high-yielding dairy cows may not be met from forage-only rations. The aim of this study was to verify how supplementation changes the behaviour and production of grazing dairy cows under organic conditions. The two Holstein cow strains investigated in this short-term study are equally suited for an organic, pasture-based farming system with no concentrate supplementation.

Introduction

In Switzerland, the milk yield per cow has increased steadily over the last years (Bundesamt für Landwirtschaft, 2015), as has the cows' demand for nutrients and energy. This leads to the question of whether high-yielding dairy cows are still able to meet their energy requirements for efficient milk production on pasture-only diets. Farming systems with a reduced or absent concentrate supplementation, as postulated by organic agriculture associations, require adapted dairy cows. Studies suggest that high producing Holstein-Friesian (HF) cows are less suited for grazing systems, particularly under high stocking rates and seasonal calving systems and without concentrate supplementation (Kolver and Muller, 1998). In New Zealand, HF cows are bred for efficient pasture use with very little concentrate supplementation and seasonal calving (Washburn and Mullen, 2014). They differ in BW, average body condition score (BCS) and milk yield from other HF cow strains (Piccand *et al*., 2013) and have a lower milk production response to concentrate supplementation compared with HF cow strains with a high genetic merit for milk production (Horan *et al.,* 2005). In addition, they may be able to use pasture more efficiently for milk production (Macdonald *et al.,* 2008) and therefore may be better suited for organic milk production systems. According to Penning and Rutter (2004), behaviour exhibited by animals is an indication of the relationship between their internal state (e.g. nutritional requirements, health) and their environment (e.g. sward state, supplementation and climate). Therefore, grazing behaviour such as grazing duration, herbage intake and intake rate may provide some evidence about well-suited dairy cows and should be examined in detail, as the efficient use of pasture is a priority. According to the knowledge of the authors, this is the first study that investigated the effect of concentrate supplementation on the eating and rumination behaviour, metabolic states and milk production of grazing dairy cows under organic farming conditions.

The aim of this study was to examine the milk yield and milk composition, grazing and rumination behaviour, physical activity and metabolic profile of grazing cows with and without concentrate supplementation under organic conditions. Furthermore, this study aimed to identify differences between two HF cow strains, especially on grazing and rumination behaviour, which could indicate a better suitability for pasture-based milk production systems with restricted concentrate supplementation for organic farming.

Material and methods

Animals and experimental design

The experiment was a 2 × 2 cross-over study with two concentrate levels and two cow strains. All experimental procedures were in accordance with the Swiss guidelines for animal welfare and were approved (no. 2012_10_FR) by the Animal Care Committee of the Canton of Fribourg, Switzerland. Before selecting the cows for the experiment, a medical examination was performed. The experiment consisted of two measurement periods, each consisting of a 21-day adaption period and a 7-day measurement period. For the flow of work and equipment reasons, the cows were equally divided into two consecutive data collection periods of 7 days/measurement period. The experiment took place on the organic farm 'Ferme École de Sorens', located 824m above sea level in Sorens, Switzerland. A total of 24 HF cows, including 12 Swiss Holstein-Friesian (HCH) cows and 12 HF cows of New Zealand origin (HNZ), were used for the experiment. In all, 14 of them were multiparous and 10 were primiparous. Matched pairs of HCH and HNZ cows were formed according to the number of lactation, days in milk (DIM) and age for primiparous cows. The average economic breeding value (ISEL; Swiss Holstein Breeding Association, Posieux, Switzerland) was 985 (SD 74) for HCH cows. The average economic breeding value for HNZ cows was 806 (SD 70), but this excluded four animals as no breeding value was available. At the start of the first data collection period, HCH cows had an average number of lactations of 2.3 (SD 1.6), had been 109 (SD 17.9) DIM, had an average BW of 609 (SD 90.1) kg, a BCS of 2.4 (SD 0.28) and were producing 27.6 (SD 3.77) kg milk/day. The HNZ cows had an average number of lactations of 2.7 (SD 2.0), had been 114 (SD 16.7) DIM, had an average BW of 560 (SD 72.3) kg, a BCS of 2.9 (SD 0.24) and were producing 24.1 (SD 5.27) kg milk/day.

Grazing management, concentrate supplementation and weather conditions

The experiment was carried out in a rotational grazing system from 7 May to 8 July 2012. All 24 experimental cows were managed as a single group separated from the rest of the lactating herd and grazed on pasture from 0800 to 1400 h and from 1800 to 0430 h the following morning. Meanwhile, cows were milked and housed in a free-stall barn. Paddocks were rotationally grazed for 2 to 5 days based on decision rules considering sward height with a reference of 130-mm pre-grazing equivalent to an herbage mass of ~1000 kg dry matter (DM)/ha above 50mm until a post-grazing sward surface height of 50mm from ground level. The sward surface height was measured with a pasture meter (C-Dax pasture meter; C-Dax Ltd, Turitea, New Zealand) before cows entered a new paddock and after leaving the paddock. The average pre-grazing sward height was 129 (SD 16.4; n = 5) mm, corresponding to 1024 (SD 209.3) kg DM/ha above 50mm in the first measurement period and 122 (SD 11.2; n = 6) mm, corresponding to 941 (SD 143.8) kg DM/ha for the second measurement period. The average post-grazing sward surface height was 57 (SD 6.6; n = 5) mm in the first measurement period and 71 (SD 19.2; n = 6) mm in the second measurement period. Herbage mass above 50mm (kg DM/ha) was calculated according to: −624.5+12.8 × sward height (mm). This regression was calibrated for the pastures of the organic farm 'Ferme École de Sorens' (R^2 = 0.84; n = 89). The pastures were long established and composed predominantly of grasses (mainly *Lolium perenne, Dactylis glomerata* and *Phleum pratense*), but also of clover (mainly *Trifolium repens*) and herbs (mainly *Taraxacum officinale*). The pastures were fertilized once per year with 25m^3/ha of farm-produced manure (corresponding approximately to 80 kg N/ha, 22 kg P/ha and 108 kg K/ha). The chemical composition of the pasture during the measurement periods is presented in Table 1.

During an adaptation period of 21 days before both measurement periods, a step-wise provision towards the targeted amount of concentrate (UFA 275 Bio; UFA AG, Herzogenbuchsee, Switzerland), 0 or 6 kg (as-fed basis), took place. The pelleted concentrate was offered to six HCH–HNZ cow pairs in two equal meals (3 kg at 0600 h and 3 kg at 1700 h) after milking in the free-stall barn using separate buckets for each cow. During the measurement period, all 6 kg of concentrate was ingested by all cows with no refusals. The other six HCH–HNZ cow pairs received no concentrate in addition to pasture. Fresh water was always available and a mineral block was available in the barn. The ambient outdoor temperature was recorded daily by the meteorological station in Grangeneuve (MeteoSchweiz, Station Grangeneuve, Switzerland), located about 15 km north of the experimental pastures. During the first measurement period, the average temperature was 16°C (minimum 13, maximum 19) and 19°C (minimum 15, maximum 24) in the second measurement period.

Table 1: *Average chemical composition of concentrate (n = 2) and pasture (n = 14) samples (mean ± SD).*

Items	Concentrate				Pasture			
	Period 1	SD	Period 2	SD	Period 1	SD	Period 2	SD
DM (g/kg of wet weight)	884	1.8	882	1.0	174	30.3	166	20.1
Analysed nutrients and mineral composition (g/kg of DM)								
OM	944	0.3	944	0.2	894	8.6	892	6.2
CP	115	1.3	117	0.3	174	23.0	172	24.5
Ether extract	59	0.1	61	1.2	47	5.3	49	5.0
Starch	502	0.7	504	0.2				
ADF	79	2.2	76	0.7	249	20.8	270	23.4
NDF	221	5.7	228	3.8	397	41.8	427	48.2
Crude fibre	55	1.6	52	0.5	199	25.4	217	23.6
Ca	8.4	0.11	8.7	0.06	8.1	1.40	8.6	2.2
P	6.2	0.03	6.3	0.07	5.1	0.46	5.2	0.54
Mg	3.2	0.01	3.2	0.03	2.1	0.23	2.2	0.24
Na	1.8	0.03	1.8	0.00	0.2	0.05	0.1	0.05
K	7.9	0.05	7.9	0.00	38	3.4	35	1.8
Calculated energy and protein supply[1] per kg of DM								
NEL (MJ)	8.1	0.02	8.2	0.01	6.3	0.24	6.1	0.32
APDE (g)	79	2.2	76	0.7	104	5.1	102	6.3
Analysed *n*-alkane contents (mg/kg of DM)								
HC32	0.5	0.50	0.7	0.03	5.0	0.66	5.6	1.60
HC33	2.6	0.24	2.5	0.02	59	10.0	65	15.5

DM = dry matter; OM = organic matter; NEL = net energy for lactation; APDE = absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen; HC32 = dotriacontane, $C_{32}H_{66}$; HC33 = tritriacontane, $C_{33}H_{68}$.
[1]According to Agroscope (2013).

Data recording and sample collection

Milk yield (Flo-Master Pro; DeLaval AG, Sursee, Switzerland) was recorded daily and milk composition was analysed from a pooled sample of the morning and evening milk on days 1, 4 and 7 of each data collection period. Milk samples were preserved in tubes containing Broad Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland) at 8°C. The BW was recorded twice daily after milking and BCS was assessed before each adaptation period and before and after each data collection period according to the five-point system of Edmonson *et al.* (1989).

To estimate individual feed intake on pasture, the *n*-alkane double-indicator technique was used (Mayes *et al.,* 1986). Gelatin capsules (HGK 17–60 sl; Capsula GmbH, Ratingen, Germany), containing 0.5 g (weighing accuracy 0.001) alkane marker HC32 (dotriacontane, $C_{32}H_{66}$; Argenta Ltd, Auckland, New Zealand) on a carrier of dried fruit pomace, were administered manually with an applicator twice per day starting 6 days before the data collection periods. During the data collection period, a daily spot sample of faeces was taken from each cow with or without stimulus between 0700 and 0800 h. Samples were pooled by cow and collection period and stored at −20°C. Collection of pasture samples started and ended 1 day before the faeces sampling. Pasture sample collection was carried out as described by Graf *et al.* (2005). Daily samples were chopped and stored at −20°C until further analysis. Samples of concentrate were taken daily and pooled per data collection period.

Grazing and rumination behaviour was recorded automatically using a jaw movement recorder with a pressure sensor (Datenlogger MSR145; MSR Electronics GmbH, Hengart, Switzerland; Nydegger *et al.,* 2011). The jaw movement frequency and amplitude were measured for 72 h. Data were evaluated with the software programs MSRReader (MSR 5.20.01) and MSR-Viewer (Viewer V2), as described in the MSR145 User Manual (MSR Electronics GmbH). The number of grazing and rumination bouts were counted manually from the evaluated output of the software MSR-Viewer. A bout was defined as a sequence of a behaviour not interrupted by any other element of behaviour with a duration >4 min (Metz, 1975) and an inter-bout interval >7.5 min (Dado and Allen, 1994). Grazing bouts contain only those bouts during grazing on pasture not considering bouts when concentrate was eaten in the barn.

Physical activity, including time spent standing, lying and walking was determined using the IceTagTM pedometer (IceRobotics Ltd, Edinburgh, Scotland, UK). The pedometer was attached to the right hind leg of the cow at the metatarsus level and recorded acceleration in

three dimensions at 0.1 s intervals for 72 h. Using the software program IceTag-Analyser (V 4.005; IceRobotics Ltd), the data were downloaded and compiled over 60 s intervals. Walking was defined as >3 steps/min, as suggested by Thanner *et al.* (2014).

On days 4 and 5 of each data collection period, blood was collected at 0700 and 1400 h by puncture of the jugular vein, using the Vacuette® System (Greiner Bio-One GmbH, Kremsmünster, Austria). Plasma for the analysis of hormones was retrieved using Vacuette® EDTA tubes (Greiner Bio-One GmbH). After sampling, these tubes were cooled in ice water until they were centrifuged at room temperature (20°C) for 5 min at 3000×g. For analyses of blood metabolites and enzymes, Vacuette® serum tubes (Greiner Bio-One GmbH) were stored upside down for 1 h at room temperature (20°C) before centrifugation at 3000×g for 15min and then at 4000×g for an additional 5min (Thanner *et al.,* 2014). The retrieved serum and plasma samples were stored at −20°C until they were analysed for hormones, metabolites and enzymes.

Laboratory analysis

Milk samples were analysed by IR spectrometry (CombiFoss FT+; Foss, Hillerød, Denmark) for contents of fat, protein and lactose (International Dairy Federation, 2000; method number 141C). Urea was determined with a differential pH analyser (Eurochem, Ardea, Italy) before and after hydrolysis with urease (International Dairy Federation, 2004; method number 195). For milk acetone determination, acetone and an internal standard (2-butanone) were transferred via static headspace directly from the milk into the gas phase. The composition of the gas phase was determined with a flame ionization detector on a gas chromatograph (HP 5890 Series II; Agilent Technologies, Santa Clara, CA, USA).

Pasture and faeces samples were lyophilized (Christ, Delta 1-24LSC; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Concentrate, pasture and faeces samples were milled through a 1.0-mm screen (Brabender mill with titanium blades; Brabender, Duisburg, Germany). Subsamples were dried for 3 h at 105°C to determine DM and subsequently incinerated at 550°C until they reached a stable mass to assess the ash contents. Mineral residues in the ash were dissolved with nitric acid and analysed for Ca, Na, P, Mg and K with inductively coupled plasma optical emission spectrometry (ICP-OES Optima 2000 DV; PerkinElmer, Shelton, CT, USA with system ICP-OES Optima 7300) based on European Standard: EN 155510:2008. The contents of *n*-alkanes HC32 and HC33 (tritriacontane, $C_{33}H_{68}$) were determined as described by Peiretti *et al.* (2006). The N content was determined using the Dumas method (Association of Official Analytical Chemists (AOAC), 1995) on a C/N analyser (type FP-2000; Leco Instruments, St. Joseph, MI, USA)

and then multiplied by 6.25 to determine the CP content. The ether extract was determined using the Soxtec Avanti 2050 apparatus (Foss, Hillerød, Denmark) for extraction following the guidelines of Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten 5.1.1. (1993). Acid-detergent fibre (procedure 973.18; AOAC, 1995) was determined with correction for residual ash obtained after incineration at 500°C for 1 h. Crude fibre was analysed in pasture and concentrate (procedure 978.10; AOAC, 1995) and NDF (Mertens, 2002) was assessed with the addition of heat-stable amylase and sodium sulphite. Starch content was determined based on the polarimetric method (method 6493; International Organization for Standardization, 2000).

Metabolite concentrations and enzyme activities were determined using the following commercial test kits: albumin (no. 11970909; Roche Diagnostics, Rotkreuz, Switzerland), alkaline phosphatase (AP, no. 12173107; Roche Diagnostics), alanine aminotransferase (ALAT, no. 63212; bio- Mérieux, Marcy-l'Etoile, France), aspartate aminotransferase (ASAT, no. 63212; bioMérieux), β-hydroxybutyrate (BHBA; no. RB1007; Randox Laboratories, Crumlin, UK), cholesterol (no. 61218; bioMérieux), creatinine (no. 11489291216; Roche Diagnostics), creatine kinase (CK, no. 61141; bioMérieux), γ glutamyltransferase (GGT, no. 2016788; Roche Diagnostics), glutamate dehydrogenase (GLDH, no. 1929992; Roche Diagnostics), total protein (no. 1553836; Roche Diagnostics), urea (no. 61974, UV 250; bioMérieux), triglyceride (no. 61236; bioMérieux), nonesterified fatty acids (NEFA, no. FA 115; Randox Laboratories) and glucose (no. 1447513; Roche Diagnostics). Plasma insulin and IGF-1 concentrations were quantified using radioimmunoassay as described by Vicari *et al.* (2008). 3,5,3'-triiodthyronine (T3) and thyroxin (T4) were measured by radioimmunoassay using the Coat-A-Count® Total T3 kit and Coat-A-Count® Total T4 kit, respectively (Siemens Schweiz AG, Zurich, Switzerland).

Calculations and statistical analyses

Net energy for lactation (NEL) and the absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen were calculated according to Agroscope (2013). The energy-corrected milk yield (ECM) was calculated based on a 4% fat, 3.2% protein and 4.8% lactose according to Agroscope (2013). Feed intake was calculated using the ratio of the n-alkanes HC32 and HC33 on the basis of the equation proposed by Mayes *et al.* (1986).

To double check the intake estimation, the pasture intake without (equation (1), DMIconc0) and with concentrate supplementation (equation (2), DMIconc6) was additionally calculated

according to Baker (2004) based on the recommendations of Agroscope (2013). Changes in BW were not considered, as a period of 1 week is too short to estimate these accurately.

1) $DMI_{conc0} = (0.293 \times BW^{0.75} + 3.14 \times ECM) / NEL\ pasture$, or

2) $DMI_{conc6} = (0.293 \times BW^{0.75} + 3.14 \times ECM - NEL\ Conc \times 5.3\ kg\ DM) / NEL$ pasture

where $BW^{0.75}$ = metabolic body size (kg BW to the power 0.75), ECM (kg/d) the energy-corrected milk yield, NEL Conc = NEL content of the concentrate (MJ/kg DM), and NEL pasture the NEL content of the pasture (MJ/kg DM).

The statistical analyses were carried out with SYSTAT 13 (Systat Software Inc., Chicago, USA). Data for milk yield and composition, rumination and grazing behaviour, physical activity, feed intake and blood traits were collected over several days and averaged per cow and measurement period. They were analysed using following linear mixed model:

$$Y_{ijklm} = \mu + \tau_i + \varphi_j + P_k + (\tau p)_{ik} + (\tau\varphi)_{ij} + P_l + K_m (P_l) + \varepsilon_{ijklm}$$

where, Y_{ijklm} is the response, μ is the least squares mean, τ_i is the fixed effect of cow strain i (i = HCH, HNZ), φ_j is the fixed effect of the treatment j (j = Conc0, Conc6), P_k is the fixed effect of the period (k = period 1, period 2), $(\tau p)_{ik}$ is the effect of the interaction between cow strain i and period k, $(\tau\varphi)_{ij}$ is the effect of the interaction between cow strain i and treatment j, P_l is the random effect of cow pair l (1,…,12), K_m is the random effect of the cow m (1,…, 24) and ε_{ijklm} is the random error. Models of this type were recommended by Tempelman (2004) with variance components as variance-covariance structure for the repeated measurements. As there were only two periods no alternative variance-covariance structure was envisaged in this study.

Not normally distributed data were either logarithmically transformed to fit with normal distribution (NEFA and creatinine kinase) or analysed using R (R Core Team, 2012) with permutation tests for linear models (lactose; Good, 2005). Data presented in the tables were back transformed.

Due to the high proportion of insulin 'non-detects' (i.e. values below 3 µU/ml), descriptive statistics was performed as described by Helsel (2012) using R (R Core Team, 2012). Inferential statistics were based on rank methods (Brunner *et al.,* 2002). The model was of F1-LD-F2 type (one between subject factor, two within-subject factors) and ANOVA-type test statistics (Brunner *et al.,* 2002) were applied.

The effects were considered significant at $P \leq 0.05$. A value of $0.05 < P < 0.10$ was considered a trend.

Results

Milk yield and milk composition and feed intake

Non-supplemented cows produced less (P <0.001) milk and less (P <0.001) ECM compared with supplemented cows and HNZ cows had a lower (P = 0.04) milk yield compared with HCH cows, but no difference between cow strains was observed for ECM (Table 2). An interaction (P = 0.02) between cow strain and supplementation was reported for milk yield. The HNZ cows produced less (P = 0.04) milk per kg concentrate than HCH cows.

Milk fat content was greater (P <0.001) for non-supplemented cows, but milk protein content was not influenced by supplementation. The HNZ cows had a greater (P <0.01) protein content and in tendency a greater milk fat content than HCH cows. No differences were recorded for the lactose content relative to concentrate supplementation or cow strain. The milk contents of acetone and urea of non-supplemented cows were greater (P <0.001) compared with supplemented cows, but no differences were observed between cow strains.

For non-supplemented cows, pasture dry matter intake (DMI) estimated with *n*-alkanes was greater (P <0.001) than pasture DMI for supplemented cows, but total DMI estimated with alkanes was lower (P <0.001) for non-supplemented cows than for supplemented cows. New Zealand Holstein cows tended to have lower pasture DMI and total DMI compared with HCH cows. Calculated pasture DMI based on the requirements was greater (P <0.001) for non-supplemented cows, but supplementation had no effect on calculated total DMI and no differences between cow strains were observed for calculated pasture and total DMI.

Grazing and rumination behaviour and physical activity

Grazing time, mastication (*n*) and mastication rate (*n*/min) were greater (P <0.001) for non-supplemented cows compared with supplemented cows and no differences occurred between cow strains (Table 3). The number of grazing bouts was not affected by supplementation, but HNZ cows tended to have greater numbers compared with HCH cows. Non-supplemented cows had a greater (P <0.001) duration of eating bouts compared with supplemented cows, but no difference between cow strains was reported.

Concentrate supplementation had no effect on traits describing rumination behaviour, but HNZ cows spent more (P <0.005) time ruminating, had a greater (P <0.005) number of mastication and a greater (P <0.005) number of boli compared with HCH cows. No difference between cow strains was observed for mastication rate, mastication per boli and rumination bouts. New Zealand Holstein cows tended to have longer duration of rumination bouts compared with HCH cows.

Non-supplemented cows spent less (P <0.001) time lying down, but stood, moved and walked more (P <0.001). There was a trend recorded for HNZ to stand and move less and lie down more, but no difference was observed for walking. No significant interaction of concentrate supplementation and cow strain was observed.

Table 2*: Effect of concentrate supplementation and cow strain and their interactions on milk production performance and feed intake*

Items	Conc0		Conc6			*P* -Value		
	HCH	HNZ	HCH	HNZ	SD	Treatment	Cow strain	Interaction
Milk production performance								
Milk yield (kg/d)	24.9^{A}	23.0^{B}	30.0^{C}	26.0^{D}	4.8	<0.001	0.04	0.02
Milk yield concentrate[1] (kg/kg)			0.84^{a}	0.50^{b}	0.30		0.04	
ECM[2] (kg/d)	22.8^{A}	22.6^{A}	25.3^{B}	23.9^{B}	3.8	<0.001	0.41	0.16
Fat (%)	3.7^{A}	4.0^{A}	3.0^{B}	3.4^{B}	0.6	<0.001	0.08	0.50
Protein (%)	3.2^{A}	3.4^{B}	3.2^{A}	3.5^{B}	0.3	0.13	<0.01	0.96
Lactose[3] (%)	4.4	4.4	4.5	4.5	0.3	0.39	0.50	0.74
Acetone (mg/L)	2.87^{A}	2.53^{A}	1.52^{B}	1.36^{B}	0.79	<0.001	0.27	0.68
Urea (mg/L)	239^{A}	226^{A}	178^{B}	176^{B}	30.0	<0.001	0.49	0.34
Feed intake								
Pasture DMI (kg DM/d)	12.5^{A}	11.7^{A}	9.7^{B}	9.0^{B}	1.7	<0.001	0.09	0.83
Total DMI (kg DM/d)	12.5^{A}	11.7^{A}	15.0^{B}	14.3^{B}	1.7	<0.001	0.09	0.83
Calculated pasture DMI[4] (kg DM/d)	17.3^{A}	16.9^{A}	12.0^{B}	11.0^{B}	2.4	<0.001	0.23	0.17
Calculated total DMI[5] (kg DM/d)	17.3	16.9	17.3	16.3	2.4	0.23	0.23	0.17

Conc0 = cows without concentrate supplementation; Conc6 = cows with concentrate supplementation; HCH = Swiss Holstein-Friesian; HNZ = New Zealand Holstein-Friesian; DMI = dry matter intake; DM = dry matter.
^{ABCD} Means with different subscript letters within the same row differ ($P < 0.01$).
^{ab} Means with different subscript letters within the same row differ ($P < 0.05$).
[1]Milk yield per ingested concentrate (kg/kg).
[2]ECM = energy corrected milk yield (Agroscope, 2013).
[3]Log_{10} transformed for statistical analyses.
[4]According to Agroscope (2013) and Baker (2004) without BW chances and activity.
[5]According to Agroscope (2013) and Baker (2004) without BW chances and activity plus 5.3 kg DM of concentrate.

Table 3: *Effect of concentrate supplementation and cow strain and their interaction on grazing and rumination behaviour and physical activity over 24 h*

Items	Conc0		Conc6			*P*-Value		
	HCH	HNZ	HCH	HNZ	SD	Treatment	Cow strain	Interaction
Grazing behaviour over 24 h								
Time (min)	547.1^{A}	568.7^{A}	442.9^{B}	453.0^{B}	43.9	<0.001	0.20	0.63
Mastications (*n*)	41 232^{A}	42 102^{A}	32 070^{B}	32 865^{B}	3 859	<0.001	0.51	0.97
Mastication rate (*n*/min)	75.3^{A}	74.0^{A}	72.5^{B}	72.7^{B}	4.0	<0.001	0.73	0.06
Grazing bouts (*n*)	5.2	5.7	5.5	6.0	0.94	0.24	0.10	0.81
Duration grazing bouts (min)	110^{A}	104^{A}	81^{B}	78^{B}	18.8	<0.001	0.42	0.77
Rumination behaviour over 24 h								
Time (min)	381^{a}	405^{b}	398^{a}	423^{b}	40.8	0.15	<0.05	0.99
Mastications (*n*)	27 661^{a}	29 796^{b}	28 888^{a}	31 204^{b}	3 932	0.20	0.04	0.93
Mastication rate (*n*/min)	72.4	73.4	72.7	73.7	5.3	0.47	0.48	0.92
Rumination boli (*n*)	522^{a}	578^{b}	551^{a}	597^{b}	69.2	0.22	0.04	0.79
Mastications boli (*n*/boli)	51.5	53.8	54.9	53.4	5.0	0.26	0.83	0.17
Rumination bouts (*n*)	13.1	12.2	13.1	12.7	1.58	0.56	0.14	0.48
Duration rumination bouts (min)	29	35	31	34	7.0	0.95	0.08	0.40
Activity over 24 h								
Lying (min)	492^{A}	541^{A}	564^{B}	590^{B}	62	<0.001	0.05	0.41
Standing + moving (min)	949^{A}	900^{A}	877^{B}	851^{B}	62	<0.001	0.05	0.41
Walking (min)	421^{A}	419^{A}	373^{B}	362^{B}	59	<0.001	0.75	0.73

Conc0 = cows without concentrate supplementation; Conc6 = cows with concentrate supplementation; HCH = Swiss Holstein-Friesian; HNZ = New Zealand Holstein-Friesian.

^{AB} Means with different subscript letters within the same row differ ($P < 0.01$).

^{ab} Means with different subscript letters within the same row differ ($P < 0.05$).

Blood traits

Non-supplemented cows had lower (P <0.01) serum glucose concentration but greater (P <0.001) concentration of serum BHBA, NEFA and urea compared with supplemented cows (Table 4). Concentration of total protein in serum indicated a tendency to be affected by supplementation, with a greater concentration for non-supplemented cows. Concentrate supplementation had no effect on concentration of serum albumin, triglycerides, cholesterol and creatinine. The activity of creatinine kinase, GLDH and GGT was not affected by concentrate supplementation, but activity of ASAT and ALAT were greater (P = 0.04) and activity of AP was lower (P <0.001) for non-supplemented cows.

Concentrate supplementation had an impact on the plasma concentration of the hormones T3 and T4 with lower (P <0.001 and P = 0.04, respectively) concentrations for non-supplemented cows. Non-supplemented cows had a lower (P = 0.03) plasma insulin and lower (P <0.001) IGF-1 concentration compared with supplemented cows.

Differences between cow strains were recorded for serum glucose concentration with lower (P <0.01) concentration for HCH cows. Furthermore, HCH cows had a lower (P <0.01) activity of ALAT and a lower (P <0.05) concentration of IGF-1. For other blood traits no differences between cow strains were recorded. No interactions of concentrate supplementation and cow strain for blood traits were observed.

Table 4: *Effect of concentrate supplementation and cow strain and their interaction on blood metabolites, enzymes and hormones*

Items	Conc0		Conc6			*P*-Value		
	HCH	HNZ	HCH	HNZ	SD	Treatment	Cow strain	Interaction
Glucose (mmol/L)	3.15^{A}	3.31^{B}	3.25^{C}	3.46^{D}	0.18	<0.01	<0.01	0.54
BHBA (mmol/L)	0.91^{A}	0.82^{A}	0.68^{B}	0.69^{B}	0.17	<0.001	0.45	0.30
NEFA[1] (mmol/L)	0.12^{A}	0.14^{A}	0.08^{B}	0.09^{B}	0.05	<0.001	0.48	0.62
Urea (mmol/L)	4.86^{A}	4.77^{A}	3.68^{B}	3.73^{B}	0.90	<0.001	0.95	0.72
Total protein (g/L)	73.6	72.9	72.0	72.2	4.20	0.10	0.88	0.48
Albumin (g/L)	38.8	39.2	38.0	38.9	2.40	0.27	0.32	0.56
Triglycerides (mmol/L)	0.30	0.33	0.30	0.31	0.08	0.44	0.28	0.74
Cholesterol (mmol/L)	6.28	6.26	6.08	6.43	1.10	0.94	0.70	0.24
Creatinine (µmol//L)	73.8	70.7	76.2	69.5	8.2	0.66	0.07	0.19
Creatine kinase[1] (U/L)	153	191	146	176	55.2	0.26	0.13	0.79
GLDH (U/L)	14.4	16.1	13.1	16.7	4.11	0.71	0.08	0.34
GGT (U/L)	22.8	24.7	22.8	24.8	4.60	0.90	0.20	0.97
ASAT (U/L)	71.2^{a}	74.8^{a}	67.6^{b}	72.8^{b}	7.38	0.04	0.15	0.52
AP (U/L)	39.1^{A}	50.6^{A}	48.9^{B}	61.2^{B}	21.20	<0.001	0.17	0.86
ALAT (U/L)	29.2^{A}	31.7^{B}	26.6^{C}	29.5^{D}	2.60	<0.001	<0.01	0.65
IGF-1 (ng/mL)	91^{a}	109^{b}	113^{c}	141^{d}	31.5	<0.001	<0.05	0.25
T3 (nmol/L)	2.28^{A}	2.36^{A}	2.45^{B}	2.60^{B}	0.31	<0.001	0.18	0.34
T4 (nmol/L)	49.0^{a}	48.7^{a}	51.6^{b}	53.5^{b}	12.01	0.04	0.84	0.51
Insulin (µU/mL)	4.31^{a}	3.81^{a}	5.82^{b}	5.92^{b}	3.54	0.03	0.94	0.58

Conc0 = cows without concentrate supplementation; Conc6 = cows with concentrate supplementation HCH = Swiss Holstein-Friesian; HNZ = New Zealand Holstein-Friesian; NEFA = non-esterified fatty acids; GLDH = glutamate dehydrogenase; GGT = gamma-glutamyltransferase; ASAT = aspartate aminotransferase; AP = alkaline phosphatase; ALAT = alanine aminotransferase; T3 = 3,5,3'-trijodthyronine; T4 = thyroxin.

^{ABCD} Means with different subscript letters within the same row differ ($P < 0.01$).

^{abcd} Means with different subscript letters within the same row differ ($P < 0.05$).

$^{11}\text{Log}_{10}$ transformed for statistical analyses.

Discussion

Many studies investigated the effects of concentrate supplementation on intake, milk production, body condition, grazing behaviour and digestion under non-organic farming conditions, reviewed by Bargo *et al.* (2003) for grazing dairy cows. Studies under organic farming conditions focussed more on the effects on health and fertility on farm level without considering the basic effects of concentrate supplementation (Sehested *et al.,* 2003; Ertl *et al.*, 2014; Ivemeyer *et al.,* 2014). According to the knowledge of the authors no other study investigated the effect of concentrate supplementation on the eating and rumination behaviour, intake, metabolic states and milk production of grazing dairy cows under organic farming conditions. Changes in organic herbage quality, as found by Spann *et al.* (2007), might be partly due to the principles of organic agriculture (International Federation of Organic Agriculture Movements, 2015), for instance, prohibition of synthesized fertilizers and pesticides, as well as with the general promotion of natural, multispecies pastures. As the nutritive value and the fibre content could influence the outcome of concentrate supplementation on grazing dairy cows, studies like ours are needed to close the gap. Finally, only few studies investigated the grazing and rumination behaviour, especially bites per rumination bolus, when investigating the effects of concentrate supplementation of grazing dairy cows.

Effect of concentrate and consequences for pasture based organic farming

In accordance with Bargo *et al*. (2003), milk yield and ECM increased for supplemented cows, but to a smaller extent. The observed interaction between concentrate supplementation and cow strain for milk production indicate the different genetic potential for milk production between the two cow strains (Bargo *et al.,* 2002; Horan *et al.,* 2005). Supplemented HCH cows responded to the extra energy supply with greater milk yield compared with HNZ cows, but ECM was similar.

The reduced milk fat content of supplemented cows is in agreement with other studies, when cows received >4 kg/day of concentrate (Bargo *et al.,* 2002; Horan *et al.,* 2005). Furthermore, the increased activity of plasma AP in supplemented cows may indicate acidotic stress. To ensure rumen health, rumination is a key factor as it influences salivation and therefore rumen buffering. In the current study, concentrate supplementation had no effect on rumination behaviour. It would have been expected that with increasing pasture DMI, time ruminating, rumination mastication and number of boli increased (Beauchemin and Rode, 1997; McCarthy *et al.,* 2007b). The absence of differences in rumination mastication

per bolus and number of boli may indicate a similar ease of bolus formation and swallowing for both supplemented and non-supplemented cows, although higher grain diets reflect a greater ease of bolus formation (Beauchemin and Rode, 1997). Rumination mastication per bolus was in the normal range. This poses the question of whether characteristics of rumination behaviour are a suitable indicator of sufficient fibre supply and therefore rumen health, at least in grazing dairy cows.

In agreement with other studies, supplementation caused a substantial reduction in grazing time (Bargo *et al.,* 2002; McCarthy *et al.,* 2007b) and therefore reduced grazing mastication and grazing mastication rate. This implies the lower motivation of supplemented cows to graze and is supported by the reduced duration of grazing bouts for supplemented cows, as grazing time or grazing time coupled with intake rate (bite rate and size) at the same BW might be indicators for the feeding drive (McCarthy *et al.,* 2007b; Prendiville *et al.,* 2010).

In accordance with other studies, pasture DMI decreased with concentrate supplementation, which is expressed as substitution rate (Bargo *et al.,* 2002; McCarthy *et al.,* 2007b). Both calculated and estimated (using *n*-alkanes) pasture DMI were lowered by concentrate supplementation. In contrast, total DMI of supplemented cows was greater or at least the same as the total DMI of non-supplemented cows. However, with reduced pasture DMI for supplemented cows, cheap pasture is substituted by expensive concentrate which is an economic aspect for the farmer. Furthermore, importing ingredients of a commercial organic concentrate mix disturbs the holistic approach of organic guidelines. In the current study, the energy:protein ratio may be balanced as concentrations of urea in blood and milk were in the normal range, with elevated values for non-supplemented cows. In organic farming, the risk of excessive protein intake from pasture might be reduced as the CP content of organic pasture is lower compared with conventional pasture (Spann *et al.,* 2007).

Current results indicate a strong relationship between physical activity and grazing behaviour. As supplemented cows spent less time grazing, they spent less time standing and walking. In a pasture-based feeding system, where energy might be the first limiting nutrient, physical activity on pasture is an important factor to be considered. Grazing cows have a greater energy expenditure compared with cows fed indoors, as grazing cows take more steps, spend less time lying down and spend more time eating (Kaufmann *et al.,* 2011). According to the Commonwealth Scientific and Industrial Research Organisation (2007), energy requirements for maintenance may increase in the range of 10% to 50% depending on grazing conditions, digestibility of pasture, distance walked, weather, topography and interactions between these factors. Thus, supplemented cows on pasture did not only ingest

more energy, but also presented improved energy balance due to energy savings in relation to shorter grazing and physical activities.

A lack of energy in early lactation can cause metabolic problems such as ketosis. In the current study, the increased acetone concentration in milk for non-supplemented cows indicates a small risk of ketosis. This is confirmed by an increased concentration of BHBA and NEFA and decreased glucose and insulin concentration for non-supplemented cows. The increased blood glucose, insulin and IGF-1 concentrations for supplemented cows indicate an increased energy status, whereas increased concentration of blood NEFA and BHBA for non-supplemented cows suggest a lower energy status (Reist *et al.,* 2002). As the concentration of T3 and T4 decreases with stronger negative energy balance (NEB) (Huszenicza *et al.,* 2002), non-supplemented cows in current study had lower concentration of T3 and T4 and therefore had a lower energy status compared with supplemented cows. Furthermore, the increased activity of ALAT of non-supplemented cows indicates an increased use of amino acids as an energy source or for gluconeogenesis (Garber *et al.,* 1976). The greater activity of ASAT of non-supplemented cows alone should be interpreted with caution. As the activities of GLDH, GGT and CK were not increased for non-supplemented cows, the occurrence of fatty liver syndrome in cows of this study is unlikely.

Results of blood traits indicate that non-supplemented cows were not in strong NEB. Supplementation may not be necessary to balance an organic pasture-based diet, at least for cows in mid-lactation and therefore more severe, but more flexible restrictions over the whole lactation for supplementation are favourable. Finally, it can be stated that the effects of concentrate supplementation on milk yield, milk composition, grazing behaviour and intake are similar in the present organic study compared with the cited conventional studies.

Effect of cow strain and consequences for pasture based organic farming

Another aspect of organic farming is the choice of the breed or strain. This implies the selection of cows adapted to lowinput pasture or forage-based feeding system for organic dairy production. In the present study, the milk yield responses obtained for HNZ and HCH cows were similar to the results of Horan *et al*. (2005). Swiss Holstein cows reached almost the overall milk yield response of 1 kg milk/ 1 kg concentrate as published by Bargo *et al*. (2003), but not in ECM terms. The lower genetic potential for milk production of HNZ cows might be the reason for their lower response of 0.5 kg milk/kg of ingested concentrate, as pasture allowance and mass was the same for both strains. Cows with high genetic potential for milk yield have a greater milk yield response to concentrate supplementation (McCarthy *et al.,* 2007b). This phenomenon might be attributed to greater nutrient partition to milk

production in high genetic merit cows compared with lower genetic merit cows (Dillon *et al.,* 2006). This is supported by the results of IGF-1, with greater plasma concentration in HNZ cows. Greater IGF-1 plasma concentration indicates the coupling of the somatotropic axis, as the liver responds to increased growth hormone concentration and therefore nutrient partitioning favours the build-up of body tissue instead of milk production (Lucy *et al.,* 2009).

In contrast to results for milk yield, no difference for ECM between cow strains was observed which can be explained by the greater milk protein and in trend greater milk fat content of the HNZ cows. Similarity of ECM yields of the two cow strains indicate a similar efficiency in milk production for HCH and HNZ cows in the present study.

New Zealand Holstein cows ruminated longer than HCH cows, which was also observed in previous studies (Schori and Münger, 2014; Thanner *et al.,* 2014). In line with this result HNZ cows tended to spend more time lying down compared with HCH cows as rumination of cows on pasture is associated with lying down (Kilgour, 2012). Furthermore, HNZ cows had a greater number of boli and greater rumination mastication per day compared with HCH cows. Anatomical differences of the muzzle and incisor breadth might explain this (Rook, 2000). Prendiville *et al.* (2010) observed smaller bolus size for the smaller Jersey cows compared with HF cows, indicating that anatomical differences influence the pattern of bolus movement during rumination. Although greater chewing activity during grazing and rumination is associated with a greater salivary secretion and therefore a better fibre digestibility (Domingue *et al.,* 1991), HNZ cows could not benefit from longer rumination time in terms of milk production.

The trend for lower pasture DMI by HNZ cows and the lack of clear differences in grazing behaviour do not confirm a greater feeding drive for HNZ cows (McCarthy *et al.,* 2007b). It could have been expected that HCH cows had a greater pasture DMI, as DMI and BW are positively correlated (Kertz *et al.,* 1991), as BW is usually positively linked to rumen size and therefore intake capacity. Similar pasture DMI indicates a greater DMI per kg BW for HNZ cows compared with HCH cows. Furthermore, HNZ cows spent longer time ruminating. Digestion rate and discharging of the rumen might be increased as rumination determines digestion rate and therefore controls voluntary intake (Bae *et al.,* 1983; Gregorini *et al.,* 2012).

The substitution rate of pasture DMI is linked to milk response with a lower substitution rate for cows with high genetic potential for milk yield (Bargo *et al.,* 2003). However, in the current study, substitution rate, estimated using n-alkane, was the same for both cow strains (0.5 kg/kg). The results are in accordance with missing differences for ECM.

Because no differences were observed between cow strains in blood concentration of insulin, NEFA and BHBA, which are indicators of the energy balance of dairy cows (Reist *et al.,* 2002), no difference between cow strains are obvious in energy status. However, a trend for an increased concentration of creatinine in blood plasma for HCH cows may indicate a higher skeletal muscle breakdown and the mobilization of more protein as an energy source or for gluconeogenesis. In accordance with results from McCarthy *et al.* (2007a), HNZ cows had greater serum glucose concentration compared with HCH cows. The greater glucose concentration for HNZ cows during breeding season (60 to 150 DIM) represents an important source for energy for the ovary, as Forshell *et al.* (1991) reported an effect of glucose concentration on conception rates. Therefore, greater glucose concentration might indicate greater conception rates which is in line with the differences in conception rates reported between HCH cows and HNZ cows (Piccand *et al.,* 2013).

The increase in ALAT activity is in accordance with the results of Thanner *et al.* (2014), which might suggest the elevated use of amino acids of the HNZ cows for purposes other than protein synthesis.

Minor differences between cow strains in the current study indicate that both cow strains are equally suited for an organic, pasture-based feeding system. However, the current study was a short-term experiment performed with a small number of animals after the peak of lactation without considering BCS losses, fertility and health traits during a whole lactation, which are important aspects for continuous and successful organic farming. Just choosing a different cow breed or cow strain may not ensure a well-working, low-input organic system. Careful selection of cows that have a high grazing drive ensures the efficient use of pasture for milk production, whereas cows that can deal with lower energy intake at the beginning of lactation and show properties of good health and fertility may be well-suited organic cows.

Acknowledgements

This study was funded by the AGFF (Zürich, Switzerland) research fund and the Fondation Sur-la-Croix (Basel, Switzerland). The authors gratefully acknowledge the help afforded to them by the staff of the farm “Ferme École de Sorens” and C. E. Pardo for sampling and assistance with the cows during experiment. We thank the staff of Agroscope in Posieux and Vetsuisse faculty of Berne for assistance in the laboratory analyses and W. Luginbühl for statistical advice.

References

Agroscope 2013. Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer (Grünes Buch). Forschungsanstalt Agroscope Liebefeld-Posieux ALP, Posieux, Switzerland.

Association of Official Analytical Chemists (AOAC) 1995. Official methods of analysis, 16th edition, AOAC, Arlington, VA, USA.

Bae DH, Welch JG and Gilman BE 1983. Mastication and rumination in relation to body size of cattle. Journal of Dairy Science 66, 2137-2141.

Baker RD 2004. Estimating herbage intake from animal performance. In Herbage intake handbook (ed. PD Penning), pp. 95-120. The British Grassland Society, Reading, UK.

Bargo F, Muller LD, Delahoy JE and Cassidy TW 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. Journal of Dairy Science 85, 1777-1792.

Bargo F, Muller LD, Kolver ES and Delahoy JE 2003. Invited Review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86, 1-42.

Beauchemin KA and Rode LM 1997. Minimum versus optimum concentrations of fiber in dairy cow diets based on barley silage and concentrates of barley or corn. Journal of Dairy Science 80, 1629-1639.

Bundesamt für Landwirtschaft 2015. Auswertung der Daten über die Milchproduktion. Eidgenössisches Department für Wirtschaft, Bildung und Forschung WBF. BLW. Bern, Switzerland.

Brunner E, Domhof S and Langer F 2002. Nonparametric analysis of longitudinal data in factorial experiments. Wiley, New York, NY, USA.

Commonwealth Scientific and Industrial Research Organisation 2007. Energy. In Nutrient requirements of domesticated ruminants (ed. Freer M, Dove H and Nolan JV), pp. 1–69. Commonwealth Scientific and Industrial Research Organisation, Collingwood, Australia.

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

Dillon P, Berry DP, Evans RD, Buckley F and Horan B 2006. Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production. Livestock Science 99, 141-158.

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

Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G 1989. A body condition scoring chart for holstein dairy cows. Journal of Dairy Science 72, 68-78.

Ertl P, Knaus W and Steinwidder A 2014. Comparison of zero concentrate supplementation with different quantities of concentrates in terms of production, animal health, and profitability of organic dairy farms in Austria. Organic Agriculture 4, 233–242.

Forshell KP, Andersson L and Pehrson B 1991. The relationships between the fertility of dairy cows and clinical and biochemical measurements, with special reference to plasma glucose and milk acetone. Journal of Veterinary Medicine Series A 38, 608-616.

Garber AJ, Karl IE and Kipnis DM 1976. Alanine and glutamine synthesis and release from skeletal muscle. II. The precursor role of amino acids in alanine and glutamine synthesis. Journal of Biological Chemistry 251, 836-843.

Good P 2005. Permutation, parametric and bootstrap tests of hypotheses. 3rd edition. Springer, Huntington Beach, CA, USA.

Graf CM, Kreuzer M and Dohme F 2005. Effects of supplemental hay and corn silage versus full-time grazing on ruminal pH and chewing activity of dairy cows. Journal of Dairy Science 88, 711-725.

Gregorini P, DelaRue B, McLeod K, Clark CEF, Glassey CB and Jago J 2012. Rumination behavior of grazing dairy cows in response to restricted time at pasture. Livestock Science 146, 95-98.

Helsel DR 2012. Statistics for censored environmental data using Minitab and R, 2nd edition. Wiley, Hoboken, NJ, USA.

Horan B, Dillon P, Faverdin P, Delaby L, Buckley F and Rath M 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. Journal of Dairy Science 88, 1231-1243.

Huszenicza G, Kulcsar M and Rudas P 2002. Clinical endocrinology of thyroid gland function in ruminants. Veterinarni Medicina Czech 47, 199–210.

International Dairy Federation 2000. Determination of milk fat, protein and lactose content. Guide for the operation of mid-infra-red instruments. IDF Standard method No. 141C, Brussels, Belgium.

International Dairy Federation 2004. Milk. Determination of urea content. Enzymatic method using difference in pH. IDF Standard method No. 195, Brussels, Belgium.

International Federation of Organic Agriculture Movements 2015. Principles of organic agriculture. Retrieved on 20 August 2015 from http://www.ifoam.org/en/organic-landmarks/principles-organic-agriculture

International Organization for Standardization 2000. Animal feeding stuffs - determination of starch content - polarimetric method. ISO, Geneva, Switzerland.

Ivemeyer S, Walkenhorst M, Holinger M, Maeschli A, Klocke P, Spengler Neff A, Staehli P, Krieger M and Notz C 2014. Changes in herd health, fertility and production under roughage based feeding conditions with reduced concentrate input in Swiss organic dairy herds. Livestock Science 168, 159-167.

Kaufmann LD, Münger A, Rérat M, Junghans P, Görs S, Metges CC and Dohme-Meier F 2011. Energy expenditure of grazing cows and cows fed grass indoors as determined by the 13C bicarbonate dilution technique using an automatic blood sampling system. In Journal of Dairy Science, 1989-2000.

Kertz AF, Reutzel LF and Thomson GM 1991. Dry matter intake from parturition to midlactation. Journal of Dairy Science, 2290-2295.

Kilgour RJ 2012. In pursuit of normal: A review of the behaviour of cattle at pasture. Applied Animal Behaviour Science 138, 1-11.

Kolver ES and Muller LD 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science 81, 1403-1411.

Lucy MC, Verkerk GA, Whyte BE, Macdonald KA, Burton L, Cursons RT, Roche JR and Holmes CW 2009. Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. In Journal of Dairy Science, 526-539.

Macdonald KA, Verkerk GA, Thorrold BS, Pryce JE, Penno JW, McNaughton LR, Burton LJ, Lancaster JAS, Williamson JH and Holmes CW 2008. A comparison of three strains of Holstein-Friesian grazed on pasture and managed under different feed allowances. Journal of Dairy Science 91, 1693-1707.

Mayes RW, LAMB CS and Colgrove PM 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. The Journal of Agricultural Science 107, 161-170.

McCarthy S, Berry DP, Dillon P, Rath M and Horan B 2007a. Effect of strain of Holstein-Friesian and feed system on calving performance, blood parameters and overall survival. Livestock Science 111, 218-229.

McCarthy S, Horan B, Rath M, Linnane M, O'Connor P and Dillon P 2007b. The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. Grass and Forage Science 62, 13-26.

Mertens DR 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 85, 1217-1240.

Metz JHM 1975. Time patterns of feeding and rumination in domestic cattle. PhD thesis, University of Wageningen, Wageningen, Netherlands.

Nydegger F, Gygax L and Egli W 2011. Automatisches Messer der Kaubewegungen bei Wiederkäuern mit Hilfe eines Drucksensors. Agrarforschung Schweiz 2, 60–65.

Penning PD and Rutter SM 2004. Ingestive behaviour. In Herbage intake handbook (ed. PD Penning), pp. 151-175. The British Grassland Society, Reading, UK.

Peiretti PG, Meineri G, Miraglia N, Mucciarelli M and Bergero D 2006. Intake and apparent digestibility of hay or hay plus concentrate diets determined in horses by the total collection of feces and n-alkanes as internal markers. Livestock Science 100, 189-194.

Piccand V, Cutullic E, Meier S, Schori F, Kunz PL, Roche JR and Thomet P 2013. Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system. Journal of Dairy Science 96, 5352-5363.

Prendiville R, Lewis E, Pierce KM and Buckley F 2010. Comparative grazing behavior of lactating Holstein-Friesian, Jersey, and Jersey x Holstein-Friesian dairy cows and its association with intake capacity and production efficiency. Journal of Dairy Science 93, 764-774.

R Core Team 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved on 20 August 2015, from http://www.R-project.org/

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N and Blum JW 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. Journal of Dairy Science 85, 3314-3327.

Rook AJ 2000. Principles of foraging and grazing behaviour. In Grass - its production & utilization (3rd edition) (ed. A. Hopkins), pp. 229-241. Blackwell Science, Oxford, UK.

Schori F and Münger A 2014. Intake, feed conversion efficiency and grazing behaviour of two Holstein cow strains in a pasture-based production system under organic farming in Switzerland. Organic Agriculture 4, 175–186.

Sehested J, Kristensen T and Søegaard K 2003. Effect of concentrate supplementation level on production, health and efficiency in an organic dairy herd. Livestock Production Science 80, 153-165.

Spann B, Diepolder M, Schmidtlein EM, Tusch S, Hermüheim A and Spengel D 2007. Umstellung zur ökologischen Milchviehhaltung - Vergleich verschiedener Leistungsparameter. In Angewandte Forschung und Beratung für den ökologischen Landbau in Bayern (volume 3) (ed. K Wiesinger), pp 65-77. Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft, Freising, Germany.

Tempelman RJ 2004. Experimental design and statistical methods for classical and bioequivalence hypothesis testing with an application to dairy nutrition studies. Journal of Animal Science 82, E162-172.

Thanner S, Schori F, Bruckmaier RM and Dohme-Meier F 2014. Grazing behaviour, physical activity and metabolic profile of two Holstein strains in an organic grazing system. Journal of Animal Physiology and Animal Nutrition 98, 1143–1153.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten 1993. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III: Die Chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Vicari T, van den Borne JJGC, Gerrits WJJ, Zbinden Y and Blum JW 2008. Postprandial blood hormone and metabolite concentrations influenced by feeding frequency and feeding level in veal calves. Domestic Animal Endocrinology 34, 74-88.

Washburn SP, Mullen KAE, 2014. Invited review: Genetic considerations for various pasture-based dairy systems. Journal of Dairy Science 97, 5923-5938.

Chapter 4

Using plant wax markers to estimate the diet composition of grazing Holstein dairy cows

C. Heublein[*†], K.-H. Südekum[†], F. L. Gill[‡], F. Dohme-Meier[*], F. Schori[*1]

[*]*Agroscope, Institute for Livestock Sciences, ILS, Tioleyre 4, 1725 Posieux, Switzerland*
[†]*University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany*
[‡] *University of Leeds, School of Earth and Environment, LS2 9JT, Leeds, United Kingdom*

Heublein, C., Südekum, K.-H., Gill, F., Dohme-Meier, F., Schori, F.
Using plant wax markers to estimate the diet composition of grazing Holstein dairy cows.
J. Dairy Sci. 100:1019-1036 (https://doi.org/10.3168/jds.2016-11494).

ABSTRACT

The objective of this study was to test whether diet selection of dairy cows under grazing conditions could be estimated using plant wax markers. Furthermore, differences between 2 cow strains and the effect of concentrate supplementation on plant species selection were investigated. The experiment was a study with a crossover design performed on an organic farm with 12 Swiss Holstein cows and 12 New Zealand Holstein cows. Both experimental periods consisted of a 21-d adaptation and a 7-d measurement period. All cows grazed full time in a rotational stocking system and received either no concentrate or 6 kg/d of a commercial cereal-grain mix. Representative herbage samples of each grazed paddock were taken and botanical composition of subsamples was manually determined. The average proportions of the plant species were 27.8% *Lolium perenne*, 6.1% *Dactylis glomerata*, 10.4% *Trifolium repens*, and 9.0% *Taraxacum officinale*. Other grass species were merged as "other grass" (38.2%) and other forb species as "other forbs" (8.5%). *n*-Alkanes, long-chain fatty acids, and long-chain alcohols (LCOH) were analyzed in the samples of plant species, concentrate, and feces from each cow. A linear discriminant analysis indicated that diet components were differentiated best with LCOH (96%) and worst with the combination of all marker groups together (12%). For each marker, the fecal marker recovery (FR) relative to dosed ytterbium was determined in 2 ways. Estimation of diet composition was performed with the software "EatWhat," and results were compared with botanical composition with the Aitchison distance. The results indicate that the diet composition of grazing dairy cows can be estimated using plant wax markers. Additionally, the calculation of FR led to mostly reliable results, yet this approach needs further validation. The most accurate estimation was achieved with the marker combination of *n*-alkanes and LCOH with a correction for FR. Less accurate estimations were achieved with long-chain fatty acids alone or in combination with *n*-alkanes. No difference relating to diet selection between the 2 cow strains was recorded, but supplemented cows apparently ingested higher proportions of *T. repens* than nonsupplemented cows. Awareness that supplementation influences selection behavior of grazing dairy cows may lead to adaptations in botanical composition of the pasture according to the demand of the animals.

Keywords: alkane, long-chain fatty acid, long-chain alcohol, concentrate supplementation

INTRODUCTION

The benefits of grassland communities with a higher diversity of species and functional groups, such as higher productivity, increased resources utilization, higher uptake of nitrogen, and increased occupation of available space, are well known (Spehn et al., 2005). Recently, the considerable features of multi-species, legume-based grassland-livestock systems at different stages in the soil-plant-animal-atmosphere system were summarized by Lüscher et al. (2014). They stated that legume-based grassland-livestock systems would constitute one of the pillars for more sustainable and competitive ruminant production systems and will become more important in the future. Concentrate supplementation of dairy cows in a pasture-based feeding system causes substitution of herbage and grazing time is reduced [McCarthy et al., 2007; C. Heublein, F. Dohme-Meier, K.-H. Südekum, R. M. Bruckmaier (Vetsuisse Faculty, Bern, Switzerland), S. Thanner (Agroscope, Posieux, Switzerland), and F. Schori, unpublished data], but no certainties exist about whether it influences plant species selection in multispecies pastures. According to Villalba et al. (2015), the knowledge of the effects of feed context on preference of grazing animals should pioneer innovative management strategies to enhance forage intake, productivity, and animal welfare. Previous studies examined the suitability of different cow strains or breeds for a pasture-based feeding system [McCarthy et al., 2007; Piccand et al., 2013; C. Heublein, F. Dohme-Meier, K.-H. Südekum, R. M. Bruckmaier (Vetsuisse Faculty, Bern, Switzerland), S. Thanner (Agroscope, Posieux, Switzerland), and F. Schori, unpublished data], but to the authors' knowledge, no studies considered differences in diet selection on pasture. In New Zealand, Holstein cows are bred for an efficient use of pasture and have a higher feeding drive (McCarthy et al., 2007). Therefore, differences might exist in plant species selection between New Zealand and other Holstein cow strains. Such investigations are needed in natural grazing situation with a greater number of plant species, as requested by Villalba et al. (2015).

Plant wax markers, such as *n*-alkanes (hereafter called alkanes), long-chain fatty acids (LCFA), and long-chain alcohols (LCOH), are used for diet composition estimation of grazing ruminants (Ali et al., 2005; Lin et al., 2012). With the combination of alkanes and LCFA (Ferreira et al., 2009, 2011) or with alkanes and LCOH (Boland et al., 2012; Ferreira et al., 2015), diet composition estimations provided reasonable results for diets with between 2 and 6 components. The combination of all 3 marker groups might be applicable to situations with more complex diets (Ferreira et al., 2015). Supplementary feeds, such as concentrates, can be labeled and considered as an additional component in the diet (Dove and Charmley, 2008; Elwert et al., 2008). However, several studies included shrubs (Ali et al., 2005) or heather-gorse plant species (Ferreira et al., 2015) in the diets, which are not typical plant

species occurring on pastures for dairy cows. Various grasses, legumes, and forbs are the main plants growing on pastures grazed by dairy cows, and studies to estimate plant species selection on multispecies pastures with dairy cows are rare. In one of the few studies on this kind of multispecies pasture, using alkanes alone led to erroneous diet composition estimations of dairy cows (Schori et al., 2012). Therefore, we tested whether the approach of estimating diet composition of grazing dairy cows using plant wax markers is applicable under farming conditions and if reasonable results are obtained with different breeds and concentrate supplementation.

The basic precondition for estimating diet selection of ruminants on a multispecies pasture is the sufficient differentiation of marker profiles between plant species. Identification of markers that contribute most to the differentiation between plant species may reduce workload and contribute to a more accurate differentiation as low concentration of markers and large within-species variation may limit their use for diet estimation (Mayes and Dove, 2000). As the recovery of the markers in the feces is incomplete, an important element for gaining accuracy of diet composition estimation is the fecal recovery (FR) correction (Ferreira et al., 2015). Corrections are needed for incomplete FR of alkanes (Dove and Mayes, 1991), LCFA (Ferreira et al., 2009), and LCOH (Ferreira et al., 2015), but in the aforementioned studies, FR was determined in indoor feeding experiments with similar diet composition to outdoors, with known amount of DMI, diet composition, and collection of total fecal output. This approach is labor intensive and expensive, so 2 alternative ways for calculating FR were used in the current study. The aim of the study was to test whether the approach using calculated FR to estimate diet selection of dairy cows is applicable under grazing conditions and to investigate which marker group or marker group combination, with or without FR correction, delivers the most accurate estimation. Furthermore, differences between 2 cow strains and the effect of concentrate supplementation on plant species selection were investigated.

MATERIALS AND METHODS

Experimental Design and Animals

All experimental procedures were in accordance with the Swiss guidelines for animal welfare and were approved (no. 2012_51_FR) by the Animal Care Committee of the Canton of Fribourg, Switzerland. Before selecting the cows for the experiment, a medical checkup including vital parameters as well as udder and claw health was performed. The experiment was a 2 × 2 factorial design, which was conducted as a crossover design with 2 concentrate

levels and 2 cow strains. It was divided into 2 measurement periods, each consisting of a 21-d adaption period and a 7-d data collection period (Figure 1). For the flow of work and equipment reasons, the cows were equally divided into 2 consecutive data collection periods of 7 d per measurement period, resulting in 4 data collection periods. The experiment took place on the organic farm “Ferme École de Sorens” located 824 m above sea level in Sorens, Switzerland.

A total of 24 Holstein cows, including 12 Swiss Holstein cows (HCH) and 12 Holstein cows of New Zealand origin (HNZ), were used for the experiment. Sixteen of them were multiparous and 8 were primiparous. Matched pairs of HCH and HNZ cows were formed according to the number of lactation and DIM for multiparous cows. For primiparous cows, age was considered beside DIM. At the start of the first data collection period, HCH cows had an average number of lactations of 2.1 (SD 1.0), had been 101 (SD 23.7) DIM, had an average BW of 580 (SD 56.3) kg, a BCS of 2.6 (SD 0.31), and were producing 34.9 (SD 6.08) kg of milk/d. The HNZ cows had an average number of lactations of 2.1 (SD 1.0), had been 102 (SD 22.0) DIM, had an average BW of 513 (SD 75.5) kg, a BCS of 2.8 (SD 0.25), and were producing 29.1 (SD 4.44) kg of milk/d.

During the first measurement period, one of the supplemented HCH cows was excluded from the experiment because of health problems.

Grazing Management, Pasture, and Weather Conditions

The experiment was carried out in a rotational grazing system from May 6 to July 14 in 2013. All 24 experimental dairy cows were managed as a single herd and grazed on pasture from 0800 to 1400 h and from 1800 to 0430 h the following morning. In the meantime, cows were housed in a freestall barn. For certain work steps, cows were briefly tethered in the cubicles. Indoors, cows had no access to roughages, but concentrate was distributed to supplemented cows. Cows were milked twice a day at 0500 h in the morning and 1600 h in the afternoon. Paddocks used were rotationally grazed for 1 to 5 d based on decision rules considering sward height with a reference of 130 mm pre-grazing equivalent to an herbage mass of approximately 1,000 kg of DM/ha above 50 mm until a postgrazing sward height of 50 mm from ground level. The sward surface height was measured with a pasture meter (C-Dax pasture meter, C-Dax Ltd., Turitea, New Zealand) before cows entered a new paddock and after leaving the paddock. The average pre-grazing sward height was 72 (SD 6.6; n = 10) mm, corresponding to 295 (SD 84.5) kg of DM/ha above 50 mm in the first measurement period, and 124 (SD 20.3; n = 5) mm, corresponding to 958 (SD 260.3) kg of DM/ha for the second measurement period. The average postgrazing sward surface height was 56 (SD 3.1; n = 10) mm in the first measurement period and 64 (SD 10.1; n = 5) mm in the second

measurement period. Herbage mass above 50 mm (kg of DM/ha) was calculated according to −625 + 12.8 × sward height (mm). This regression was calibrated on the same paddocks during the vegetation period 1 yr before the current study (R2 = 0.84, n = 89). The pastures were long established and composed predominantly of grasses (mainly *Lolium perenne*, Dactylis glomerata, and *Phleum pratense*) but also of clover (mainly *Trifolium repens*) and forbs (mainly *Taraxacum officinale*). The pastures were fertilized once per year with 25 m3/ha of farm-produced manure (corresponding to approximately 80 kg of N, 22 kg of P, and 108 kg of K per ha). The ambient outdoor temperature and rainfall were recorded daily by the meteorological station in Grangeneuve (Meteo-Schweiz, Station Grangeneuve, Switzerland), located about 15 km north of the experimental pastures. During the first measurement period, the average temperature was 12°C (minimum 6°C, maximum 17°C) and in the second measurement period 19°C (minimum 16°C, maximum 20°C). On 7 out of the 14 d, scattered rain showers occurred with an average daily precipitation of 7 (SD 10.1) mm in the first measurement period, whereas on 2 out of the 14 d in the second measurement period the average daily precipitation was 1 mm.

Concentrate Supplementation

Figure 1 shows the description of the experiment setup. A step-wise adaptation to the targeted amount of concentrate, 0 or 6 kg (as-fed basis), took place during the first 14 d of the adaptation periods of 21 d before data collection periods. Six days before the data collection periods and during the whole data collection periods, the organic, commercial concentrate mix (UFA 275 Bio, UFA AG, Herzogenbuchsee, Switzerland; composition in descending order according to the delivery note: corn, wheat bran, wheat, barley, sorghum mill feed, barley mill feed, rye, sugar-beet molasses, oats, vegetable oil, minerals, and sunflower seed press cake), was mixed with 10% of labeled barley (50 g/kg of octacosane HC28, $C_{28}H_{58}$, Acros Organics BVBA, Geel, Belgium). The concentrate was fed after milking in 2 equal meals (3 kg at 0600 h and 3 kg at 1700 h) at the feed fence in the freestall barn using separate buckets for each cow. During the data collection periods, all 6 kg/d of concentrate was ingested by all cows with no refusals. The nonsupplemented cows received no concentrate in addition to pasture. Fresh water was always available and a mineral block was available in the barn.

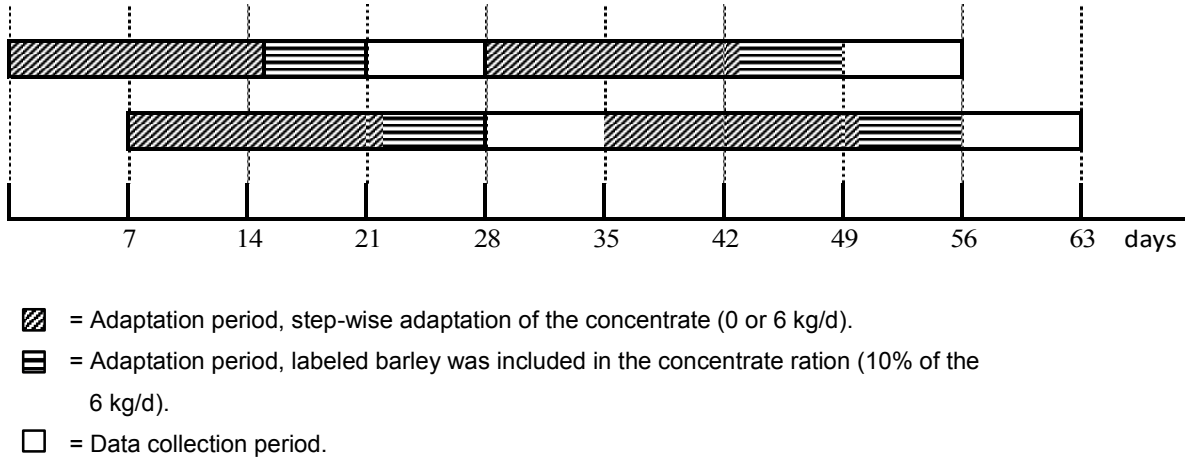


Figure 1: Description of the experiment set-up.

Data Recording and Sample Collection

Individual feed intake was estimated with the *n*-alkane double indicator technique (Mayes et al., 1986). Gelatin capsules (HGK 17–60 sl; Capsula GmbH, Ratingen, Germany), containing 0.5 g (weighing accuracy 0.01) dotriacontane (HC32, C32H66, Argenta Ltd., Auckland, New Zealand) on a carrier of dried fruit pomace and 1.0 g of ytterbium(III) oxide (purity: 99.99%, REacton, Alfa Aesar GmbH & Co KG, Karlsruhe, Germany), were administered twice per day starting 6 d before the data collecting periods. The ytterbium(III) oxide was added to calculate relative FR of alkanes, LCFA, and LCOH. During the data collection periods, a daily spot sample of feces was taken from each cow with or without stimulus between 0700 and 0800 h. Samples were pooled by cow and data collection period and stored at −20°C. For estimating feed intake, herbage samples were collected starting 1 d before the feces sampling. Herbage sample collection was carried out every morning and evening as described by Graf et al. (2005). Samples were chopped and stored at −20°C until further analysis. Samples of commercial concentrate mix and labeled barley were taken daily, pooled per data collection period and analyzed separately.

Milk yield (Flo Master Pro, DeLaval AG, Sursee, Switzerland) was recorded daily and milk composition was analyzed from a pooled sample of aliquot proportion of morning and evening milk on d 1, 4, and 7 of each data collection period. Milk samples were preserved in tubes containing Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland) at 8°C. Grazing and rumination behavior was recorded automatically using jaw movement recorders (Datenlogger MSR 145, MSR Electronics GmbH, Hengart,

Switzerland), and for recording physical activity IceTag pedometers (IceRobotics Ltd., Edinburgh, UK) were used. Further details are described by Thanner et al. (2014).

During the data collection periods, 2 per measurement period, 2 strips of herbage per paddock were cut before the cows entered the new paddock for estimation of the botanical composition and to collect plant species or groups for the marker analysis. In the first data collection period, cows grazed on 10 different paddocks and in the second period cows grazed on 5 different paddocks. The strips, 7 to 9 m long and 1 m wide, were cut with a motor mower Rapid BM 117 (Rapid Technic AG, Killwangen, Switzerland). The harvested biomass was collected in plastic bags and subsamples were manually separated for botanical analysis. Dominant plant species were *Lolium perenne*, *D. glomerata*, *T. repens*, and *Taraxacum officinale*. Other grass species were merged as "other grass" representing *Phleum pratense, Poa pratensis, Poa annua, Festuca pratensis, Agrostis spp*., and *Holcus lanatus*. Further forbs were merged as "other forbs" representing *Plantago lanceolata, Ranunculus acris,* and *Rumex acetosa*. Plant species or group samples of every data collection period, 4 in total, were collected, weighed, and chemically analyzed. The average proportion of the plant species or groups over all subsamples of all data collection periods was 27.8% L. perenne, 6.1% *D. glomerata*, 38.2% other grass, 10.4% *T. repens*, 9.0% *T. officinale*, and 8.5% other forbs.

Extraction and Analysis of n-Alkanes, Long-chain Fatty Acids, and Long-chain Alcohols

Samples of plant species, concentrate, and feces were analyzed for the concentrations of alkanes, LCFA and LCOH according to the methods of Dove and Mayes (2006) without the steps for further purification of LCFA and LCOH fractions. Samples of alkanes, LCOH, and LCFA were dissolved in dodecane before analysis by gas chromatography, using a Trace 1300 GC fitted with an AS 1300 series autosampler and a flame ionization detector (Thermo Scientific, Hemel Hempstead, UK) equipped with a nonpolar-fused silica capillary column (CPSil-5CB, 50 m × 0.32 mm × 0.12 mm, Agilent Technologies, Santa Clara, CA). The temperature program used for alkanes and LCFA was initial oven temperature 170°C, hold for 4 min; first gradual increase 30°C/min to 215°C, 1 min hold; second gradual increase 6°C/min to 300°C, 10 min hold. The temperature program used for LCOH was initial oven temperature 40°C, first gradual increase 20°C/min to 130°C; second gradual increase 4°C/min to 250°C; third gradual increase 1.5°C/min to 300°C, hold for 10 min. Mixed standard solutions were run regularly to enable corrections for variation in detector response. To allow peak identification several samples of the diet components and feces were also analyzed using GC described above, equipped with an identical column, coupled to an ISQ

mass spectrometer (Thermo Scientific). The ion source was maintained at 300°C and the transfer line at 300°C. The emission current was set to 50 mA and the electron energy to 70 eV. The analyzer was set to scan at 50 to 650 m/z with a scan cycle time of 0.6 s.

The alkanes with a carbon chain length (CCL) of 24 to 33 were analyzed. The LCFA with the even number CCL of 22 to 34 and the LCOH with the even number CCL of 20 to 30 were analyzed, as they occur in higher concentrations compared with odd chain substances.

Further Laboratory Analysis

Milk samples were analyzed by infrared spectrometry (Combifoss FT+, Foss, Hillerød, Denmark) for contents of fat, protein, and lactose (International Dairy Federation, 2000; method number 141C). Urea in milk was determined with a differential pH-analyzer (Eurochem, Ardea, Italy) before and after hydrolysis with urease (International Dairy Federation, 2004; method number 195). For milk acetone determination, acetone and an internal standard (2-butanone) were transferred via static headspace directly from the milk into the gas phase. The composition of the gas phase was determined with a flame ionization detector on a GC (HP 5890 Series II, Agilent Technologies, Santa Clara, CA).

Herbage, plant species, and feces samples were lyophilized (model Delta, 1–24 LSC, Christ, Osterode, Germany). Thereafter, concentrate, herbage, plant species, and feces samples were milled through a 1.0-mm screen (Brabender mill with titanium blades, Brabender, Duisburg, Germany). Subsamples of the lyophilized samples were dried for 3 h at 105°C to determine DM and subsequently incinerated at 550°C until they reached a stable mass to assess the ash contents. The contents of alkanes HC32 and tritriacontane (HC33, $C_{33}H_{68}$) were determined as described by Peiretti et al. (2006). The N content was determined using the Dumas method (AOAC International, 1995) on a C/N analyzer (type FP-2000, Leco Instruments, St. Joseph, MI) and then multiplied by 6.25 to determine the CP content. The ether extract was determined using the Soxtec Avanti 2050 apparatus for extraction following the guidelines of VDLUFA (2012, method 5.1.1.). Acid detergent fiber (procedure 973.18; AOAC International, 1995) was determined with correction for residual ash obtained after incineration at 500°C for 1 h. For analyzing Yb, subsamples were dissolved in HNO_3 before analyzed with an inductively coupled plasma optical emission spectrometry (ICP-OES Optima 2000 DV, Perkin Elmer, Shelton, CT, with system ICP-OES Optima 7300). Crude fiber was analysed only in herbage, plant species, and concentrate samples according to the procedure 978.10 (AOAC International, 1995) and NDF (Mertens, 2002) was assessed with the addition of heat-stable amylase and sodium sulfite. Starch content was determined based on the polarimetric method (ISO, 2000; method 6493) in concentrate samples. Water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) were analyzed according to

Hall et al. (1999) and determined with the Thermo Scientific Genesys 10S Vis Spectrophotometer (Thermo Scientific, Waltham, MA). Lignin was analyzed according to the procedure 973.18 (AOAC International, 1995).

Calculation of Fecal Recovery and Estimation of Diet Composition

The correction for incomplete FR was performed in 2 different ways. To estimate average marker concentration in the herbage (HM) for the subsequent calculation of individual FR of each marker substance (FRM), the average botanical composition of the pasture was assessed in 2 different ways.

Method 1: With the alkane concentrations of the representative herbage samples and from the plant species, the average botanical composition for each data collection week was calculated using a nonnegative leastsquares procedure in R (R Core Team, 2012). Mean FR rates were subsequently calculated with the formula described below for alkanes, LCOH, and LCFA across all data collection weeks (FR1).

Method 2: The manually assessed botanical composition was used to calculate HM (FR2).

The relative FR of alkanes (CCL: 24–33), LCFA (CCL: 22, 24, 26, 28, 30, 32, and 34), and LCOH (CCL: 20, 22, 24, 26, 28, and 30) to Yb were calculated with the following equation:

$$FR_M = (D_{Yb} + DMI_H * H_{Yb}) * FR_{Yb} * F_M / ((D_{HC32} + DMI_H * H_M + DMI_{Conc} * Conc_M) * F_{Yb}),$$

where D is the dosed amount of Yb (D_{Yb}) and HC32 ($D_{HC32;}$ just used for FR calculation of HC32), H is the concentration of Yb (H_{Yb}) in herbage, FR_{Yb} is the FR of Yb fixed to 0.95, F is the concentration of Yb (F_{Yb}) and marker (F_M) in feces, and $Conc_M$ is the concentration of markers in the concentrate (only included for supplemented cows). Total DMI was separated into herbage DMI (DMI_H) and concentrate DMI (DMI_{Conc}).

With the average FR_M of all cows over both measurement periods, concentrations of alkanes, LCOH and LCFA in feces were corrected for the diet composition estimation.

Diet composition of each animal was estimated using a non-negative least-squares procedure included in the software “EatWhat” (Dove and Moore, 1995). Estimations were performed with alkanes, LCFA, and LCOH alone, and their combination. Furthermore, all diet composition estimations were performed with data not corrected for FR (FR0), with FR1, and FR2 resulting of 21 combinations (7 marker and marker combinations and 3 FR). For supplemented cows, concentrate was included as a diet component. The alkanes administered with the capsules were considered in diet estimations with alkanes or in marker combination with alkanes.

Other Calculations and Statistical Analyses

The NE_L and the absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen were calculated according to Agroscope (2013). The ECM was calculated based on a 4% fat, 3.2% protein and 4.8% lactose basis (Agroscope, 2013). Feed intake was estimated with the equation proposed by Mayes et al. (1986) using the alkanes HC32 and HC33. The statistical analyses for milk yield and composition, rumination and grazing behavior, physical activity, and feed intake were carried out with SYSTAT 13 (Systat Software Inc., Chicago, IL). The data were collected over several d and averaged per cow, d and measurement period. The averages were analyzed using the following linear mixed model:

$$Y_{ijklm} = \mu + \tau_i + \varphi_j + P_k + (\tau p)_{ik} + (\tau\varphi)_{ij} + P_l + K_m (P_l) + \varepsilon_{ijklm}$$

where, Y_{ijklm} is the response (respectively its logarithm), μ is the least squares mean, τ_i is the fixed effect of cow strain i (i = HCH, HNZ), φ_j is the fixed effect of the treatment j (j = non-supplemented, supplemented cows), P_k is the fixed effect of the period (k = period 1, period 2), $(\tau p)_{ik}$ is the effect of the interaction between cow strain i and period k, $(\tau\varphi)_{ij}$ is the effect of the interaction between cow strain i and treatment j, P_l is the random effect of cow pair l (1,…,12), K_m is the random effect of the cow m (1,…, 24) and ε_{ijklm} is the random error. The effects were considered significant at $P \leq 0.05$. A value of $0.05 < P < 0.10$ was considered a trend.

Linear discriminant analyses were performed with SYSTAT 13 for evaluating the differentiation of marker profiles of the plant species and the concentrate. Results are summarized in a jackknifed classification matrix where the percentage of correct allocations of marker profiles to the plant species is presented. The concentrate percentage of the diet of supplemented cows was subtracted from the results of entire diet estimated with “EatWhat” and compared to the manually assessed botanical composition. Based on the Aitchison distance measure (Aitchison et al., 2000) the similarity between botanical compositions and diet estimations was tested with the R package “compositions” to figure out the marker group and FR with the most accurate diet estimation. Using the R package PCS (Wilson, 2013) the marker group combinations with the most accurate diet estimation were determined. A parametric linear mixed model was applied to the Aitchison distances of the 2 best marker and FR combinations to test whether they differ significantly from each other (SYSTAT 13). The most accurate marker group combination for diet estimation was selected and the effects of concentrate supplementation and of cow strain were tested with the R package “composition” as described in van den Boogaart et al. (2013). Zeroes in the estimated compositions were first replaced by the nonparametric imputation procedure proposed by

Martin-Fernandez et al. (2003). For each plant variety a robust linear mixed model (Koller, 2015) was applied to the logarithms of the estimated compositional results of the most accurate diet estimation to test the effects of concentrate supplementation and of cow strain.

RESULTS

Chemical Composition of Herbage and Concentrate

In Table 5, the average chemical composition of the herbage samples from the paddocks for each measurement period is presented. All analyzed components are similar in both measurement periods. The average chemical composition of plant species and concentrate is displayed in Table 6. Small differences were recorded between plant species; for example, *T. repens* had the highest concentration of CP, but the lowest concentration of WSC. Grass species, including *L. perenne, D. glomerata*, and the group of other grass, had a higher concentration of crude fiber, ADF, and NDF than the other plant species. As the concentrate was a commercial mix based on grain, the chemical composition was similar to the labeled barley with high NE_L concentration (8.0 MJ/kg of DM for both) and medium CP concentration (127 and 138 g/kg of DM, respectively).

Table 5. Average chemical composition of herbage samples (n = 28; mean ± SD)

Item	Measurement period 1	SD	Measurement period 2	SD
DM (g/kg of wet weight)	201	35.4	192	35.5
Analyzed nutrients composition (g/kg of DM)				
OM	889	21.3	906	6.3
CP	159	17.9	162	18.2
Ether extract	36	4.7	42	5.6
ADF	222	15.1	246	22.0
NDF	376	22.6	375	41.5
Crude fiber	184	11.8	195	21.7
Lignin	26	4.4	33	8.9
WSC[1]	241	24.4	200	28.5
ESC[2]	124	23.7	112	23.4
Calculated energy and protein supply [3] (per kg of DM)				
NE_L (MJ)	6.0	0.3	6.1	0.2
APDE[4] (g)	100	5.1	101	4.5
Analyzed *n*-alkane contents[5] (mg/kg of DM)				
HC28[5]	3	0.4	3	0.5
HC32[6]	5	0.8	5	1.0
HC33[7]	51	6.9	50	10.5

[1]WSC = water soluble carbohydrates.
[2]ESC = ethanol soluble carbohydrates.
[3]According to Agroscope (2013).
[4]APDE = absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen.
[5]HC28 = octacosane, $C_{28}H_{58}$; HC32 = dotriacontane, $C_{32}H_{66}$; HC33 = tritriacontane, $C_{33}H_{68}$.

Table 6. Average chemical composition of plant species and groups (n = 4), concentrate (n = 4) and labeled concentrate (n = 4).

Item	LP[1]		DG[2]		OG[3]		TR[4]		TO[5]		OF[6]		Conc[7]		lConc[8]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	215	17.4	211	10.9	232	25.3	165	26.9	137	20.2	159	21.2	880	1.4	870	0.7
Analyzed nutrient composition (g/kg of DM)																
OM	905	10.9	904	15.5	897	16.7	870	29.4	855	29.5	854	48.3	945	0.6	978	0.2
CP	142	18.6	152	15.4	151	16.5	204	13.1	145	19.6	142	20.2	127	2.6	138	2.9
Ether extract	29	2.9	38	5.2	35	1.1	44	4.7	42	7.0	34	3.8	63	3.7	33	1.4
Crude fiber	222	22.2	247	3.5	241	15.3	148	21.5	142	17.2	156	18.6	49	0.9	53	1.8
ADF	248	30.2	270	6.4	266	19.9	207	25.9	209	21.8	229	34.9	73	8.1	78	8.9
NDF	436	46.1	484	5.3	469	30.5	246	19.8	223	19.6	284	38.5	274	31.7	287	47.3
Lignin	24	5.7	28	4.0	31	5.8	53	1.0	53	4.4	65	9.6	24	5.2	16	2.6
WSC[9]	213	61.1	180	22.6	175	40.1	95	5.5	182	31.9	161	26.0				
ESC[10]	99	19.1	84	7.6	77	11.1	93	6.0	123	12.6	124	30.7				
Starch													488	15.3	552	4.4
Calculated energy and protein supply[11] per kg of DM																
NE_L	6.3	0.3	6.2	0.2	5.9	0.1	5.9	0.3	5.9	0.3	5.6	0.4	8.2	0.1	8.0	0.0
APDE[12]	101	4.2	101	4.1	98	3.6	105	4.9	94	5.9	94	3.6				

[1] LP = *Lolium perenne*; [2] DG = *Dactylis glomerata*; [3] OG = other grass species; [4] TR = *Trifolium repens*; [5] TO = *Taraxacum officinale*; [6] OF = other forb species; Conc[7] = concentrate; lConc[8] = labelled concentrate.
[9]WSC = water soluble carbohydrates; [10]ESC = ethanol soluble carbohydrates.
[11]According to Agroscope (2013).
[12]APDE = absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen.

Milk Yield, Milk Composition, and Dry Matter Intake

Nonsupplemented cows had lower ($P < 0.001$) milk production compared with supplemented cows (Table 7). Swiss Holstein cows had higher milk yield, but no difference between cow strains was recorded for additional milk yield per kilogram of concentrate. Concentrate supplementation had no effect on ECM yield and no difference between cow strains occurred. Nonsupplemented cows had a higher ($P < 0.001$) milk fat content and a lower ($P < 0.01$) milk protein content. Swiss Holstein cows had a lower ($P < 0.05$) milk fat and a lower ($P < 0.001$) milk protein content compared with HNZ cows. Concentration of acetone and urea in milk were influenced by concentrate supplementation with greater ($P < 0.001$) concentrations for nonsupplemented cows. Nonsupplemented cows had a higher ($P < 0.001$) herbage DMI compared with supplemented cows, but total DMI was lower ($P < 0.001$) for nonsupplemented cows. No further difference between cow strains and no interactions for aforementioned traits were observed.

Grazing and Rumination Behavior and Physical Activity

Grazing time, grazing mastication, and grazing mastication rate were higher ($P < 0.001$) for nonsupplemented cows, but no differences between cow strains were recorded (Table 8). Concentrate supplementation had no influence on rumination behavior, but nonsupplemented cows tended to have a lower number of mastications per bolus than supplemented cows ($P = 0.08$). Swiss Holstein cows spent slightly ($P = 0.09$) less time ruminating and made less rumination mastications. A trend ($P = 0.06$) for lower number of boli per day was recorded for HCH cows compared with HNZ cows. Physical activity was not influenced by concentrate supplementation. No difference between cow strains was recorded for time spent lying or standing. The HCH cows had a tendency to walk less ($P = 0.07$) and made fewer ($P = 0.03$) steps compared with HNZ cows. No interactions were recorded.

Table 7. Effect of concentrate supplementation[1] and cow strain[2] on milk production performance and feed intake

Item	Conc0		Conc6			*P* -Value		
	HCH	HNZ	HCH	HNZ	SD	Cow strain	Treatment	Interaction
Milk production performance								
Milk yield (kg/d)	23.4	21.3	26.4	23.6	4.14	0.05	<0.001	0.58
Milk yield (kg/ kg concentrate)			0.49	0.40	0.44	0.55		
ECM (kg/d)	21.7	20.9	22.6	21.6	4.05	0.44	0.18	0.86
Fat (%)	3.7	4.0	3.0	3.3	0.45	0.03	<0.001	0.92
Protein (%)	3.1	3.4	3.2	3.5	0.16	<0.001	<0.01	0.35
Lactose (%)	4.6	4.5	4.6	4.6	0.24	0.38	0.65	0.91
Acetone (mg/L)	2.7	2.3	1.6	1.5	0.64	0.26	<0.001	0.35
Urea (mg/L)	248	245	222	210	30.7	0.48	<0.001	0.55
Feed intake								
Herbage DMI (kg/d)	15.4	14.7	12.2	10.9	2.30	0.10	<0.001	0.28
Total DMI (kg/d)	15.4	14.7	17.4	16.1	2.30	0.10	<0.001	0.28

[1]Conc0 = non-supplemented cows; Conc6 = cows supplemented with 6 kg/d concentrate.
[2]HCH = Swiss Holstein cows; HNZ = New Zealand Holstein cows.

Table 8. Effect of concentrate supplementation[1] and cow strain[2] on grazing and rumination behavior as well as on physical activity over 24 h.

Item	Conc0		Conc6			*P*-Value		
	HCH	HNZ	HCH	HNZ	SD	Cow strain	Treatment	Interaction
Grazing behavior over 24 h								
Time (min)	541	558	463	470	47.1	0.32	<0.001	0.71
Mastications (n)	40’066	42’130	33’279	34’166	4’418	0.17	<0.001	0.58
Mastication rate (n/min)	73.8	75.4	71.6	72.7	3.58	0.30	<0.01	0.83
Rumination behavior over 24 h								
Time (min)	406	433	413	450	53.1	0.09	0.33	0.67
Mastications (n)	29’590	32’046	29’725	33’607	5’011	0.09	0.47	0.54
Mastication rate (n/min)	72.8	73.9	71.7	74.3	4.67	0.31	0.67	0.34
Rumination boli (n)	533	610	532	584	80.4	0.06	0.20	0.23
Mastications boli (n/boli)	56.3	52.8	57.3	58.4	9.79	0.76	0.08	0.20
Activity over 24 h								
Lying (min)	473	465	521	478	75.3	0.22	0.16	0.40
Standing and moving (min)	968	976	920	963	75.3	0.22	0.16	0.40
Walking (min)	355	422	353	390	93.0	0.07	0.55	0.59
Steps (n)	4’326	5’098	4’096	4’763	1’130	0.03	0.38	0.87

[1]Conc0 = non-supplemented cows; Conc6 = supplemented cows.
[2]HCH = Swiss Holstein cows; HNZ = New Zealand Holstein cows.

Composition of Diet Components, Fecal Recovery, and Diet Selection

Concentration of alkanes, LCFA, and LCOH of plant species and concentrate are given in Table 9. The LCOH had the highest average concentration with 446 mg/kg of DM compared with alkanes (21 mg/ kg of DM) and LCFA (277 mg/kg of DM). The oddchain alkanes were in higher concentration compared with even-chain numbered alkanes. For *L. perenne, D. glomerata*, other grass, and other forbs the C_{31} *n*-alkane had the highest concentration, but for *T. repens* and *T. officinale* the C_{29} *n*-alkane was most abundant. Alkane concentration in concentrate was generally low (<3 mg/kg of DM) except for the C_{28} *n*-alkane from labeled barley added to the concentrate. The C_{24} *n*-alkane had the lowest concentration of those measured with <1 mg/kg of DM in all diet components. In general, the highest overall average alkane concentration in plant species was analyzed for *L. perenne* with 36 mg/kg of DM and the lowest for *T. officinale* with 10 mg/kg of DM. The highest concentrations of LCFA occurred for those with CCL of 22, 24, 26, and 28, and the lowest concentration was recorded for the C_{34} LCFA in all diet components. The highest average LCFA concentration in plant species occurred for *T. repens* with 426 mg/kg of DM and the lowest for *L. perenne* with 163 mg/kg of DM. The C_{26} LCOH had the highest concentration and the C_{20} LCOH had the lowest concentration in all diet components, except for *T. repens*, where the C_{30} LCOH had the highest and the C_{20} and C_{22} LCOH had the lowest concentration. *Dactylis glomerata* had the highest average LCOH concentrations in plant species with 1,150 mg/kg of DM and *T. repens* had the lowest with 257 mg/kg of DM.

Table 9. Concentrations (mg/kg DM) of *n*-alkanes, long-chain fatty acids (LCFA), and long-chain alcohols (LCOH) in samples of concentrate (n = 4) and plant species (n = 4) for all data collection week

Marker	LP[1]		DG[2]		OG[3]		TR[4]		TO[5]		OF[6]		Conc[7]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n-alkanes														
C24	1	0.3	1	0.5	1	0.3	0	0.1	0	0.4	1	0.2	0	0.1
C25	16	14.0	11	5.8	15	5.9	5	1.0	3	2.1	6	1.9	1	0.3
C26	1	0.8	1	0.4	1	0.2	1	0.3	1	0.5	1	0.2	0	0.1
C27	29	17.6	9	2.5	23	6.5	18	2.3	14	10.8	24	4.7	1	0.6
C28	2	0.6	1	0.3	2	0.6	2	0.4	2	1.6	3	0.5	40	1.5
C29	95	27.5	19	1.9	81	20.9	84	26.5	40	33.7	95	23.1	2	1
C30	6	1.3	1	0.5	4	1.1	4	2.0	2	1.9	8	2.5	0	0.1
C31	151	60.0	36	4.1	125	32.7	59	39.7	29	21.4	158	40.9	2	1
C32	3	0.4	2	1.0	3	0.7	2	0.8	2	1.5	7	2.2	0	0.1
C33	57	13.2	27	6.0	40	4.6	5	2.1	5	3.1	45	10.9	0	0.2
LCFA														
C22	424	86.1	426	119	612	188	671	255	1259	1431	416	116	308	65.4
C24	295	45.5	330	105	415	123	946	278	1367	1015	518	112	188	10.3
C26	354	126	787	286	658	270	574	133	436	136	257	45.9	87	3.4
C28	201	65.8	395	369	339	193	609	182	336	91.0	183	39.3	187	3.1
C30	93	46.8	73	42.8	160	104	162	64.0	126	19.9	114	47.6	5	1.2
C32	47	19.9	55	25.0	70	38.5	19	10	71	17.0	45	23.3	3	1.5
C34	16	7.9	48	21.0	27	16.3	2	1.2	16	3.9	8	2.3	0	0
LCOH														
C20	8	2.1	3	2.0	5	1.5	19	8.4	6	4.9	7	4.3	1	0.1
C22	16	2.4	38	14.6	19	5.9	19	4.9	35	16.0	26	8.4	1	0.3
C24	108	24.4	74	20.4	83	11.7	57	7.7	285	76.9	94	14.3	4	0.5
C26	2362	871	6519	2223	3599	915	370	30.2	1010	91.9	363	49.8	7	1.1
C28	383	148	234	139	604	431	270	191	560	280	135	33.6	3	0.5
C30	23	17.0	30	22.2	56	40.0	808	63.6	454	132	51	53.7	0	0.3

[1] LP = *Lolium perenne*; [2] DG = *Dactylis glomerata*; [3] OG = other grass species; [4] TR = *Trifolium repens*; [5] TO = *Taraxacum officinale*; [6] OF = other forb species; [7] Conc = concentrate; mean value proportional with normal (90%) and labeled concentrate (10%) overall data collection wk.

Most accurate discrimination of diet composition was achieved with the LCOH, where 96% of the plant species or groups were correctly allocated (Table 10). A score of 81% correct allocations was obtained with alkanes or LCFA. The marker combination with the most accurate allocation (81%) was LCFA and LCOH. Finally, the weakest discrimination with 12% correct allocation resulted from the combination of all 3 marker groups. The most accurate diet component allocation was achieved for concentrate, where a 100% allocation was accomplished unless all 3 marker groups were used. The plant-specific correct allocation varied from 50 to 77% with the best allocation for *T. repens* (77%) followed by *D. glomerata* (71%). The least accurate average allocation was achieved for *L. perenne* as it was frequently mixed up with the group of other grass (data not shown). In Figure 2, the results of calculated FR are presented for alkanes, LCFA, and LCOH. All FR increased with increasing CCL, except for the FR2 of the LCOH, where FR increased until LCOH with a CCL of 26 and decreased for the ones with a CCL of 28 and 30. Both FR methods for alkanes and LCOH indicated an incomplete recovery (FR < 1.0) of the lower CCL alkanes (C_{24} to C_{32}) and LCOH (C_{20} to C_{28}), but were >1.0 for the C_{33} alkane and the C_{30} LCOH. The average FR of LCFA was higher than the FR of alkanes and LCOH. For the LCFA with a CCL of 26 to 32 FR was >1.0 with the highest rate for the LCFA with a CCL of 26 (FR1 = 2.6, and FR2 = 2.4).

Table 10. Correct allocation (%) of marker profiles of plant species and concentrate with linear discriminant analysis[1]

Marker group combination	LP	DG	OG	TR	TO	OF	Conc	total
n-alkanes	75	75	75	100	100	50	100	81
LCFA	50	100	50	100	100	75	100	81
LCOH	75	100	100	100	100	100	100	96
n-alkanes + LCFA	25	50	75	67	0	75	100	56
n-alkanes + LCOH	50	100	75	75	67	50	100	74
LCFA + LCOH	50	75	50	100	100	100	100	81
n-alkanes + LCFA + LCOH	25	0	0	0	0	25	33	12

[1] LP = *Lolium perenne;* DG = *Dactylis glomerata;* OG = Other grass species; TR = *Trifolium repens;* TO = *Taraxacum officinale;* OF = other forb species; Conc = Concentrate; LCFA = long-chain fatty acids; and LCOH = long-chain alcohols.

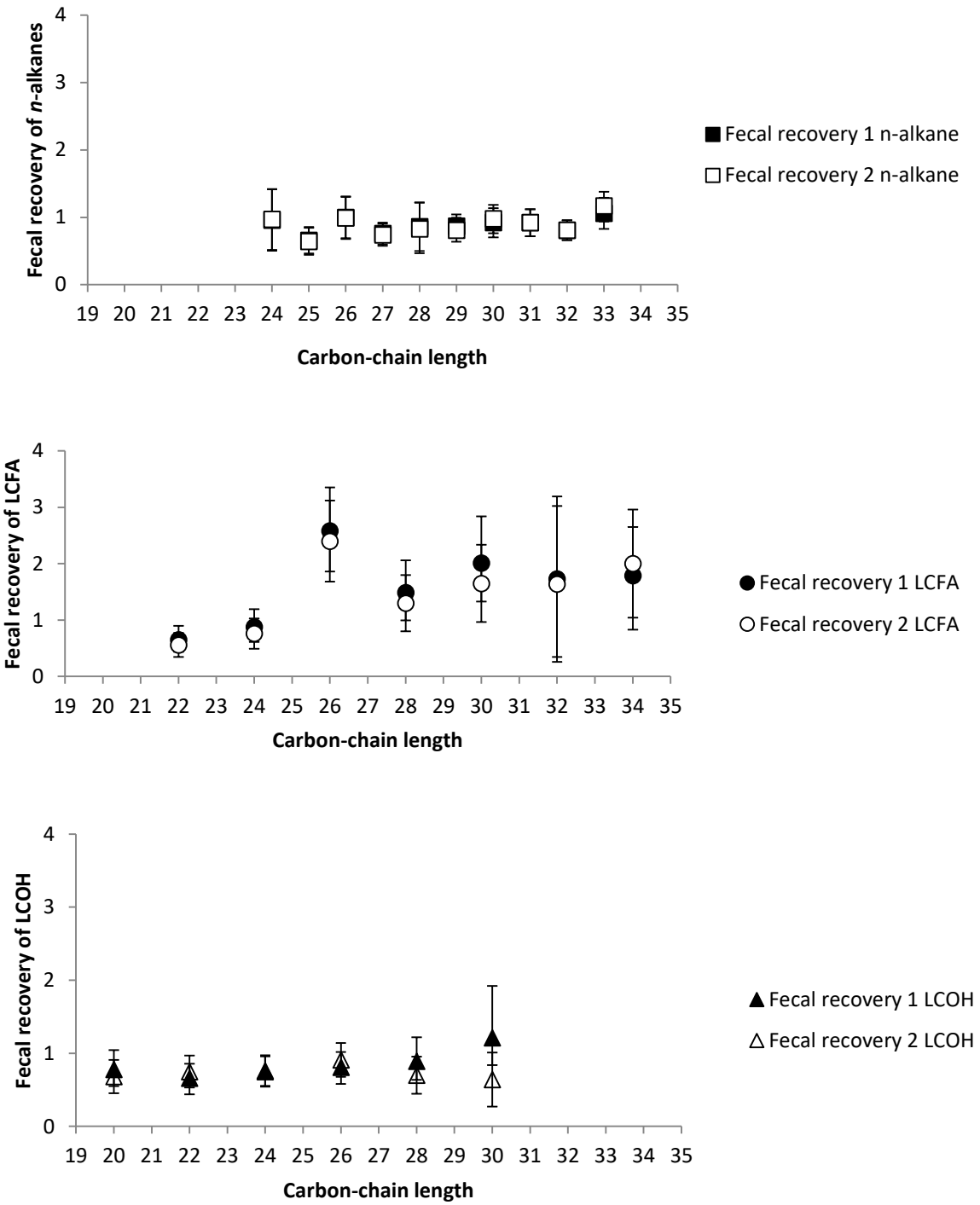


Figure 2. Calculated fecal recoveries of markers based on alkane concentration (1) and on botanical composition (2). Error bars indicate SD.
LCFA = long-chain fatty acids; LCOH = long-chain alcohols.

The accuracy of the diet composition estimation depending on the marker combinations was ranked based on the Aitchison distance (Table 11). With the combination alkanes, LCOH and FR1, the most accurate estimation was achieved (smallest Aitchison distance), as shown in Figure 3, with and without concentrate included, compared with the assessed botanical composition. The least accurate diet composition estimations were achieved with the combination alkanes and LCFA, and LCFA alone: with or without FR correction. Using the most accurate marker group combination, alkanes, LCOH, and FR1, differences between cow strains or concentrate supplementation on diet composition has been tested (Table 12). Results indicate no difference between cow strains (P = 0.49), but an effect of concentrate supplementation (P = 0.02) on diet selection. Nonsupplemented cows had a lower (P < 0.05) proportion of *T. repens* in their diet compared with supplemented cows.

Table 11. Results of diet estimation validation with Aitchison distance

Sequence	Marker combination[1]	Fecal recovery[2]	Aitchison distance
1	*n*-alkanes + LCOH	FR1	0.368[3]
2	*n*-alkanes + LCOH	FR2	0.437
3	LCOH	FR2	0.447
4	*n*-alkanes + LCOH	FR0	0.458
5	LCOH	FR0	0.463
6	LCOH	FR2	0.474
7	*n*-alkanes + LCFA + LCOH	FR2	0.583
8	LCFA + LCOH	FR2	0.630
9	*n*-alkanes + LCFA + LCOH	FR1	0.637
10	*n*-alkanes + LCFA + LCOH	FR0	0.644
11	LCFA + LCOH	FR0	0.647
12	LCFA + LCOH	FR1	0.669
13	*n*-alkanes	FR0	0.680
14	*n*-alkanes	FR1	0.734
15	*n*-alkanes	FR2	0.809
16	*n*-alkanes + LCFA	FR0	1.060
17	LCFA	FR0	1.060
18	*n*-alkanes + LCFA	FR1	1.060
19	LCFA	FR1	1.060
20	*n*-alkanes + LCFA	FR2	1.060
21	LCFA	FR2	1.060

[1]LCFA = long-chain fatty acids; LCOH = long-chain alcohols.
[2]FR0 = no correction for fecal recovery; FR1 = mean fecal recovery calculated according to alkanes in herbage samples; FR2 = mean fecal recovery according to botanical composition analysis.
[3]Value for Aitchison distance of the marker combination alkanes + LCOH and FR1 differs significantly (P < 0.001) from the value for Aitchison distance of marker combination alkanes and LCOH with FR2.

Table 12. Results of diet estimation with most accurate marker combination (alkanes and long-chain alcohols with fecal recovery 1) and their impact on concentrate supplementation and cow strain.

Item[1] (%)	Conc0[2]		Conc6[3]			*P*-Values		
	HCH[4]	HNZ[5]	HCH[4]	HNZ[5]	SD	Cow strain	Treatment	Interaction
LP	35.3	30.6	28.2	31.0	22.1	0.97	0.73	0.81
DG	8.4	9.9	7.3	10.3	10.0	0.32	0.67	0.41
OG	32.3	33.1	22.7	24.7	19.5	0.59	0.14	0.70
TR	11.4	13.4	14.9	15.4	8.7	0.23	<0.05	0.33
TO	2.2	1.9	5.7	1.6	7.3	0.87	0.88	0.48
OF	10.6	10.5	21.2	17.1	11.7	0.59	0.40	0.88

[1]LP = *Lolium perenne;* DG = *Dactylis glomerata;* OG = other grass species; TR = *Trifolium repens;* TO = *Taraxacum officinale;* OF = other forb species.
[2]Conc0 = nonsupplemented cows; [3]Conc6 = cows supplemented with 6 kg/d of concentrate.
[4]HCH = Swiss Holstein cows; [5]HNZ = New Zealand Holstein cows.

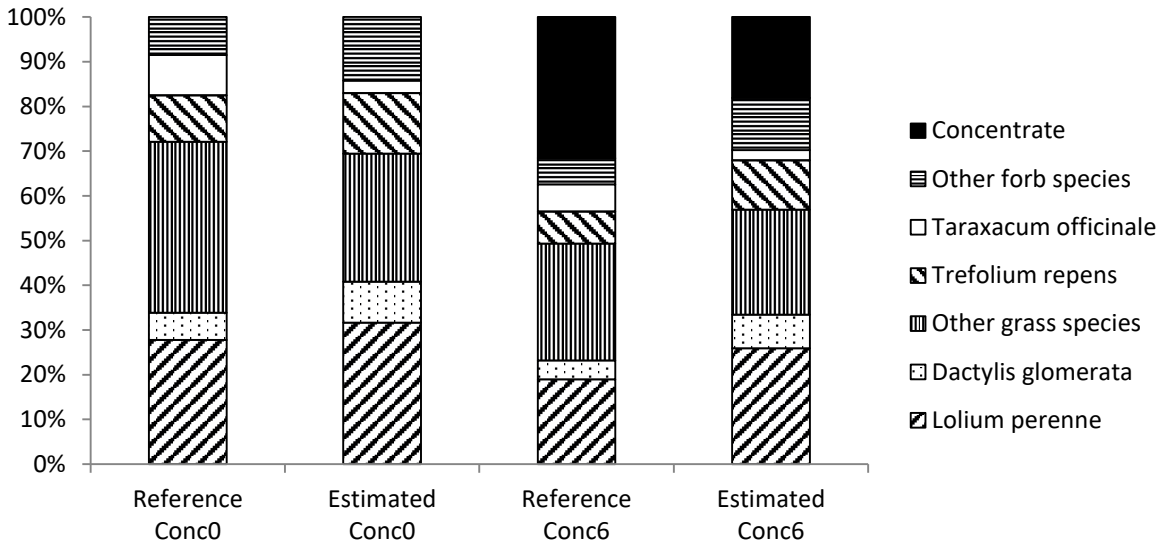


Figure 3. Diet composition assessed during manual plant species separation or estimated with "EatWhat" considering the combination of *n*-alkanes, long-chain alcohols, and fecal recovery, determined with alkane concentration in pasture and feces samples (FR1), with or without concentrate.
Reference Conc0 = diet composition of non-supplemented cows determined during manual plant species separation;
Estimated Conc0 = estimated diet composition of non-supplemented cows determined with "EatWhat";
Reference Conc6 = diet composition including concentrate of supplemented cows determined during manual plant species separation;
Estimated Conc6 = estimated diet composition including concentrate of supplemented cows determined with "EatWhat".

DISCUSSION

Plant Wax Concentration and Profiles of Plant Species

A sufficient differentiation between plant species is essential for successful diet estimation. Differentiation between plant species with alkanes, LCFA, and LCOH is feasible, but most previous studies included only a few pasture plant species (Boland et al., 2012) or studied diets containing herbaceous and heathland woody species (Ferreira et al., 2009). Similar marker profiles between different species result in incorrect allocations, which create a challenge for accurate diet composition estimation. Therefore, plant species from the same genus or plants with similar marker profile can be summarized and denoted as one diet component (Ferreira et al., 2011). However, differences in palatability and consequently intake have to be considered. For example, *D. glomerata* is less preferred than *L. perenne* when taking the whole grazing season into account (Ivins, 1952), because the decline in quality of *D. glomerata* is more rapid than that of *L. perenne*. Concentrations of all 3 marker groups varied within plant species samples, which was attributed to environmental conditions (Dove et al., 1996) and simultaneous sampling of plant species and animal feces is required. Date of sampling influences the alkanes concentration depending on the plant growth stage, as concentration differs between plant parts. The highest is in the florescence, at least for *L. perenne* (Dove et al., 1996; Ferreira et al., 2009), *T. officinale*, and *T. repens* (Gedir and Hudson, 2000). Ferreira et al. (2009, 2015) observed similar LCFA and LCOH marker profiles of *L. perenne* for leaf and stem fractions, and for the spike fraction, and there may be only minor differences between plant parts in other plant species, which should be tested in future studies. The concentration of the C_{31} alkane was high in samples of *L. perenne* and in the group of other grass, which is typical for grass species (Bush and McInerney, 2013). Furthermore, alkane and LCFA profiles of *L. perenne* and other grass were similar, probably because cognate grasses such as *Lolium multiflorum* are included in the group of other grass, resulting in frequent mixing up in the outcome of the linear discriminant analysis. The C_{29} alkane was dominant in *T. repens*, which is typical for legumes (Dove et al., 1996; Charmley and Dove, 2007), and a 100% correct assignment of *T. repens* profiles was achieved with alkanes. In accordance with Schori et al. (2012), *T. officinale* had low alkane concentrations, but achieved a 100% correct assignment with alkanes alone. Long-chain fatty acids achieved a good differentiation of plant species, but no individual LCFA was identified that contributed most for differentiation. The concentration of LCFA in samples of *L. perenne* was similar to that of Ferreira et al. (2010), except C_{30} and C_{32} LCFA, which in the current study showed lower concentration. In contrast, concentrations of the LCFA with a CCL of 22, 24, 26, and 28 in *T. repens* samples were higher in the current study compared with Ferreira et al. (2010) and those with a CCL of 30, 32, and 34 were in the same range.

Environmental conditions, variety, and plant growth stage can influence the concentration of plant wax markers. A comparison of samples from different locations and time is therefore problematic. The predominant LCOH for grass species are those with a CCL of 26 and 28 (Dove and Charmley, 2008; Ferreira et al., 2015), whereas the C_{30} LCOH was dominant in *T. repens*, which is typical for clover (Dove and Charmley, 2008). However, the C_{24} and C_{26} LCOH contributed most to the accurate allocation in the current study. Labeling the concentrate with the C_{28} alkane seems to be sufficient for discrimination, and allocation also worked well with LCFA and LCOH without labeling. However, estimations of proportion of concentrate in the diet may be underestimated (Figure 3). Hameleers and Mayes (1998) did not consider the supplemented barley in diet composition calculations because of the low alkane concentration. Without labeling, grain-based concentrate, which has low alkane concentrations, may lead to difficulties in accurate estimation of the concentrate proportion in the diet (Charmley and Dove, 2007). The advantage of using labeled concentrate is the parallel assessment of DMI of the animals, as discussed in Dove and Charmley (2008).

Fecal Recovery

Estimation of FR of grazing animals is difficult as total fecal output, composition of plant species on pasture and precise pasture DMI estimation are required. An independent feeding experiment with housed animals and total feces collection is labor intense and expensive, and feed selection of cut herbage fed indoors might be different compared with selection behavior on pasture. Rectal grab samples collected once or twice daily provide a representative marker profile in the feces and are valid for estimating diet composition under field conditions (Dove and Charmley, 2008). Calculated FR based on the fixed FR of Yb led to appropriate results, at least for alkanes and LCOH. Further studies are necessary to investigate whether the methods for calculating FR are adequate and correlate to measured FR.

In previous studies, FR increased with increasing CCL for alkanes, LCFA, and LCOH (Dove and Charmley, 2008; Elwert et al., 2008; Ferreira et al., 2011). In the current study, all calculated FR tended to increase with CCL except the FR2 for LCOH. Similar to the study of Ferreira et al. (2009), no clear relationship was detected between alkane CCL and FR. A separate analysis of odd- and even-numbered alkanes indicated a linear increase for FR of odd-numbered alkanes and a curvilinear decrease for FR of even-numbered alkanes. Concentration of even-numbered alkanes is low compared with odd-numbered alkanes and low concentrations include more analytical uncertainties. The values of both calculated FR and their SD for LCFA were unrealistically high (up to 2.6) with the highest value for the LCFA with CCL of 26. The increase of both calculated FR occurred in a curvilinear way, as in

the study of Ferreira et al. (2011). Equally, a high concentration of the LCFA with a CCL of 26 was observed in the study of Ferreira et al. (2011), although the value did not exceed 1.0. The current method of calculating FR did not work for LCFA as evidenced by unrealistically high values but, in contrast, calculated FR for alkanes and LCOH seemed to be appropriate. Measured LCFA in feces could partly originate from endogenous sources and peaks might not be completely pure (Ali et al., 2005). However, a subset of samples was tested using GC-MS and peaks of LCFA were identified without contamination of other FA components. Nevertheless, the LCFA concentration in feces may be overestimated leading to unrealistically high FR and resulting in inaccurate diet estimations. Both methods of calculating FR achieved similar results (taking into account the relation between the difference of the means to the SD) for alkanes, LCFA and LCOH, except C_{28} and C_{30} LCOH. The FR1 increased with increasing CCL in a linear way as recorded by Dove and Charmley (2008), but FR2 increased up to the C_{26} LCOH and decreased with increasing CCL afterward. Furthermore, SD was high for the LCOH with a CCL of 30, especially for FR1. This may be related to different consumption of T. repens, which has high concentrations of C_{30} LCOH.

Ruminant species may also have an effect on FR (Ferreira et al., 2011) as well as diet composition (Elwert et al., 2008; Ferreira et al., 2010), although others reported no effect of diet composition on FR (Ali et al., 2004; Dove and Charmley, 2008). Increasing digestibility of diet components decreased the FR of alkanes, which partly explained the differences in FR between diets in the study of Elwert et al. (2008). In accordance with Ferreira et al. (2015), diet estimation was more accurate with FR than without FR, indicating that a correction of marker concentration in feces is recommended. The significant difference between the combinations of alkanes and LCOH with either FR1 or FR2 for diet estimation indicates that a correction of recoveries calculated with the alkanes (FR1) results in more precise diet estimation, at least for the combination of alkanes and LCOH. Probably, diet estimations with FR1 achieved better results as alkanes were also used to estimate botanical composition for calculating FR. The botanical composition assessed during manual separation of plant species for FR2 might be a more independent factor. Therefore, it is important that pasture is grazed evenly without systematic leftovers. This is difficult to ensure as cows avoid grazing around dung patches. Further studies with total fecal output collection would be necessary to confirm and improve the used methods for FR determination.

Diet Composition Estimations

Results of diet estimation with the program “EatWhat” (Dove and Moore, 1995) were compared using the Aitchison distance with the average botanical composition of pastures.

Cows stayed on paddocks until an average postgrazing height of 56 mm in the first and 64 mm in the second period and thus, we can assume a consumption of all plants on pasture. This assumption was supported by visual evaluations. Regarding marker groups separately, LCOH achieved best results for diet estimation followed by results with alkanes and the poorest results were reached with LCFA, as in Ali et al. (2005). The best combination for diet estimation was alkanes and LCOH with a correction of FR, followed by LCOH alone. This is in agreement with Ferreira et al. (2015), where a combination of alkanes and LCOH improved accuracy of diet estimation compared with LCOH or alkanes alone. In our study, using LCOH alone achieved a more accurate diet estimation compared with any combination with LCFA or with a combination of all 3 marker groups.

The marker group combination of alkanes and LCFA resulted in a less accurate diet estimation compared with LCOH alone, which is contrary to results of Ferreira et al. (2011). Despite reasonable differentiation between plant species, diet estimation reached poor results with LCFA, as in Ali et al. (2005), indicating difficulties in the analysis of LCFA in feces. Thus, differentiation of marker profiles between plant species is essential, but does not guarantee reasonable results for diet estimation. Other factors, such as correction of FR or relation of patterns in plant species to patterns in feces influence the method of diet estimation. Mayes and Dove (2000) mentioned that markers with the highest concentration affected diet estimation more than lower marker concentration, particularly when least squares are used. Transforming to relative terms, weighting individual marker concentration or omitting certain markers according to individual analytical uncertainties, concentration levels, utility for discrimination, or variability within plant species might be useful for a better diet composition estimation (Mayes and Dove, 2000).

With the best marker combination (alkanes and LCOH with FR1), concentrate was identified as a part of diet composition but, the proportion was underestimated (Figure 3), which is in line with the results of Dove and Charmley (2008) who calculated FR as a grand mean of all treatments. On the other hand, herbage DMI might be underestimated resulting in higher percentage of concentrate in the diet. Average herbage DMI of 15 kg/d for nonsupplemented cows seemed to be reasonable and is comparable to values presented in the review of Bargo et al. (2003). Higher herbage DMI (McCarthy et al., 2007) are possible as herbage DMI depends on intake capacity, milk production, and herbage offer (quality and quantity). Estimating DMI of grazing animals may contain difficulties (Thanner et al., 2014), but the double-alkane method was tested indoors (Berry et al., 2000) and outdoors (Bezabih et al., 2012), and considered to be accurate. Estimated percentage of *T. officinale* in the diet was lower compared with botanical composition, although dairy cows may prefer *T. officinale* over grass species (Lantinga et al., 2004). The underestimation might have resulted from low

alkane concentrations of *T. officinale*, even though LCOH concentration was high. Low marker concentrations lead to difficulties in accurate diet composition estimation (Charmley and Dove, 2007). A reason for the overestimated portion of the group other forbs may be the heterogeneous composition of the group. Forb species differ in their morphological appearance, and a separate analysis of forb species, which are included in the group other forbs, may be necessary to test the variance of marker profiles between them and decide if a different grouping of the forbs is preferable. Differences between botanical composition on pasture and estimated diet composition might occur because of individual variation of cows' selection behavior. Compared with other studies where all cows received the same diet with the same composition, diet selection and preference may play a bigger role in grazing dairy cows.

Differences between Cow Strains and Impact of Concentrate Supplementation

With the most accurate diet estimation (alkanes, LCOH, and FR1), differences between the 2 cow strains and the effect of concentrate supplementation on diet selection have been investigated. Fedele et al. (1993) recorded differences of feed preference between 2 breeds of goats grazing on pasture. In the current experiment, similar diet selection between cow strains is in accordance with their similar grazing and rumination behavior. The concentrate supplementation had a similar effect on the milk production, milk composition, grazing time, herbage, and total DMI of grazing cows as in other studies [Bargo et al., 2003; McCarthy et al., 2007; C. Heublein, F. Dohme-Meier, K.-H. Südekum, R. M. Bruckmaier (Vetsuisse Faculty, Bern, Switzerland), S. Thanner (Agroscope, Posieux, Switzerland), and F. Schori, unpublished data]. Interestingly, current results indicate that supplemented grazing dairy cows apparently select different plant species for ingestion compared with nonsupplemented cows. The reasons for plant species selection and preference in ruminants are still unclear and several assumptions exist, such as balancing nutrient intake, maintaining rumen function, and avoiding toxins (Rutter, 2006). The assumption that dairy cows balance their nutrient intake, as shown for pigs (Lin and Patience, 2016) and poultry (Denbow and Cline, 2015), may fit with results of the current study, because cows supplemented with energy-rich concentrate had a higher amount of *T. repens* in their diet compared with nonsupplemented cows. As *T. repens* had higher content of CP and lower concentrations of WSC and ESC, supplemented cows may have tried to balance their diet. Results of Bach et al. (2012) indicate that lambs can balance their CP intake according to their requirements. Grain-based concentrate has high concentrations of highly fermentable carbohydrates (high concentration of starch), which causes a decline in rumen pH and increases the risk of cows becoming acidotic (Bramley et al., 2008). The decrease in milk fat concentration of supplemented cows indicated changes in rumen VFA profiles. Animals may select plant species to reduce the

variation in ingesta composition as far as possible (Fedele et al., 1993) and might have reacted to supplementation of concentrate by avoiding plant species high in WSC concentration. Grazing cows and sheep exhibit a preference for clover over grass, but they prefer mixed diets, even when a diet of clover alone could match their nutrient requirements (Rutter, 2006; Chapman et al., 2007). In contrast to the aforementioned studies, the current study took place on an organic, multispecies sward with a *T. repens* proportion of only 10%, and thus, cows had to search more for preferred plant species. This assumption was supported by the results of similar physical activity between nonsupplemented and supplemented cows, although grazing time was significantly lower for supplemented cows. On the other hand, weather conditions in the first measurement period may have precluded supplemented cows lying down for long periods. Supplemented cows were probably more quickly satiated with a lower motivation to graze, which is supported by results of lower grazing time and lower mastication rate, but higher motivation to search for palatable plant species. Fasting sheep spend less time eating clover than *L. perenne* (Newman et al., 1994), indicating that fasting probably provokes a higher feeding drive, which results in longer grazing time and less selection. More research is needed to explore whether ruminants are able to select plant species to match their nutrient demand and which signals lead them to select. If other studies confirm that supplemented cows change their diet selection to balance carbohydrates and protein in the diet and to reduce the load of rapidly fermented carbohydrates, sward composition could be better adapted to the needs of the ruminants, for example, by increasing the percentage of *T. repens* of pasture DM. This may therefore increase efficiency.

CONCLUSION

The results of our study indicate that the diet selection of dairy cows under grazing condition can be estimated with plant wax markers. For the differentiation of plant species, LCOH performed best and the combination of all 3 marker groups achieved the worst differentiation. Diet estimation with LCFA alone or in combination gave poor results. Analytical difficulties concerning LCFA in feces might create uncertainties in the estimation of diet selection. The calculated FR relative to Yb gave mostly realistic results, but further validation is required. Using calculated FR instead of experimental measured ones would be time-saving, less expensive, and applicable for field work. The marker group combination alkanes and LCOH with FR1 achieved the most accurate results for diet composition estimation and provided evidence that HCH and HNZ cows had a similar diet selection behavior, and that concentrate supplementation influenced diet selection of grazing dairy cows. The knowledge of diet

selection and foraging behavior may allow optimization of the offer (herbage) to the demands of the cows, which is expected to improve animal health, welfare, and efficiency.

ACKNOWLEDGEMENTS

This study was funded by the AGFF (Zurich, Switzerland) research fund and the Fondation Sur-la-Croix (Basel, Switzerland). The authors gratefully acknowledge the help afforded to them by the staff of the farm “Ferme École de Sorens” and S. Thanner (Agroscope, Posieux, Switzerland) for assistance with the cows during experiment. We thank the staff of Agroscope in Posieux and F. Keay of Leeds University for assistance in the laboratory analysis and W. Luginbühl (Chemstat, Bern, Switzerland) for statistical advice.

REFERENCES

Agroscope. 2013 Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer (Grünes Buch). In: Online ed. Forschungsanstalt Agroscope Liebefeld-Posieux ALP, Posieux, Switzerland.

Aitchison, J., C. Barceló-Vidal, J. A. Martín-Fernández, and V. Pawlowsky-Glahn. 2000. Logratio analysis and compositional distance. Math. Geol. 32:271-275.

Ali, H. A. M., R. W. Mayes, C. S. Lamb, B. L. Hector, A.K. Verma, and E. R. Ørskov. 2004. The potential of long-chain fatty alcohols and long-chain fatty acids as diet composition markers: development of methods for quantitative analysis and faecal recoveries of these compounds in sheep fed mixed diets. J. Agric. Sci. 142:71-78. http://dx.doi.org/10.1017/S0021859604004034

Ali, H. A. M., R. W. Mayes, B. L. Hector, A. K. Verma, and E. R. Ørskov. 2005. The possible use of n-alkanes, long-chain fatty alcohols and long-chain fatty acids as markers in studies of the botanical composition of the diet of free-ranging herbivores. J. Agric. Sci. 143:85-95. http://dx.doi.org/10.1017/S0021859605004958

AOAC International. 1995. Official Methods of Analysis. 16th ed. AOAC International, Arlington, VA.

Bach, A., J. J. Villalba, and I. R. Ipharraguerre. 2012. Interactions between mild nutrient imbalance and taste preferences in young ruminants. J. Anim. Sci. 90:1015-1025. http://dx.doi.org/10.2527/jas.2011-4176

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. J. Dairy Sci. 86:1-42. https://doi.org/10.3168/jds.S0022-0302(03)73581-4.

Berry, N. R., Scheeder, M. R. L., Sutter, F.; Kröber, T. F., and M. Kreuzer. 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann. Zootech. 49:3–13.

http://dx.doi.org/10.1051/animres:2000104

Bezabih, M., W. F. Pellikaan, A. Tolera, and W. H. Hendriks. 2012. Estimation of feed intake and digestibility in cattle consuming low-quality tropical roughage diets using molasses-based n-alkane bolus. Anim. Feed Sci. Technol. 177:161-171. http://dx.doi.org/10.1016/j.anifeedsci.2012.08.014

Boland, H. T., G. Scaglia, D. R. Notter, A. J. Rook, W. S. Swecker, and A. O. Abaye. 2012. Diet composition and dry matter intake of beef steers grazing tall fescue and alfalfa. Crop Sci. 52:2817-2825. http://dx.doi.org/10.2135/cropsci2011.12.0638

Bramley, E., I. J. Lean, W. J. Fulkerson, M. A. Stevenson, A. R. Rabiee, and N. D. Costa. 2008. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. J. Dairy Sci. 91:308-326. http://dx.doi.org/10.3168/jds.2006-601

Bush, R. T. and F. A. McInerney. 2013. Leaf wax n-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. Geochim. Cosmochim. Acta 117:161-179.

Chapman, D. F., A. J. Parsons, G. P. Cosgrove, D. J. Barker, D. M. Marotti, K. J. Venning, S. M. Rutter, J. Hill, and A. N. Thompson. 2007. Impacts of Spatial Patterns in Pasture on Animal Grazing Behavior, Intake, and Performance. Crop Sci. 47:399-415. http://dx.doi.org/10.2135/cropsci2006.01.0036

Charmley, E. and H. Dove. 2007. Using plant wax markers to estimate diet composition and intakes of mixed forages in sheep by feeding a known amount of alkane-labelled supplement. Aust. J. Agric. Res. 58:1215-1225. http://dx.doi.org/10.1071/AR07187

Denbow D. M., and M. A. Cline. 2015. Intake regulation. Pages 469-485 in Sturkie´s Avian Physiology. Vol. 6. C. G. Scanes, ed. Academic Press, London, UK.

Dove, H. and E. Charmley. 2008. Using the alkanes and long-chain alcohols of plant cuticular wax to estimate diet composition and the intakes of mixed forages in sheep consuming a known amount of alkane-labelled supplement. Animal 2:1474-1485. http://dx.doi.org/10.1017/S1751731108002735

Dove, H. and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Aust. J. Agric. Res. 42:913-952.

Dove, H. and R. W. Mayes. 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nat. Protocols 1:1680-1697. http://dx.doi.org/10.1038/nprot.2006.225

Dove, H., R. W. Mayes, and M. Freer. 1996. Effects of species, plant part, and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. Aust. J. Agric. Res. 47:1333-1347.

Dove, H. and A. D. Moore. 1995. Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. Aust. J. Agric. Res. 46:1535-1544.

Elwert, C., H. Dove, and M. Rodehutscord. 2008. Faecal alkane recoveries from multi-component diets and effects on estimates of diet composition in sheep. Animal 2:125-134. http://dx.doi.org/10.1017/S1751731107000900

Fedele, V., M. Pizzillo, S. Claps, P. Morand-Fehr, and R. Rubino. 1993. Grazing behavior and diet selection of goats on native pasture in Southern Italy. Small Ruminant Res. 11:305-322. http://dx.doi.org/10.1016/0921-4488(93)90002-Y

Ferreira, L. M. M., S. Carvalho, V. Falco, R. Celaya, U. García, A. S. Santos, M. A. M. Rodrigues, and K. Osoro. 2009. Assessment of very long-chain fatty acids as complementary or alternative natural fecal markers to n-alkanes for estimating diet composition of goats feeding on mixed diets. J. Anim. Sci. 87:2732-2745. http://dx.doi.org/10.2527/jas.2008-1718

Ferreira, L. M. M., R. Celaya, V. Falco, M. Oliván, A. S. Santos, C. Guedes, M. A. M. Rodrigues, and K. Osoro. 2010. Evaluation of very long-chain fatty acids and n-alkane epicuticular compounds as markers for estimating diet composition of sheep fed heathland vegetation species. Anim. Feed Sci. Technol. 156:75-88.

Ferreira, L. M. M., R. Celaya, A. S. Santos, V. Falco, C. Guedes, M. A. M. Rodrigues, and K. Osoro. 2011. The utilization of long-chain fatty acids as markers for diet composition estimates in ruminants: effects of animal species, diet composition and marker combination. Grass Forage Sci. 66:183-195. http://dx.doi.org/10.1111/j.1365-2494.2010.00774.x

Ferreira, L. M. M., R. Celaya, A. S. Santos, R. W. Mayes, M. A. M. Rodrigues, and K. Osoro. 2015. Application of long-chain alcohols as diet-composition markers in sheep fed on grass–white clover and heather–gorse plant species. Grass Forage Sci. 70:30-43. http://dx.doi.org/10.1111/gfs.12083

Gedir, J. V., and R. J. Hudson. 2000. Seasonal intake determination in reproductive wapiti hinds (*Cervus elaphus canadensis*) using n-alkane markers. Can. J. Anim. Sci. 80:137-144. http://dx.doi.org/10.4141/A99-042

Graf, C. M., M. Kreuzer, and F. Dohme. 2005. Effects of supplemental hay and corn silage versus full-time grazing on ruminal pH and chewing activity of dairy cows. J. Dairy Sci. 88:711-725. http://dx.doi.org/ 10.3168/jds.S0022-03020572735-1

Hall, M. B., W. H. Hoover, J. P. Jennings and T. K. Miller Webster. 1999. A method for partitioning neutral detergent soluble carbohydrates. J. Sci. Food Agric. 79:2079-2086.

Hameleers A., and R.W. Mayes. 1998. The use of n-alkanes to estimate herbage intake and diet composition by dairy cows offered a perennial ryegrass/white clover mixture. Grass Forage Sci. 53:164-169. http://dx.doi.org/10.1046/j.1365-2494.1998.5320164.x

International Dairy Federation. 2000. Determination of milk fat, protein and lactose content. Guide for the operation of mid-infra-red instruments. IDF Standard method No. 141C, Brussels, Belgium.

International Dairy Federation. 2004. Milk. Determination of urea content. Enzymatic method using difference in pH. IDF Standard method No. 195, Brussels, Belgium.

ISO (International Organization for Standardization). 2000. Animal feeding stuffs - Determination of starch content - Polarimetric method. ISO, Geneva, Switzerland.

Ivins, J.D. 1952. The relative palatability of herbage plants. Grass Forage Sci.7:43-54. http://dx.doi.org/10.1111/j.1365-2494.1952.tb01302.x

Koller M. (2015). robustlmm: Robust linear mixed effects models. R package version 1.7-2. Accessed Apr 10, 2015. http://CRAN.R-project.org/package=robustlmm

Lantinga E. A., J. H. Neuteboom, and J. A. C Meijs. 2004. Sward methods. Pages 23-52 in Herbage Intake Handbook. Vol. 2. P. D. Penning, ed. The British Grassland Society, reading, UK.

Lin, L. J., X. Y. Zhu, C. Jiang, H. L. LUO, H. Wang, Y. J. Zhang, and F. Z. Hong. 2011. The potential use of n-alkanes, long-chain alcohols and long-chain fatty acids as diet composition markers: indoor validation with sheep and herbage species from the rangeland of Inner Mongolia of China. Animal 6:449-458. http://dx.doi.org/10.1017/S1751731111001765

Lin Q., and J. F. Patience. 2016. Factors involved in the regulation of feed and energy intake of pigs. Anim. Feed Sci. Technol. http://dx.doi.org/10.1016/j.anifeedsci.2016.01.001

Luscher, A., I. Mueller-Harvey, J. F. Soussana, R. M. Rees, and J. L. Peyraud. 2014. Potential of legume-based grassland-livestock systems in Europe: A review. Grass Forage Sci. 69:206–228. https://doi.org/10.1111/gfs.12124.

Martín-Fernández J. A., C. Barceló-Vidal, and V. Pawlowsky-Glahn. 2003. Dealing with zeros and missing values in compositional data sets using nonparametric imputation. Math. Geol. 35:253-278.

Mayes, R. W., and H. Dove. 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. Nutr. Res. 13:107-138.
http://dx.doi.org/10.1017/S0954422400000068

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107:161-170. http://dx.doi.org/10.1017/S0021859600066910

McCarthy, S., B. Horan, M. Rath, M. Linnane, P. O'Connor, and P. Dillon. 2007. The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. Grass Forage Sci. 62:13-26. http://dx.doi.org/10.1111/j.1365-2494.2007.00557.x

Mertens D., R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Intern. 85:1217-1240.

Newman, J. A., P. D. Penning, A. J. Parsons, A. Harvey, and R. J. Orr. 1994. Fasting affects intake behaviour and diet preference of grazing sheep. Anim. Behav. 47:185-193. http://dx.doi.org/10.1006/anbe.1994.1021

Peiretti, P. G., G. Meineri, N. Miraglia, M. Mucciarelli, and D. Bergero. 2006. Intake and apparent digestibility of hay or hay plus concentrate diets determined in horses by the total collection of feces and n-alkanes as internal markers. Livest. Sci. 100:189-194. http://dx.doi.org/10.1016/j.livprodsci.2005.08.016

Piccand, V., E. Cutullic, S. Meier, F. Schori, P. L. Kunz, J. R. Roche, and P. Thomet. 2013. Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system. J. Dairy Sci. 96:5352–5363.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL. Accessed Apr 10, 2015. http://www.R-project.org/

Rutter, S. M. 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Anim. Behav. Sci. 97:17-35.

Schori, F., S. Darms, and A. Münger. 2012. Diet selection under full grazing of two Holstein strains in organic farming. Pages 276-278 in Proceedings of the 24th general meeting of the EGF, Lublin, Poland.

Spehn, E. M., A. Hector, J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. Bazeley-White, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Hogberg, K. Huss-Danell, A. Jumpponen, J. Koricheva, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, C. Palmborg, J. S. Pereira, A. B. Pfisterer, A. Prinz, D. J. Read, E.-D. Schulze, A.-S. D. Siamantziouras, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 2005. Ecosystem effects on biodiversity manipulations in European grasslands. Ecol. Monogr. 75:37–63. https://doi.org/10.1890/03-4101.

Thanner, S., F. Schori, R. M. Bruckmaier, and F. Dohme-Meier. 2014. Grazing behaviour, physical activity and metabolic profile of two Holstein strains in an organic grazing system. J. Anim. Physiol. Anim. Nutr. 98:1143–1153. http://dx.doi.org/10.1111/jpn.12172

van den Boogart, K. G., R. Tolosana, and M. Bren. 2014. Compositions: Compositional Data Analysis. R-package version 1.40-1. Accessed Apr 10, 2015. http://CRAN.R-project.org/package=compositions

VDLUFA. 2012. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III: Die Chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Villalba, J. J., F. D. Provenza, F. Catanese, and R. A. Distel. 2015. Understanding and manipulating diet choice in grazing animals. Anim. Prod. Sci. 55:261–271. https://doi.org/10.1071/AN14449.

Wilson, J. 2013. PCS: Calculate the probability of correct selection (PCS). R-package version 1.2. Accessed Apr 10, 2015. https://cran.r-project.or

Chapter 5: General discussion

5.1. Milk response to concentrate

Energy is the most limiting nutrient in pasture-based feeding systems and high-yielding Holstein cows may not match their demand and exhaust their genetic potential for milk production (Kolver and Muller, 1998). Results from the trial of this thesis indicated that energy limited milk production as supplemented cows reached higher milk yields than non-supplemented cows. However, response differed between cow strains, at least in the first trial. Swiss Holstein cows (HCH) cows had higher extra milk yield per kg concentrate than New Zealand Holstein (HNZ) cows, as presented in several studies (Roche et al., 2006; McCarthy et al., 2007; Kennedy et al., 2009). In the second trial, no difference between cow strains was recorded and the response to supplemented concentrate was lower for HCH cows (0.8 vs 0.5 kg/kg in the first and second trial, respectively). Reasons for lower response can be environmental aspects (Roche et al., 2006). The lower response might be attributed to the difference in net energy for lactation (NEL, MJ/kg dry matter (DM)) concentration of pasture in the first measurement period (6.3 vs 6.0 MJ/kg DM in the first and the second trial, respectively) and might have suppressed a higher milk response to concentrate supplementation of the HCH cows. However, milk yield per kg concentrate was the same for HNZ cows in both trials indicating that they could better compensate the lower quality of pasture in the second trial.

Energy corrected milk (ECM) yield was similar for supplemented and non-supplemented cows in the second trial, which reflect that concentrate supplementation could not increase milk yield as much as in the first trial. However, in both trials no difference between cow strains was recorded. The higher milk yield of the HCH cows was probably compensated with higher milk fat and milk protein content from the HNZ cows. High milk solids are included in the New Zealand breeding index and payment to the farmer is related to milk fat and milk protein content (Harris and Kolver, 2001). Regarding efficiency, conversion of pasture, which is useless for human consumption, into animal protein, which is important for human nutrition, milk solids have an important nutritional value. Just adding concentrate to the diet of dairy cows does not guarantee high milk yields, especially when cows are not able to use it for extra milk production because of their genetic endowments or deficit in nutrient supply. Therefore the question arises if it is profitable to add concentrate to the diet, considering that milk fat content would decrease and milk protein content probably slightly increases (Bargo et al., 2003). Adding concentrate based on by-products to the diet might be

a reasonable option to balance the energy-protein ratio and increase the edible feed conversion ratio (Ertl et al., 2015).

5.2. Grazing behavior on pasture

Grazing and rumination behavior was similar in both trials, as management measures, such as time on pasture, were equal. Compared to the study of McCarthy et al. (2007) grazing time for non-supplemented cows was similar for the HCH cows compared to high durability Holstein cows, but the New Zealand Holstein cows in the experiment of McCarthy et al. (2007) spent longer time grazing than HNZ cows in our trials. Concentrate supplementation reduced grazing time, but a stronger decrease was recorded in our trials compared to the one in McCarthy et al. (2007). This stronger decrease might be related to lower available herbage mass and pre-grazing sward heights in our trials compared to the swards used in the experiment of McCarthy et al. (2007). In organic farming the use of mineral N fertilizer is prohibited, the output of N in form of manure is restricted and management measures are needed to support an optimal growth of pasture and ensure a dense sward. Organic pastures have higher plant species diversity (Gabriel et al., 2006) and the crude protein content is lower compared to conventional pastures (Spann et al., 2007). Pasture characteristics influence grazing behavior as bite mass and intake rate decrease with decreasing sward surface height (Gibb, 2006). Cows have to make more effort (more bites and mastications) to reach the same pasture dry matter intake (DMI) compared to cows on pastures with higher sward surface heights. Motivation for grazing is lower for cows that receive additional food in the barn, especially in pastures with lower sward surface height. Grazing time coupled with intake rate (bite rate and size) at the same body weight (BW) are indicators for the feeding drive, which means how effective the cow harvests the grass (Prendiville et al., 2010; McCarthy et al., 2007). The recorded reduction in grazing time and lower grazing mastications for supplemented cows suggested a lower feeding drive for supplemented cows. The reduced pasture DMI for supplemented cows is well documented and called substitution, as supplement is replacing pasture of the total DMI due to reduction in energy deficit of the cow (Baudracco et al., 2010). Dairy cows have three to five grazing bouts (Gregorini, 2012) as recorded in current study. Although number of grazing bouts was the same for non-supplemented and supplemented cows, duration of grazing bouts differed with longer duration for non-supplemented cows, which is in accordance to longer grazing time of non-supplemented cows. The major grazing events occur in the morning and in the evening, and shorter bouts can occur flexible due to external environment and farming management measures (Kennedy et al., 2009; Gregorini, 2012). As cows returned to barn because of the work flow at 14:00 h and stayed until 18:00 h after milking, cows compensated missing time

on pasture and grazing bouts during night were recorded, although cows prefer to ruminate during night (Gregorini et al., 2012).

5.3. Estimation of diet composition on pasture

5.3.1. Approach and method

Understanding grazing behavior is important in pasture-based feeding systems, but nearly no observations exist about the selection behavior of dairy cows on pasture. Understanding what cows are eating on pasture helps to adapt sward composition to the needs of the animals and might help to improve efficiency and animal health.

Several studies exhibited that plant wax markers, such as alkanes, long-chain fatty acids (LCFA) and long-chain alcohols (LCOH) are suitable for estimation diet composition in ruminants (Ali et al., 2005; Lin et al., 2011), but only few studies exist with cows or other cattle under grazing conditions (Fraser et al., 2009; Boland et al., 2012). There are several challenges that influence estimations of diet composition of cows on pasture. One of the challenges is the correct estimation of the botanical composition of the pasture, especially on multispecies pastures with a rotational grazing system. To assess the correct percentage of each plant species is difficult, as they are not evenly distributed over all paddocks. Further, organic pastures have a higher plant species diversity compared to conventional pastures (Gabriel et al., 2006), and some plant species occur in small quantities, which makes it difficult to collect sufficient material for sample analysis. Grouping several plant species from the same genus seems reliable (Ferreira et al., 2011), although it might have contributed in this trial to high variance of marker concentration (within the plant species group). Another approach is the grouping according to similar marker profiles. Further studies are required to figure out which approach of grouping reaches the most accurate results.

Concentration of plant wax markers differs between plant species (Ali et al., 2005; Lin et al., 2011) and therefore, every plant species has a specific marker profile. The pre-condition for successful diet composition estimation is the sufficient differentiation of plant species with their marker profiles. Results of the linear discriminant analysis indicate a sufficient differentiation, except when alkanes, LCFA and LCOH were used together. Probably using too many markers (all together 23) lead to controversial results and create difficulties in differentiation and diet composition estimation. Not all markers are perhaps suited, because of inaccuracy in determining concentration (especially in concentrations <1 mg/kg DM) or because no variation between plant species occurred, which leads to mistakes in differentiation. Using only the markers that contribute most to differentiation might increase accuracy of diet composition estimation.

Another challenge is the determination of fecal recovery (FR). Studies performed in the barn can assess FR with known amount of DMI, composition of the diet and total fecal collection. This is not possible for ruminants on pasture, and either a parallel experiment in the barn is required or other approaches need further investigation. Boland et al. (2012) used FR from literature, but it is still unclear if diet composition (Ferreira et al., 2009) or ruminant species (Ferreira et al., 2011) influence FR and can therefore be generalized. In the trial of this thesis FR was calculated in two different ways, which led to realistic results for alkanes and LCOH, but high recoveries for LCFA. Although a feasible differentiation of plant species was achieved with LCFA, only poor results of calculated FR and diet composition estimation were gained. In contrast, Ferreira et al (2009) reached plausible results in diet composition estimation with LCFA. Probably, feces samples in current trial were contaminated with endogenous sources (Ali et al., 2005) and influenced correct determination of LCFA concentration which led to unrealistic high FR.

Using FR related to the individual animal led to more accurate diet composition estimations (Dove and Charmley, 2008; Ferreira et al., 2015), but in the current trial mean FR over all measurement weeks and all cows were used. As mentioned above, determining correct botanical composition is difficult and plant species diversity differs to varying extent from paddock to paddock. Further, variation of marker concentration in plants was high due to environmental conditions and impact of growing status of plants, and using mean FR might balance high variance and uncertainties.

Although estimation of diet composition with the marker combination of alkanes and LCOH was more correct with FR than without correction, further validation of this approach is strongly recommended.

5.3.2. Main findings and conclusions

The most accurate diet composition estimation was achieved with the marker combination alkanes, LCOH and FR1. Interestingly, a difference in diet composition estimation between non-supplemented and supplemented cows was recorded, where supplemented cows ingested more *T. repens* compared to non-supplemented cows. Evidence exists that ruminants are able to distinguish between different diet components and that their diet choice is not random (Tolkamp et al., 1998). Probably, cows that consumed energy-rich concentrate might have tried to balance their diet and ingested higher amounts of *T. repens* which had higher concentration of crude protein than the other plant species or plant groups, but lower concentration of water-soluble carbohydrates to reduce the variation in ingesta composition (Fedele et al., 1993). Experience in effects of different diet components in young life give a distinction and ruminants learn to connect certain flavors to nutritive value of the food (Bach

et al., 2015). They seem to be able to recognize internal protein deficiencies and then decrease the preference of components with a flavor connected to negative effects of deficiencies, for example malaise and calories (Bach et al., 2015). Further, experience with secondary plant compounds, such as tannins or terpenes, in young life under different circumstances may influence future food preference (Baraza et al., 2005). Experiments with cows on pasture indicated that heifers on alpine pastures preferred to ingest forbs and legumes over other grass species (Dumont et al., 2007) and Chapman et al. (2007) recorded a preference of *T. repens* over *Lolium perenne*, but always a mixture of both plant species was eaten. Several theories exist why ruminants prefer to eat mixed diets, such as balancing nutrient intake, maintain rumen function and avoiding toxins (Rutter, 2006). On multi-species pastures, cows are forced to eat mixed diets and components in lower quality, as the availability of high quality alternatives may be limited (Villalba et al., 2015). In this trial, supplemented cows were probably more saturated with lower motivation to graze, but higher motivation to search for *T. repens*. Further, fasting might indicate a higher feeding drive, as fasting sheep spend more time eating *L. perenne* than clover (Newman et al., 1994). This would be in accordance with lower grazing time and lower number of mastications for supplemented cows in the trial.

Further research with cows on pasture is necessary to investigate the aforementioned results of this trial and give more information about it. A better understanding of dairy cows´ selection behavior helps to adapt botanical composition to the demand of cows and consequently ensures efficiency and animal welfare.

5.4. Rumination behavior

Rumination plays an important role as it is the key component of rumen digestion. It is the process of the postprandial regurgitation of ingesta followed by mastication, reforming the bolus and re-swallowing. Rumination is influenced by the structure and amount of fiber ingested (Mertens, 1997), and cows spend more time ruminating with increased fiber content of the diet (McCarthy et al., 2007). New Zealand Holstein cows ruminated longer and had a higher number of boli, which is in accordance with other studies performed on this farm (Schori and Münger, 2014; Thanner et al., 2014). This might indicate that HNZ cows have ingested more plant species with higher fiber content. Kunz et al. (2010) recorded that HNZ cows grazed longer around dung patches, where more over-matured herbage is growing, than other cow types. However, results from current trial could not confirm this as no differences between cow strains in the estimation of plant species selection were observed. Prendiville et al. (2010) recorded that smaller Jersey cows had smaller bolus size compared to Holstein cows. Anatomical differences of the muzzle and incisor breadth, which influences

the pattern of bolus movement, might explain why HNZ cows spent longer time ruminating to handle ingested feed (Rook, 2000). On the other hand, it could have been expected that HCH cows had a higher pasture DMI, as DMI and BW are positively correlated (Kertz et al., 1991), and BW is usually positively linked to rumen size and therefore intake capacity. Digestion rate and ruminal digesta outflow of HNZ cows might be increased as rumination determines digestion rate and therefore controls voluntary intake (Bae et al., 1983; Gregorini et al., 2012). This is in accordance with no differences in grazing time and only minor differences in DMI in both current trials.

It would have been expected that concentrate supplementation influences rumination behavior. Adding rapidly degradable concentrate to the diet of dairy cows result in changes of ruminal pH, which alters ruminal fermentation, and might cause problems such as subclinical acidosis (Bargo et al., 2002). High grain diets reflect lower fiber intake and a greater ease of bolus formation (Beachemin and Rode, 1997) and therefore less mastications per bolus would be needed. In contrast, supplemented cows had slightly more mastications per bolus in the second trial. High DMI might provoke longer duration of rumination (Gregorini et al., 2012), but no differences in time spent ruminating and rumination mastications were recorded in both trials. Results of diet composition estimation on pasture in the second trial indicated that supplemented cows selected different plant species than non-supplemented cows and different diet composition might have influenced rumination behavior. Probably, supplemented cows have regurgitated slightly bigger boli, which needed more mastications per boli. However, rumination is subject of voluntary control by the animal as they can regulate time and duration (Gregorini et al., 2012). This poses the question of whether characteristics of rumination behavior are a suitable indicator of sufficient fiber supply and therefore rumen health, at least in grazing dairy cows.

5.5. Physical activity

In pasture-based feeding systems, especially in alpine region, physical activity of dairy cows plays an important role. Cows have to cover long distances from barn to pasture or on the pasture itself to search for their food and energy requirements for maintenance may increase in the range of 10 to 50% (CSIRO, 2007). Grazing cows have higher energy expenditure as they make more steps, spend less rime lying down and graze longer time compared to cows kept indoors (Kaufmann et al., 2011). Results of the first trial supported the assumption that physical behavior is closely related to grazing behavior. Supplemented cows spent more time lying down, but less time standing and walking, which is in accordance with reduced grazing time. The extra energy intake via the supplement and reduced energy expenditure for physical activity could have been used for milk production. The results of blood analysis

(such as concentration of glucose, beta-hydroxybutyric acid and non-esterified fatty acids in blood) in the first trial indicated that supplemented cows had a more stable energy status than non-supplemented cows.

In contrast, supplementation had no influence on physical activity in the second trial. This might be related to cold and wet weather conditions in the first measurement period of the second trial and cows might have preferred to stand instead of lying down on wet ground. On the other hand, results of diet composition estimation ensured a higher intake of *T. repens* for supplemented cows, which is preferred over other grass species (Chapman et al., 2007), but only presented 10% DM of all plant species. Probably cows spent more time searching on pasture for *T. repens* resulting in the same physical activity as non-supplemented cows, but with less time spent grazing.

5.6. Comparison of both cow strains

New Zealand Holstein cows are selected for feed efficiency and survivability on a pasture-based diet with little or no concentrate supplementation (Harris and Kolver, 2001). Therefore, they should have a higher feeding drive, even under high stocking rates, compared to other Holstein cows (McCarthy et al., 2007). No differences in grazing behavior between HCH and HNZ cows were recorded and only a tendency for different DMI in the trials could not support this. The differences in rumination behavior might be explained by anatomical differences of the muzzle (Rook, 2000), but HNZ cows could not benefit from a possible better fiber digestibility with increased milk yield. In accordance with other studies (Horan et al., 2005; McCarthy et al., 2007) HNZ cows had lower milk yield than HCH cows, but higher milk fat and milk protein content. In the first trial an interaction of cow strain and treatment was recorded for milk yield as in Horan et al. (2005), but none was recorded in the second trial, where also the milk yield per kilogram concentrate was lower for HCH cows. An explanation for this may be the lower NEL concentration of pasture in the first measurement period of the second trial, as discussed above. The same amount of kilogram milk to kilogram concentrate of the HNZ cows indicated that they were not affected by difference pasture quality and could probably better compensate. Unfortunately, no blood samples were taken in the second trial to give an indicator for the energy status. In the first trial blood analysis indicated that HCH cows were not in a strong NEB and had a stable energy status with no concentrate supplementation. Probably, greater differences would have been recorded in the second trial and HNZ cows could have profited more from their genetic potential when pasture in low quality and quantity was available.

In accordance with similar grazing behavior of HCH and HNZ cows, no difference in plant species selection between cow strains was observed. Villalba et al. (2015) asserted that

experience in utero and young life engraves the attitude to unpalatable foods with lower nutritive value and animals may become more efficient at extracting nutrients from foods than inexperienced animals. Probably, grazing behavior is influenced from learning and experience in young life, and as all cows used for the trials have been grown up on the same farm in a pasture-based feeding system, all cows are more or less adapted to pasture-based system with varying nutrient content of the pasture.

However, trials considered only mid-lactation. Long-term experiments including health and fertility traits would give further information about differences in efficiency between cow strains on a pasture-based feeding system.

Chapter 6: General conclusion and outlook

Grazing behavior is influenced by many factors and understanding the interactions between environmental aspects and reactions of cows is essential. Cows are partly able to adapt their behavior to environmental issues and, therefore, management measures should direct this adaptation to optimize production and ensure animal health and welfare but, at the same time allow for sustainable and efficient farming. Just using a different cow strain or breed does not guarantee successful farming, as results indicate that in general divergently bred cow strains can cope with pasture-only diets, and only small differences were recorded between individual animals. Therefore, it poses a challenge for the farmer to balance the needs of the animals and a profitable farming. A better knowledge of diet selection and grazing behavior may allow optimization of the pasture to the demands of the cows. Therefore, using and establishing seed mixtures that are adjusted to the preference and demand of dairy cows, may lead to higher feeding drive and increase the efficient use of pasture by dairy cows for milk production. The approach estimating diet selection of grazing dairy cows with plant wax markers appeared promising and results are coherent with results from previous studies. The advantage with the calculated fecal recovery would allow the removal of an indoor experiment with known diet composition to determine fecal recovery. If validations confirm a successful operation of this approach, the realization of experiments would be easier for further research. In addition, just using alkanes and LCOH, but skip LCFA for estimation of plant composition saves work and costs.

The decision whether or not adding supplements to the diet at pasture are related to several factors: milk yield potential of the cows, animal welfare and health, efficiency, environmental issues, grazing behavior and production costs, as well as social aspects, such as competition for cereal grains or arable land. Organic farming should be a pioneer for a sensitive use of valuable foods and questioning the application of high amounts of grain to ruminants. Both cow strains seemed to be suitable to pasture-based feeding systems with none or only little concentrate supplementation. Long-term studies with different dairy cow strains or breeds in early lactation, with and without concentrate supplementation are required to substantiate and potentially revise the organic farming guidelines. A change to a model, as in Switzerland, with a stronger restriction of concentrate supplementation in organic milk production, based on the annual ration (10%) may still be flexible enough for the farmer to feed dairy cows according to their requirements but will call attention to a meaningful and reliable use of grain-based concentrate.

References

Ali, H. A. M., R. W. Mayes, B. L. Hector, A. K. Verma, and E. R. Ørskov. 2005. The possible use of n-alkanes, long-chain fatty alcohols and long-chain fatty acids as markers in studies of the botanical composition of the diet of free-ranging herbivores. J. Agric. Sci. 143:85-95.

Bach, A., J. J. Villalba, and I. R. Ipharraguerre. 2012. Interactions between mild nutrient imbalance and taste preferences in young ruminants. J. Anim. Sci. 90:1015-1025.

Bae, D. H., J. G. Welch, and B.E. Gilman. 1983. Mastication and rumination in relation to body size of cattle. J. Dairy Sci. 66:2137–2141.

Baraza, E., J. J. Villalba, and F. D. Provenza. 2005. Nutritional context influences preferences of lambs for foods with plant secondary metabolites. Appl. Anim.l Behav. Sci. 92:293-305.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. J. Dairy Sci 85:1777–1792.

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1-42.

Baudracco, J., N. Lopez-Villalobos, C. W. Holmes, and K. A. Macdonald. 2010. Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: a review. N. Z. J. Agric. Res. 53:109–133.

Beauchemin, K. A., and L. M. Rode. 1997. Minimum versus optimum concentrations of fiber in dairy cow diets based on barley silage and concentrates of barley or corn. J. Dairy Sci. 80:1629–1639.

Boland, H. T., G. Scaglia, D. R. Notter, A. J. Rook, W. S. Swecker, and A. O. Abaye. 2012. Diet composition and dry matter intake of beef steers grazing tall fescue and alfalfa. Crop Sci. 52:2817-2825.

Chapman, D. F., A. J. Parsons, G. P. Cosgrove, D. J. Barker, D. M. Marotti, K. J. Venning, S. M. Rutter, J. Hill, and A. N. Thompson. 2007. Impacts of Spatial Patterns in Pasture on Animal Grazing Behavior, Intake, and Performance. Crop Sci. 47:399-415.

CSIRO. 2007. Nutrient requirements of domesticated ruminants. M. Freer, H. Dove, and J.V. Nolan, ed. CSIRO, Collingwood, Australia.

Dove, H. and E. Charmley. 2008. Using the alkanes and long-chain alcohols of plant cuticular wax to estimate diet composition and the intakes of mixed forages in sheep consuming a known amount of alkane-labelled supplement. Animal. 2:1474-1485.

Dumont, B., J. P. Garel, C. Ginane, F. Decuq, A. Farruggia, P. Pradel, C. Rigolot, and M. Petit. 2007. Effect of cattle grazing a species-rich mountain pasture under different stocking rates on the dynamics of diet selection and sward structure. Animal 1:1042-1052.

Ertl, P., Q. Zebeli, W. Zollitsch, and W. Knaus. 2015. Feeding of by-products completely replaced cereals and pulses in dairy cows and enhanced edible feed conversion ratio. J. Dairy Sci. 98:1–9.

Fedele, V., M. Pizzillo, S. Claps, P. Morand-Fehr, and R. Rubino. 1993. Grazing behavior and diet selection of goats on native pasture in Southern Italy. Small Ruminant Res. 11:305-322.

Ferreira, L. M. M., S. Carvalho, V. Falco, R. Celaya, U. García, A. S. Santos, M. A. M. Rodrigues, and K. Osoro. 2009. Assessment of very long-chain fatty acids as complementary or alternative natural fecal markers to n-alkanes for estimating diet composition of goats feeding on mixed diets. J. Anim. Sci. 87:2732-2745.

Ferreira, L. M. M., R. Celaya, A. S. Santos, V. Falco, C. Guedes, M. A. M. Rodrigues, and K. Osoro. 2011. The utilization of long-chain fatty acids as markers for diet composition estimates in ruminants: effects of animal species, diet composition and marker combination. Grass Forage Sci. 66:183-195.

Ferreira, L. M. M., R. Celaya, A. S. Santos, R. W. Mayes, M. A. M. Rodrigues, and K. Osoro. 2015. Application of long-chain alcohols as diet-composition markers in sheep fed on grass–white clover and heather–gorse plant species. Grass Forage Sci. 70:30-43.

Fraser, M. D., V. J. Theobald, J. B. Griffith, S. M. Morris, and J. M. Moorby. 2009. Comparative det selection by cattle and sheep grazing two contrasting heathland communities. Agric. Ecosyst. Environ. 129:182-192.

Gabriel, D., I. Roschewitz, T. Tscharntke, and C. Thies. 2006. Beta diversity at different spatial scales: plant communities in organic and conventional agriculture. Ecol. Appl. 16:2011-2021.

Gibb, M. 2006. Grassland management with emphasis on grazing behavior. Pages 141-157 in Fresh Herbage for Dairy Cattle. A. Elgersma, J. Dijkstra, and S. Tamminga, ed. Springer, Wageningen, Netherlands.

Gregorini, P. 2012. Diurnal grazing pattern: its physiological basis and strategic management. Anim. Prod. Sci. 52:416–430.

Gregorini, P., B. DelaRue, K. McLeod, C. E. F. Clark, C. B. Glassey, and J. Jago. 2012. Rumination behavior of grazing dairy cows in response to restricted time at pasture. Livest. Sci. 146:95–98.

Harris, B. L. and E. S. Kolver. 2001. Review of holsteinization on intensive pastoral dairy farming in New Zealand. J. Dairy Sci. 84:56-61.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. J. Dairy Sci. 88:1231–1243.

Kaufmann, L. D., A. Münger, M. Rérat, P. Junghans, S. Görs, C. C. Metges, and F. Dohme-Meier. 2011a. Energy expenditure of grazing cows and cows fed grass indoors as determined by the 13C bicarbonate dilution technique using an automatic blood sampling system. J. Dairy Sci. 94:1989–2000.

Kennedy, E., M. McEvoy, J. P. Murphy, and M. O´Donovan. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. J. Dairy Sci. 92:168-176.

Kertz, A. F., L. F. Reutzel, and G. M. Thomson. 1991. Dry matter intake from parturition to mid-lactation. J. Dairy Sci. 74:2290–2295.

Kolver, E. S. and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403-1411.

Kunz P, Wetter A, Roth N, Thomet P (2010) Comparison of the eating behaviour of two cow types on pasture. Pages 30-32 in: Book of Abstract 61st Annual Meeting of the EAAP, Heraklion, Greece.

Lin, L. J., X. Y. Zhu, C. Jiang, H. L. LUO, H. Wang, Y. J. Zhang, and F. Z. Hong. 2011. The potential use of n-alkanes, long-chain alcohols and long-chain fatty acids as diet composition markers: indoor validation with sheep and herbage species from the rangeland of Inner Mongolia of China. Animal. 6:449-458.

McCarthy, S., B. Horan, M. Rath, M. Linnane, P. O'Connor, and P. Dillon. 2007. The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. Grass Forage Sci. 62:13-26.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463–1481.

Newman, J. A., P. D. Penning, A. J. Parsons, A. Harvey, and R. J. Orr. 1994. Fasting affects intake behaviour and diet preference of grazing sheep. Anim. Behav. 47:185-193.

Prendiville, R., E. Lewis, K. M. Pierce, and F. Buckley. 2010. Comparative grazing behavior of lactating Holstein-Friesian, Jersey, and Jersey x Holstein-Friesian dairy cows and its association with intake capacity and production efficiency. J. Dairy Sci. 93:764–774.

Roche, J. R., D. P. Berry, and E. S. Kolver. 2006. Holstein-Friesian strain and feed effects on milk production, body weight, and body condition score profiles in grazing dairy cows. J. Dairy Sci. 89:3532-3543.

Rook, A. J. 2000. Principles of foraging and grazing behaviour. In: Hopkins A. (ed.), Grass - its production & utilization. 3rd ed., Blackwell Science, Oxford, UK, pp. 229–241.

Rutter, S. M. 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Anim. Behav. Sci. 97:17-35

Schori F., and A. Münger. 2014. Intake, feed conversion efficiency and grazing behaviour of two Holstein cow strains in a pasture-based production system under organic farming in Switzerland. Org. Agric. 4:175–186.

Spann, B., M. Diepolder, E. M. Schmidtlein, S. Tusch, A. Hermüheim, and D. Spengel. 2007. Umstellung zur ökologischen Milchviehhaltung - Vergleich verschiedener Leistungsparameter. Pages 65-77 in Angewandte Forschung und Beratung für den ökologischen Landbau un Bayern. Vol. 3. K. Wiesinger, ed. Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft, Freising, Germany.

Thanner, S., F. Schori, R. M. Bruckmaier, and F. Dohme-Meier. 2014. Grazing behaviour, physical activity and metabolic profile of two Holstein strains in an organic grazing system. J. Anim. Physiol. Anim. Nutr. 98:1143–1153.

Tolkamp, B. J., R. J. Dewhurst, N. C. Friggens, I. Kyriazakis, R. F. Veerkamp, and J. D. Oldham. 1998. Diet Choice by Dairy Cows. 1. Selection of feed protein content during the first half of lactation. J. Dairy Sci. 81:2657-2669.

Villalba, J. J., F. D. Provenza, F. Catanese, and R. A. Distel. 2015. Understanding and manipulating diet choice in grazing animals. Anim. Prod. Sci. 55:261-27

DANKSAGUNG

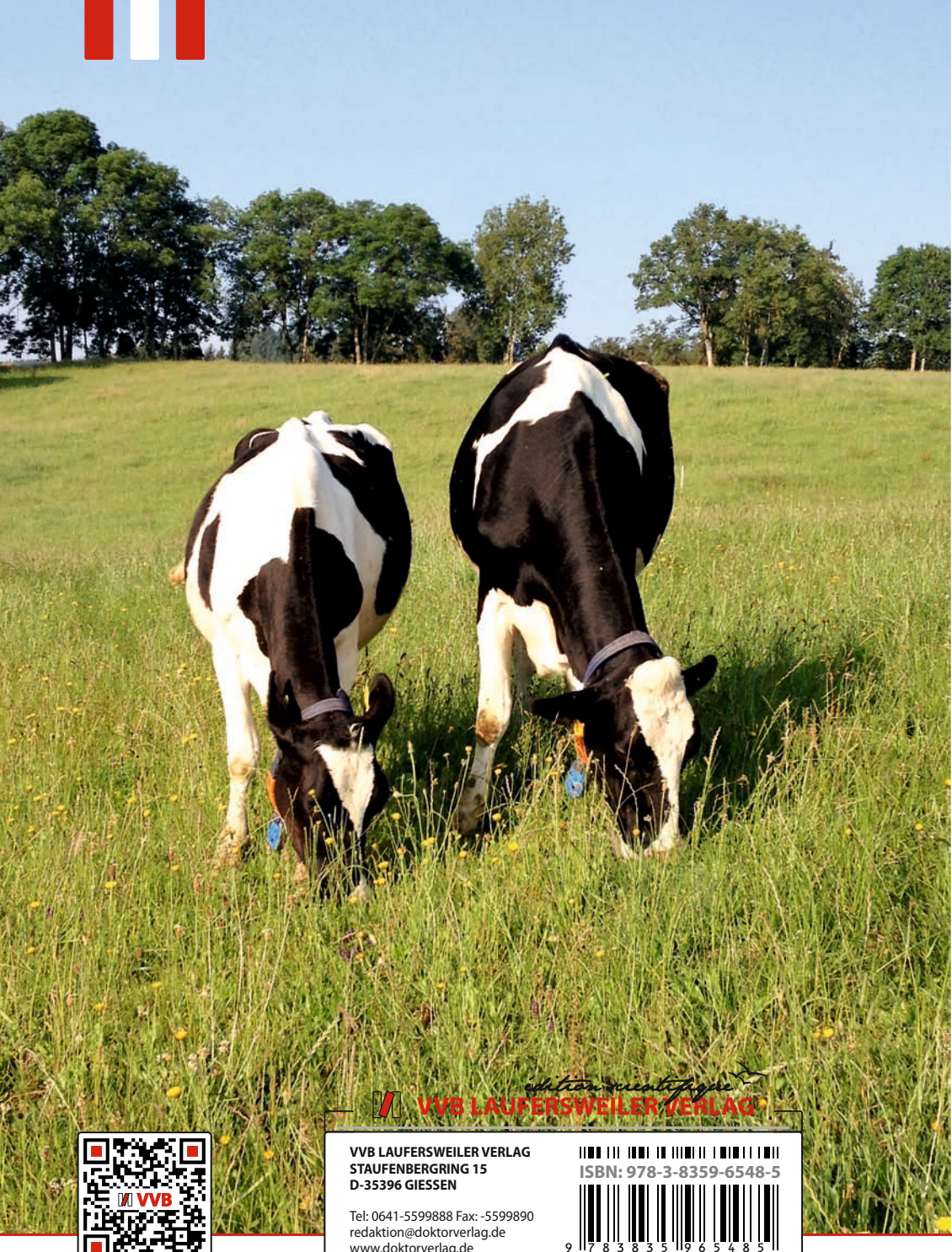
An dieser Stelle möchte ich mich ganz herzlich bei allen bedanken, die zum Gelingen dieser Arbeit beigetragen haben.

Zuerst ein großes Dankeschön an Herrn Prof. Karl-Heinz Südekum für die sehr gute und angenehme Betreuung, auch während meines Aufenthalts in der Schweiz. Außerdem danke ich Herrn Prof. Michael Kreuzer für die Übernahme des Korreferats, Herrn PD Dr. Jürgen Schellberg für die Übernahme des Amtes als fachnahes Prüfungsmitglied, sowie Frau Prof. Helga Sauerwein für das Amt als Prüfungsvorsitzende.

Ganz besonders möchte ich mich bei Fredy Schori für die tolle Betreuung bedanken. Du hast meine Fragen immer bereitwillig beantwortet und mich motiviert, wenn es mal nicht so gut lief. Auch ein großes Dankeschön an Dr. Frigga Dohme-Meier für die Betreuung und die Hilfe, vor allem bei wichtigen Entscheidungsfindungen.

Des Weiteren möchte ich mich bei den Mitarbeitern und Helfern im Stall von Sorens und Agroscope bedanken für die Hilfsbereitschaft und die gute Fürsorge der Kühe während der Versuche. Außerdem danke ich ganz herzlich den Mitarbeitern im Labor von Agroscope für die Analyse der vielen Kot und Grünfutter Proben. Dr. Fiona Gill und Fiona Keay von der Universität Leeds danke ich für die freundliche Aufnahme und Unterstützung im Labor in England. Außerdem danke ich Herrn Dr. Werner Luginbühl für die Beantwortung aller Fragen rund um die statistische Auswertung.

Zu guter Letzt gilt ein großes Dankeschön an Georg, meiner Familie und allen Freunden. Ihr seid immer für mich da und habt mich motiviert und aufgebaut, wenn es mal nicht gut lief.



edition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN: 978-3-8359-6548-5