MTT SCIENCE 25

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Doctoral Dissertation

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Milk production and physiological responses to concentrate supplementation of dairy cows grazing timothy-meadow fescue swards

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Abstract

This thesis comprises reports on experiments in which the effects of concentrate supplementation and part-time grazing on milk production and rumen physiology were studied. Cows were fed timothy-meadow fescue (Phleum pratense-Festuca pratensis) grass in confinement (one experiment) or by grazing at pasture (eight experiments). Part-time grazing studies (two experiments) included feeding grass silage and grazing at pasture. The amount of concentrate varied between 0 and 12 kg/d and it consisted of grain (mainly barley and oats, three experiments) or pelleted concentrate mixture (five experiments).

The main objectives of the study were to determine the overall marginal response functions of full-time grazed cows to increasing amounts of concentrate supplementation, and to clarify differences in rumen physiology between silage and grazed-pasture diets. The additional objectives were to study part-time grazing as an alternative summer-feeding strategy, and to study limiting factors for intake under grazing conditions.

Grass intake at pasture was defined using a sward-cutting method. The measured amount of grass intake in milk-production studies at pasture was smaller than the amounts based on estimated intake according to feed recommendations or modelled intake. Low-substitution rate with increasing concentrate supplementation at pasture suggests that forage intake could be limited in grazing situations despite the high quality and availability of fresh grass. Unnecessarily high crude protein (CP) content of the pasture diet could limit the herbage intake, but the more probable reason for limited intake may be related to pasture-management factors.

Rumen dry matter (DM) pool size increased with increasing grass indigestible neutral detergent fibre (iNDF) content, and with decreasing degradation rate (k_d) of potentially digestible neutral detergent fibre (pdNDF). High-digestibility forage at pasture is low in iNDF content and high in k_d of pdNDF, which enables high intake without rumen-fill limitation. Rumen iNDF pool size was possibly not a limiting factor with high digestibility pasture diets, at least for the mid-lactation cows used in this study.

Grass iNDF content described well the differences in rumen fermentation when the results between fresh and ensiled grass were compared. High rumen ammonia concentration, originating from high CP content, was typical for herbage of grazed-pasture diets. The use of low iNDF-content grass increased molar proportions of butyrate and propionate in the rumen fluid, whereas that of acetate decreased. The changes in acetate and butyrate occurred in parallel with increasing concentrate supplementation. The physiological part of this study, however, did not include high amounts of concentrates and this limits the overall conclusions. Microbial protein synthesis was high with pasture diets and this decreases the need for protein supplementation. The benefits of protein supplementation to cows at pasture may, therefore, be limited.

The milk yield increased quadratically with increasing amount of concentrate supplementation. Both the maximum milk yield and energy corrected milk (ECM) yield was reached with 12 kg concentrate, which was the highest level used. The responses to concentrate supplementation did not differ markedly between the silage diet used as the reference and that of grazed grass of comparable forage digestibility. The effect of milk production level on the response of concentrate supplementation was so small during most of the lactation period that it is reasonable to use a flat-rate concentrate feeding strategy for full-time grazed cows.

Supplementing silage with grazed grass at pasture increased the milk yield of cows in the part-time grazing experiments. The difference was most evident with the night-time grazed group, whereas the day-time grazed group had higher milk yields than the silage group only during the last half of the grazing season. The digestibility of grazed grass at the beginning of the day-time grazing experiment was lowered, and the grazing time was also shorter than in the case of the night-time grazing experiment. These results showed that part-time grazing maintained or increased milk yield when concentrate and silage were fed separately, a moderate amount of concentrate was used (less than 40% of total feed on a DM basis), and the energy content of grazed grass was higher than silage. Part-time grazing seems to be an appropriate strategy for Finnish conditions.

Keywords:
Dairy cattle, Grazing, Concentrate feeding, Restricted grazing, Rumen fermentation

Laiduntavien lehmien väkirehuvaste ja syöntiä rajoittavat tekijät timoteinurminatalaitumella

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Tiivistelmä

Tutkimus sisältää yhdeksän erillistä lypsylehmien ruokintakoetta, joiden tavoitteena oli tutkia väkirehun tuotosvastetta, laidunruohon syöntiä rajoittavia pötsifysiologisia tekijöitä sekä osa-aikalaidunstrategiaa. Kuudessa kokeessa lehmät laidunsivat kokoaikaisesti, kahdessa kokeessa käytettiin osa-aikalaidunta ja yhdessä kokeessa käytettiin niittoruokintaa. Osa-aikalaidunkokeissa sisäruokintana oli vapaa nurmisäilörehu. Kaikissa kokeissa käytetty nurmi oli timotei-nurminataseosta. Lypsyn yhteydessä jaettava väkirehu (0-12 kg/pv) oli viidessä kokeessa täysrehua ja kolmessa kokeessa ohra-kaura- rypsirouhe seosta.

Laidunrehun syönti mitattiin määräalaniittotekniikalla kahdessa kokeessa. Mitattu määrä oli pienempi verrattuna karkearehun syönnin ennustemalliin tai ruokintanormeihin perustuvaan syönninennusteeseen. Samoin määräalaniitolla mitattu laitumen korvaussuhde väkirehumäärän lisääntyessä oli suhteellisen matala. Laidunrehun syönnin rajoitus suhteessa maitotuotokseen selittäisi osaltaan näitä havaintoja. Tämä siitä huolimatta, että nurmen sulavuus oli korkea ja nurmirehua oli periaatteessa riittävästi saatavilla. Laidundieetin korkea raakavalkuaispitoisuus voi olla yksi selittävä tekijä syönnin rajoitukseen, mutta todennäköisin syy löytyy laiduntamisen käytännön toteutuksesta.

Fysiologisen osatutkimuksen perusteella pötsin kuiva-ainepooli lisääntyi nurmen sulamattoman kuidun (iNDF) pitoisuuden noustessa. Samoin kasvuasteen vanhetessa kuidun sulatusnopeuden aleneminen lisäsi pötsin kuiva-ainepoolia. Laidunruokinnalla varhaisesta kasvuasteesta johtuen nurmen iNDF pitoisuus on matala ja kuidun sulatusnopeus puolestaan on korkea. Pötsin kuiva-ainepooli tai iNDF pooli ei täten todennäköisesti muodostu syöntiä rajoittavaksi tekijäksi, mikä mahdollistaa runsaan laidunrehun syöntimäärän.

Nurmen iNDF pitoisuus selitti eroja pötsifermentaatiossa verrattaessa laidunruokintaa ja kirjallisuuskatsaukseen perustuvaa nurmisäilörehuruokintaa. Laidunruokinnan korkea raakavalkuaispitoisuus selitti korkeaa pötsin ammoniakkipitoisuutta. Matalakuituinen laidunnurmi lisäsi voihapon ja propionihapon mooliosuuksia pötsinesteessä verrattuna kirjallisuuden perusteella saatuihin säilörehuruokinnan mooliosuuksiin. Samansuuntaiset muutokset saatiin väkirehun osuutta nostamalla. Tämän tutkimuksen fysiologisessa osiossa valitettavasti ei käytetty korkeita väkirehumääriä, jotka olisivat olleet tärkeitä johtopäätösten kannalta. Mitattu mikrobiproteiinisynteesi pötsissä oli niittoruokinnalla korkea. Korkea mikrobisynteesi yhdessä laidunnurmen korkean raakavalkuaisen kanssa puoltavat mahdollisuutta vähentää lisävalkuaisruokintaa laitumella.

Maitotuotos nousi käyräviivaisesti väkirehutason noustessa. Suurin maitotuotos sekä suurin energiakorjattu maitotuotos saavutettiin korkeimmalla väkirehutasolla, 12 kg/pv. Tässä tutkimuksessa mitatut väkirehuvasteet laidunruokinnalla ja kirjallisuuteen perustuvat väkirehuvasteet säilörehuruokinnalla eivät poikenneet merkittävästi toisistaan, kun nurmirehun sulavuus otetaan huomioon. Laktaatiovaiheen merkitys väkirehuvasteisiin oli pieni aivan loppulypsykautta lukuun ottamatta, mikä puoltaa tasaväkirehumallin käyttöä ympärivuorokautisessa laidunruokinnassa.

Maitotuotokset osa-aikalaitumella olivat joko korkeampia tai samaa tasoa säilörehuruokintaan verrattuna. Erot laidunrehun sulavuudessa kesän eri aikoina selittivät maitotuotoseroja laidun- ja säilörehuruokinnan välillä. Laidunruokinnan maitotuotosta lisäävä tulos on yleistettävissä silloin, kun käytössä on enimmillään tämän tutkimuksen mukainen, korkeintaan 40 prosentin väkirehuosuus ruokinnassa ja laidunrehun sulavuus on säilörehua korkeampi. Osa-aikalaidun on tämän tutkimuksen mukaan toimiva laidunnusmuoto Suomen olosuhteissa.

Avainsanat:
Lypsylehmät, laiduntaminen, väkirehuruokinta, osa-aikalaidun, pötsifysiologia

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Finally I wish to thank my family and especially my dear wife Sari. You asked a few years ago what was the real purpose of the publication I was writing at that time. An excellent question and I am still searching the right answer to that.

In Siilinjärvi, February 2014.

List of original publications

The thesis is a summary of the following articles referred to by their Roman numerals:

I Khalili, H. & Sairanen, A. 2000. Effect of concentrate type on rumen fermentation and milk production of cows at pasture. Animal Feed Science and Technology 84: 199–212.

II Sairanen, A., Khalili, H., Nousiainen, J.I., Ahvenjärvi, S. & Huhtanen, P. 2005. The effect of concentrate supplementation on nutrient flow to the omasum in dairy cows receiving freshly cut grass. Journal of Dairy Science 88: 1443–1453.

III Sairanen, A., Khalili, H. & Virkajärvi, P. 2006. Concentrate supplementation responses of the pasture-fed dairy cow. Livestock Science 104: 292–302.

IV Sairanen, A., Khalili, H., Virkajärvi, P. & Hakosalo, J. 2006. Comparison of part-time grazing and indoor silage feeding on milk production. Agricultural and Food Science 15: 280–292.

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A reprint of the original article IV is published with the kind permission of Agricultural and Food Science

The author participated as co-author in the publication I and was the main responsible for publications II, III and IV. The author took full responsibility for the calculation of all works.

Contents

Abbreviations

CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DOM	Digestible organic matter
ECM	Energy corrected milk
FPR	Milk fat to protein ratio
HA	Herbage allowance
iNDF	Indigestible neutral detergent fibre
k_p iNDF	Passage rate of iNDF
k_d pdNDF	Digestion rate of potentially digestible NDF
LW	Live weight
ME	Metabolizable energy
milk MR	Marginal milk response, kg milk/kg additional concentrate DM
MPS	Microbial protein synthesis
MR	Marginal energy corrected milk response, kg milk/kg additional concentrate DM
NDF	Neutral detergent fibre
pdNDF	Potentially digestible NDF
R^2	Coefficient of determination
RDP	Rumen degradable protein
RMSE	Root-mean-square error
RUP	Rumen undegradable protein
SD	Standard deviation
SEM	Standard error of mean
TMR	Total mixed ration
VFA	Volatile fatty acids

"We are all familiar with the expression that grazing dairy cows is an art."
James E. Delahoy and Lawrence D. Muller

1 Introduction

1.1 Background

Grazing is a common and traditional summer feeding practice in Finland. Grazed grass is widely reported to be the cheapest source of feed available to dairy farmers (Finneran et al., 2010). In many cases, grazed grass is also a cost-effective alternative to feeding silage in the summer period on farms at Nordic latitudes. Seppälä et al. (2006) have shown that full-time grazing is economically a more profitable strategy, compared to silage feeding, at least for herds of up to 60 cows, at times of year when grazing is possible.

Welfare considerations also support the use of traditional grazing of pastures. This is justified because grazing is an integral part of cows' natural behaviour. Many studies have shown that grazing is associated with animal health improvements and it reduces the risk of lameness (Herlin, 1995; Rodriguez-Lainz et al., 1999; Washburn et al., 2002; Olmos et al., 2009) although some aspects of health benefits are not always so clear (Virkajärvi et al., 2004; Chapinal et al., 2010). Heat stress, insects and insufficient knowledge about grazing management are some potential disadvantages of grazing.

It is, however, generally accepted that grazing increases the welfare of cows, and this has also been reflected in legislation and organic farming regulations. During the grazing season all the tie-stall housed cows and heifers must be allowed access to a pasture or exercise yard, at least for 60 days (VNA 2010). An exercise yard is one possibility for compliance with demands of legislation but it usually requires more expensive investments compared to the costs of grazing. According to the regulations of organic farming, cows must be let out both during summertime and wintertime (Evira, 2009).

In Finland, the proportion of the annual energy consumption of dairy cows that is provided by grazing at pasture has decreased during the last decades and in 2011 it was as low as 6% (ProAgria, 2012). The proportion of energy supplied by grazing during three grazing months is, of course, much higher than this annual value. Increasing herd size and the use of automatic milking systems are factors leading to low usage of grazing. The increasing herd size is essential for economically profitable milk production, and grazing practices have to be developed taking this reality into account. Pasture feeding needs labour for fencing and for cow transfer, both of which increase with herd size, and it requires suitable land close to the cowhouse. Different grazing strategies, such as part-time grazing, should therefore be studied, so that large herds do not need to give up grazing completely.

Grass growth rate varies between 30 and 200 kg dry matter (DM)/ha during the grazing season (Virkajärvi, 2004) in Nordic latitudes and this variation presents difficulties for planning the grazing rotation. A grazing strategy based on continuous stocking is not suitable for swards based on timothy meadow-fescue (Phleum pratense-Festuca pratensis) because of their poorer rate of regrowth compared with ryegrass pastures. Ryegrasses do not overwinter properly in Finland and thus their usage is limited. Part-time grazing has been successfully used in Ireland to extend the grazing season into early spring or late autumn (Dillon et al., 2002). In Finland, however, grass growth starts very rapidly in spring and thus it is not possible to extend the grazing season. The strategy where silage and concentrate supplementation make up part of the diet during summer is also applicable in the conditions of Northern latitudes. The proportion of the energy in the diet supplied as silage is low when the grass growth rate is high at the beginning of the summer, and it increases depending on weather and grass growing conditions as the summer season advances.

A large number of studies have been conducted to quantify the milk-production responses to supplementary feeding of grass silage-based diets. Experiments with grazing cows, however, have seldom been conducted using high amounts of concentrates with timothy-meadow fescue pastures as the basal feed. The main difference between the herbage of timothy-meadow fescue swards and that of the widely used ryegrass is the maturity stage and fibre content. The herbage of rotationally grazed grass pastures in Northern latitudes contains quite high amounts of fibre and occasionally the growth stage can be advanced (Virkajärvi, 2004). In these conditions it is possible to use relatively high amounts of concentrates.

Production responses to concentrate supplementation may be highly variable. This is because responses depend on a wide range of factors, involving the cows, feeds and management systems. The milk yield responses to concentrate supplementation (kg milk/kg concentrate DM; milk MR) of cows in grazed pasture vary from 0.4 kg/kg (Leaver et al., 1968; Kennedy et al., 2001) to 1.0 kg/kg (Ettala et al., 1986; Bargo et al., 2003; Kennedy et al., 2003). Ettala et al. (1986) included experiments conducted between 1969 and 1979 at MTT. Cows (480 in total) were supplemented with 0-6 kg cereal concentrate. They found average milk MR of 0.8 kg/kg below 4 kg concentrate supplementation but the milk MR was negligible above that.

In their review, Bargo et al. (2003) reported the highest average linear milk MR of 1.0 kg/kg, and up to 10 kg concentrate DM per day for the high yielding group. They suggested that concentrate of 10 kg DM per day, or less than 50 % of the total diet, would be the upper limit of concentrate supplementation for avoiding metabolic health problems. In a more recent review study, Baudracco et al. (2010) reported an average of 0.88 kg energy corrected milk (ECM) response per kg concentrate DM (MR). The experiments were conducted using ryegrass-dominant pasture swards using 0-6 kg DM amount of concentrates. They concluded that the milk MR depends mainly on the size of the relative energy deficit between potential energy demand and actual energy supply. An Irish review including 10 studies, reported an average milk MR of 0.48 kg/kg (O'Neill et al. 2013). They observed the lowest milk MR, 0.36 kg/kg, during spring time. The only quadratic milk MR among the reported reviews was published by Bargo et al. (2003) for a group of three experiments where the maximum milk yield was below 22.3 kg at the beginning of lactation. According to Ettala et al. (1986) it can also be supposed to be a quadratic milk MR but it was not tested statistically. Higher proportion of concentrate to supplement the fibrous grasses under Nordic conditions may be needed and the MR function may be extended to quadratic area.

Kennedy et al. (2001) speculated that the increase in milk yield in response to supplementary concentrate feeding in recent decades stems partly from genetic improvement of cows. The interaction between MR and milk production level may be difficult to prove. High genetic merit cows partition supplementary feed energy towards milk production instead of body reserves at the beginning of lactation. On the other hand, the stage of lactation can affect MR because energy is partitioned more to body reserves at the end of lactation (Broster and Broster, 1984). These two factors are usually confounded. However, the MR and factors affecting MR in different conditions have to be quantified before reliable economic comparisons between various diets can be conducted.

In addition to animal factors, herbage allowance (HA) and quality (Mayne and Peyraud, 1996), physical distension of the rumen and metabolic intake regulation (Mertens, 1994; Allen, 1996) have to be taken into account. It is impossible to make a universal model which is suitable for all conditions. Thus, there is a need for studies to be conducted under local conditions.

Physiological studies are needed to quantify factors that limit nutrient intake and utilization, and also for determining differences between fresh-grass and ensiled-grass diets. It is possible to carry out marker infusions on cows at pasture as tested in MTT. However, accurate measurement of parameters of rumen kinetics needs reliable intake measurement, which is an extremely demanding task to perform for cows at pasture. In these conditions, the feeding of freshly cut grass in confinement may be used as a feasible alternative to grazing at pasture.

Direct physiological comparisons between pasture and silage diets are difficult, or even impossible, to conduct due to practical reasons. Silage requires time for fermentation and the maturity of herbage in a pasture sward changes continuously during the growing season. Thus, it is impossible to conduct an experiment with the same cows and the same forage (fresh and ensiled) simultaneously. Cushnahan et al. (1995) reported one indirect comparison between fermented grass and a fresh-grass diet, where only minor differences between the diets were observed. The experiment was conducted using successive periods for fresh and ensiled grass. Cushnahan and Gordon (1995) also reported the same effective forage degradability between frozen and bunker-silo ensiled grass, which indicates that ensiling itself has little effect on grass nutritive value.

Physiological studies including rumen kinetics measurements with fresh-grass diets are also rare. For example, Krizsan et al. (2010) generated an empirical prediction equation for fibre passage from the rumen and, out of 49 experiments in the dataset, only two were based on fresh grass. As physiological studies provide fundamental knowledge about digestive processes in the rumen, there is a need for such studies to be conducted under grazing conditions. Only then would it be possible to make at least indirect comparisons about differences between pasture (i.e. grazed grass) and silage diets. This information is, however, essential for establishing practical recommendations for farmers on feeding strategies under grazing.

1.2 Hypothesis and objectives of the study

The major part of Finnish research on dairy cow feeding has focused on silage-based diets and most of the feeding recommendations have been made for indoor feeding. Understanding the differences between pasture (grazed grass) and silage diets helps us to apply the knowledge based on conserved-feed studies to pasture circumstances. The general aim of this study was to provide information about grazed-grass pastures, under rotational stocking, as a summer feeding strategy in Nordic conditions.

It can be assumed that the quality of fresh grass is at least equal to or better than (due to inevitable preservation losses) the quality of ensiled grass made from the same sward. Owing to potentially high quality of grazed grass the three hypotheses of the study are:

- Grazed grass has no intrinsic limitations compared to ensiled grass, so grazing does not limit DM intake (DMI). Grass maturity stage also explains differences between silage and grazed-grass diets and the forage type itself has little effect on this.

- Secondly, that in this study the milk-production responses to concentrate supplementation of cows grazing at pasture are comparable to equations based on indoor feeding studies, under conditions when the digestibility values of grazed and ensiled grass are equal.

- Thirdly, that part-time grazing would maintain high milk yield and it would provide a feasible alternative to zero grazing.

The specific objectives of this work were:

- To study rumen physiology of dairy cows fed grazed-grass diets (I, II) for identifying the typical rumen fermentation and digestion characteristics on grazed-grass diets. This enables indirect comparisons in digestive physiology between grazing and silage diets.

- To measure grass intake and to determine the overall marginal response function to increasing amount of concentrate supplementation (kg ECM/kg concentrate DM) with full-time grazed cows (I, III).

- To study part-time grazing as an alternative summer-feeding strategy to full-time silage feeding (IV).

2 Material and methods

2.1 Description of experiments

The study comprised nine experiments (Table 1). Experiments 1–6 (I, II, III) included variable amounts of concentrate (0–12 kg per cow/d). Concentrates consisted of grain (three experiments) or pelleted concentrate mixture including barley, oats, cereal by-products and rapeseed meal (five experiments). Protein supplementation (rapeseed meal) as a treatment was used in experiments 6 (unpublished, see appendix 1) and 8 (IV). Experiment 2 (II) was conducted using fresh cut grass and all other experiments included grazing at pasture as a treatment. Studies of part-time grazing (experiments 7 and 8, IV) included both grazed-grass and silage. Three experiments included physiological measurements (experiments 1 (I), 2 (II) and 9 (unpublished, see appendix 2). The two sets of unpublished data were used as additional data for meta-analyses.

All other experiments have been described in detail in publications I-IV, except experiments 6 and 9. In brief, the unpublished experiments included the effect of concentrate supplementation on milk production (experiment 6) and the effect of season on rumen physiology (experiment 9).

Experiment 6 included three levels of concentrate (a barley-rapeseed meal mixture at 6, 9 and 12 kg/d, crude protein (CP) 160 g/kg DM) in a cross-over design with three periods and four weeks in each. The first period began at the beginning of June. The experiment included 36 cows and used the same protocol and measurements as described in experiment 5, with the exception that HA was unrestricted in this experiment. The available herbage mass was estimated using a sward-cutting method.

Experiment 9 included five rumen-fistulated cows with a diet that consisted solely of grazed grass. The experiment was designed to clarify the differences in rumen content and fermentation pattern during the grazing season and the only treatment was period (four in total). The first data collection started on 10 June. Every period lasted three weeks including 5 days of data collection at the end. The measurements included milk yield, grass intake, rumen pool size, rumen fermentation and the digestibility of the diet. The rumen sampling was conducted on a pasture using a movable stall. Marker dosing and rumen evacuation was conducted in the barn. The physiological measurements were conducted using methods described in experiment 1. The DMI was measured using chromium oxide as an external marker to estimate faecal output and indigestible neutral detergent fibre (iNDF) as an internal marker to determine diet digestibility. The faecal collection was conducted after morning and evening milking by sampling individual dung patches on the pasture.

The grass species used on pastures were mixture of timothy and meadow fescue. The main soil type was fine sand. Fertilization was carried out according to recommendations, which followed maximum amount of nutrients limited by environmental legislation. Pastures were typically fertilized three times during the growing season, and received a total of 220 kg N/ha per year. The first fertilization was conducted in the middle of May, about one week before the start of grazing. The age of pastures varied between 1 and 4 years and they were topped with a forage harvester to a stubble height of approximately 10 cm typically three times during the summer: after the first (approx. 10 June), second (25 June) and third rotation (15 July). Rotations four and five lasted 4 weeks each until the grazing season finished, which was typically in mid September. The last rotation was typically conducted with a part-time grazing strategy. The daily herbage allowance was estimated visually to be over 25 kg DM/ha between experimental periods. The distance from the barn to pastures varied between 0.1 km and 1 km.

All the experiments were conducted using Holstein-Friesian cows and either different amount or type of concentrates. Cows were kept in a tie-stall barn and milked twice per day. They were taken to the pasture as one herd except in experiments 3 and 4, where the cows grazed separately according to treatments. Milk yield, the amount of silage, concentrates and concentrate refusals were measured individually every day. Concentrates were given in four equal proportions: after being brought in from the pasture and before turning out to pasture, both morning and evening. The HA, was fixed in experiments 3, 4, 5 (III) and 7 (IV), and free (estimated HA > 30 kg DM/cow/d) for the rest of the experiments. The HA was 21 kg DM in experiments 3 and 4, 25 kg DM in experiment 5, and 13 kg DM in experiment 7. Experiment 2 included fresh-cut grass in confinement and experiments 7 and 8 included both grazed grass and grass silage.

The intensive physiological study 2 (II) was conducted in tie-stall conditions. The stall feeding was chosen because of the requirement for accurate intake measurement. It would have been possible to carry out marker infusions on the pasture but the intake measurement with markers includes a great amount of variation. Timothy-meadow fescue grass was harvested three-times daily with a Haldrup 1500 plot harvester. Grass was offered to 6 rumen-cannulated cows as 6 equal meals daily, and concentrates were fed as 2 equal meals daily. The digesta flow from the rumen was measured using an omasal sampling technique in combination with a triple marker method (CoEDTA, Yb, and iNDF as markers).

Where appropriate, grazing terminology used in this paper conforms with Allen et al. (2011).

Table 1. Description of experiments.

Publ.	Exp.	Treatments	Cows, n	Objective
I	1	a) Concentrate mixture, 4 kg b) Milled Barley, 4 kg c) Grazed Pasture alone	15	To evaluate the effects of concentrate supplementation type on rumen fermentation and milk production.
II	2	a) Fresh-cut grass alone b) Grass + 3 kg concentrate mixture c) Grass + 6 kg concentrate mixture	6	To determine the effects of concentrate supplementation on intake and nutrient flow to the omasum.
III	3	0-9 kg concentrate mixture	28	To evaluate marginal responses to concentrate supplementation using fixed herbage allowance.
	4	3-9 kg concentrate mixture	45	
	5	6-12 kg concentrate mixture	36	
	6	6-12 kg barley/oats/rapeseed meal concentrate, CP 160 g/kg DM	36	Unpublished (see appendix 1). Additional data for the concentrate response study.
IV	7	a) Full-time indoor silage feeding b) Night-time grazing	34	To compare the effects of full-time indoor feeding and part-time grazing on milk production.
	8	a) Full-time indoor silage feeding with protein supplementation 130 vs 185 g CP/kg DM. b) Day-time grazing with protein supplementation 130 vs 185 g CP/kg DM.	32	
	9	Grazing season: four periods between early June and mid August	5	Unpublished (see appendix 2). Additional data to physiological studies to clarify differences in rumen content during the grazing season.

DM = dry matter, CP = crude protein

2.2 Statistical methods

The statistical methods for each experiment have been described in the articles I-IV and appendices 1-2. The SAS GLM procedure was used in experiments 1 and 2, and the SAS Mixed procedure (version 9.3, SAS Institute Inc., Cary, NC, USA) was used in experiments 3-9. The experiment 6 was analysed with the same method as experiment 5. The treatment in experiment 9 was period and it was included to the model as a fixed variable.

Typical experimental design was a cross-over trial with a two-week transition period and a one-week data collection period. Experiments 4, 7, 8 and 9 were conducted using completely randomized or randomized block design. The cows were blocked according to parity, combination of pre-experimental milk yield and days in milk (DIM). Treatments were assigned randomly to cows within blocks. Treatment differences were declared at $P < 0.05$ using a multiple comparison test (adjusted Tukey).

Meta-analysis to determine milk production responses to concentrate supplementation

The data collected from experiments 1 and 3-6 were analysed using the random coefficient regression model (St-Pierre, 2001) to estimate the milk response function to increasing amount of concentrate with the SAS MIXED procedure. Dependent variables were modelled by a fixed intercept, a fixed slope, a random intercept clustered by experiment, and a random slope also clustered by experiment. The model included an unstructured covariance matrix for the intercepts and slopes. Adjusted observations were calculated by adding the residual from each individual observation to the predicted value of the experiment regression. Coefficient of determination (R^2) and root-mean-square error (RMSE) were performed between the adjusted dependent variable and independent variable using the REG procedure of SAS.

The model in the SAS MIXED regression analysis included:

- the amount of milk or the amount of ECM as a dependent variable
- the amount of concentrate as a continuous independent variable (both linear and quadratic effects)
- experiment was used as a random class variable

The analysis for testing the effect of interaction between production level and concentrate supplementation on the milk production response was conducted separately for every experiment. The cows within each experiment were blocked to three blocks according to pre-experimental milk yield: low-yielding cows (milk yield under 28 kg/d), moderate-yielding cows (milk yield 28-35 kg/d) and high-yielding cows (milk yield over 35 kg/d). Experiment 1 included late-lactating cows and thus included only a low-yielding block. Experiment 5 included only moderate and high-yielding blocks. The aim of the blocking was to determine interaction between production level and concentrate supplementation on the milk production response.

The models within each experiment included:

- period, the level of concentrate, block and the interaction between the amount of concentrate and block as fixed-class variables. In experiment 4 (III) the period was used as a repeated measurement.
- cow as a random variable

Meta-analysis for rumen fibre kinetics

The physiological inspection included both grazed-grass and silage studies (Table 2). The selection criteria for silage studies in the comparison were different grass maturity stages within the study. The grass species included were timothy-meadow fescue mixtures and perennial ryegrass. The data set included 10 observations from grazed-grass diets and 18 observations from silage diets.

The variables in the comparison were rumen pools of DM, neutral detergent fibre (NDF) and potentially digestible NDF (pdNDF), digestion rate (k_d) of pdNDF and passage rate (k_p) of iNDF. The pool sizes were calculated per 100 kg live weight (LW), except for those presented in Table 2 which includes the description of the experiments. Comparisons between the silage and the grazed-grass diets were made either by comparison between the average values or via regression analysis. When the regression analysis was used, the analysis was conducted with the same method as described earlier in the chapter "Meta-analysis to determine milk production responses". The best fitting model was chosen by the highest R^2 and the lowest RMSE.

The model in the SAS MIXED regression analysis included:

- concentrate DM intake, DM intake, forage NDF content and forage iNDF content as continuous variables
- experiment as a random class variable
- forage type (fresh grass vs silage) was also tested, but it was not included to the final model due to lack of significance

Rumen fermentation

Rumen fermentation was studied using comparisons between results published in literature and own results. The summary included experiment 1(I) and the Finnish experiments presented in Table 2. The regression analysis included:

- forage type: fresh grass or ensiled grass as fixed variables
- forage iNDF content as a continuous variable
- experiment as a random factor

Table 2. Description of the physiological experiments used in the grass vs silage comparison.

		Intake, kg		In the rumen, kg			% per hour	
	Forage type	Forage DM	Total DM	DM	NDF	pdNDF	k_d pdNDF	k_p iNDF
Grass and pasture diets								
Experiment 2 (II)	Fresh grass	16.1	18.7	11.0	5.3	3.3	8.9	1.8
Experiment 9	Grazed grass	15.1	15.1	8.6	4.5	3.1	10.6	2.7
Virkajärvi et al. (2002)	Grazed grass	14.9	14.9	10.3	5.5	3.7	6.8	1.8
Silage diets								
Bosch et al. (1992)	Grass silage	11.4	14.9	10.7	6.3			
Kuoppala et al. (2009)	Grass silage	12.6	20.6	12.7	8.0	5.7	3.4	2.4
Kuoppala et al. (2010)	Grass silage	12.0	19.0	12.1	7.2	5.1	3.8	2.6
Rinne et al. (2002)	Grass silage	11.8	18.0	10.5	6.5	4.5	5.6	2.5

DM = dry matter, NDF = neutral detergent fibre, pdNDF = potentially digestible neutral detergent fibre, k_d pdNDF = the digestion rate of pdNDF, k_p iNDF = the passage rate of indigestible neutral detergent fibre

3 Results and general discussion

3.1 Grass intake on pasture

Grass quality, sward structure and herbage allowance

Dry matter intake explains most of the variation observed in milk production (Mertens, 1994) and therefore it is essential to know the amount of the consumed grass at pasture when examining limiting factors for milk production during the grazing season. The supply of energy has been reported to be the first limiting factor for the production of dairy cows fed on grazed grass (Kolver and Muller, 1998). Energy intake from grass is a combination of the amount and quality of the ingested forage. The energy content of a well-managed pasture sward is high (Table 3), which enables high energy intake and subsequently high milk production.

It has been debated whether milk production is driven by intake or intake is driven by milk production. According to the energy-intake regulation theory, cows are supposed to consume enough feed to meet their energy needs (NRC, 2001). A cow's genetic merit defines the individual production potential and, furthermore, the maximum amount of feed consumed. A physical feed-intake regulation limits the maximum intake when low digestibility feed is offered, whereas metabolic-intake regulation limits intake with high-digestibility diets. In addition, there are several factors which can limit DMI on pasture swards and which can prevent the cow from reaching its milk production potential. The most important herbage-based factors that limit intake are herbage mass, and height and density of the grass (Mayne and Peyraud, 1996).

Table 3 presents the average chemical composition of herbage of pasture swards, the range in NDF concentration being 486–582 g NDF/kg DM during the data collection periods in this study. High-quality herbage contains 500–550 g NDF/kg DM (Virkajärvi, 2004). The value of 580 g/kg DM in experiment 1 represents an advanced maturity stage, which is not recommendable for grazing. Silage is harvested 2–3 times during the growing season whereas herbage on a pasture sward is typically grazed 5 times under Finnish conditions. Long intervals of growing time between harvests increase the grass NDF content and decrease the grass energy value when calculated as an average over the growing season. Therefore, the milk production potential of typical grass silage should be lower compared to that of grazed grass.

Grazing rotation and the maturity stage of grass were kept near to the recommended optima (Virkajärvi, 2004) during the data collection periods, which decreased the variability (Table 3) in grass quality compared to the conditions that can commonly occur on practical dairy farms, or during whole experiments. The herbage mass varied between 1000–4000 kg DM/ha between the data collection periods in this study which describes the real grazing situation. The reason for the variation is that the growth rate of grass varies greatly during the summer (Virkajärvi, 2004) and it is difficult to maintain an optimal grazing rotation that allows the right amount and quality of grass for every day of the grazing period.

The CP content (Table 3) of grass was very high, compared to the values presented in Feed Tables (MTT, 2013). This is a consequence of the early growth stage of the grass (Rinne, 2000) and ample nitrogen fertilization. The amount of nitrogen fertilization was unnecessarily high taking into account both N recycling via urine and

Table 3. Chemical composition and amount of herbage used in Experiments 1-9 (I-IV1).

	NDF	CP	ME, MJ/kg DM	Herbage mass,	Herbage allowance,
Exp.	g/kg DM			kg DM/ha	kg DM/d/cow
1	582	209	10.9	2600	ad libitum
2	509	234	11.5	-	ad libitum
3	527	231	11.6	2400	21
4	486	206	11.3	2700	25
5	531	189	11.2	3100	25
6	519	171	11.2	2900	ad libitum
7	487	229	11.7	2600	13
8	499	214	11.4	-	ad libitum
9	568	212	10.7	2300	ad libitum
Mean	518	210	11.4	2657	
SD	31.1	21.9	0.30	276.0	

For experiments 6 and 9, unpublished data, see appendices 1-2. DM = dry matter, NDF = neutral detergent fibre, CP = crude protein, ME = metabolizable energy, SD = standard deviation

faeces (Saarijärvi and Virkajärvi, 2009) and lower herbage yield from the pasture sward compared to that of grass mown for silage. The pastures used in this study were fertilized according to recommendations valid at the time of experimentation; this, together with circulated N via faeces and urine resulted in unnecessarily high CP content in forage. The current recommended values for N fertilization have been reduced since that time.

High sward density and a high proportion of leaf material in the sward allow grazing livestock to maximize their grass intake rate (Chacon and Stobbs, 1976; Peyraud et al., 1996). In Finland, typical pasture swards differ markedly from the perennial ryegrass pastures that occur in more southern latitudes. The bulk density of perennial ryegrass varies typically between 1.7 and 5.5 kg DM/m^3 whereas timothy-dominated Finnish pasture swards have bulk density values of between 0.68 and 0.92 kg DM/m^3 (Virkajärvi, 2006). Finnish pastures also usually have a low proportion of leaves, as stem formation commonly increases in mid-summer (Virkajärvi, 2006). The low bulk density of timothy-meadow fescue swards is compensated by an increased sward height, which thus allows an increased herbage mass per unit of area. There is no direct comparison between timothy-meadow fescue and perennial ryegrass pastures, but Virkajärvi (2004) concluded that the Nordic-type of sward structure does not limit total intake, compared to that of perennial ryegrass swards.

When herbage mass per hectare is low, cows must work more to maximize their intake, and this decreases the forage availability in practice, despite the amount of total grass available being ample. Peyraud et al. (1996) concluded that when herbage mass, as measured to 5 cm stubble height, is below 2400 kg DM/ha, the DM intake declines progressively, and below 1500 kg DM/ha the intake is markedly reduced. On the other hand, increasing the herbage mass above 3300 kg DM/ha does not result in increased intake. As shown in Table 3, the herbage mass in this study was within acceptable limits.

Herbage allowance is one of the most important management factors affecting herbage intake (Mayne and Peyraud, 1996; Spörndly, 1996). Intakes were reported to increase linearly when HA increased from

15 to 40 kg DM/cow (Wales et al., 1998). However, Virkajärvi et al. (2002) reported poor utilization of offered herbage above 23 kg DM/cow despite the fact that the cows were not fed concentrates. Concentrate supplementation decreases the need for HA due to substitution of herbage DM with concentrate DM so the HA presented in Table 3 can be considered to be adequate in the experiments that comprised this study.

Intake measurements

It is a challenging task to measure grass intake on pasture. The sward-cutting method, which involves herbage mass difference before and after grazing (Mejs et al., 1982), is widely used but it is laborious (Virkajärvi et al., 2002) and it needs a large part of the offered herbage to be consumed (Smit et al., 2005). The sward-cutting method is also not a suitable method for physiological studies where it is essential to measure individual intake. Experiments 3 and 4 (III) included fixed HA for each concentrate-treatment group and consequently included grass-intake measurement with sward cutting or with rising plate (Stockdale, 1984) method at the group level.

The use of indigestible faecal markers, as used in experiment 9, would allow an individual intake estimate for physiological experiments. Possible problems in using markers include the recovery of markers (it may be incomplete), diurnal variation in faecal marker concentration, and analytical challenges, all of which decrease the reliability of the marker method in physiological studies conducted on pasture (Lippke, 2002). For example, slight overestimation in the amount of faeces and slight underestimation in digestibility can together lead to marked overestimation of DMI. Experiment 9 included an intake measurement with chromium oxide (the amount of faeces) and iNDF (diet digestibility) as markers and the average estimate of DMI was reasonable. The average DM digestibility was 756 g/kg DM (SEM 6.6 g/kg DM) and the average intake was 15.1 kg DM (SEM 0.88 kg DM) which seems reasonable for cows yielding 19.4 kg ECM on a diet that consisted solely of grazed herbage at pasture. Only the last period in experiment 9 showed unrealistic high DM intake (18.0 kg) taking account of the milk production.

Experiment 9 also included an n-alkane technique for measuring grass intake. The method has been used successfully (Smit et al., 2005) but the results were not reliable in our study. The reason for this can be found in low odd n-alkanes content in Nordic mixed grasses (Sormunen-Cristian et al., 2005)

One example of an intake evaluation model is the computer-based program Grazemore (Delagarde et al., 2004), which has been validated to give good precision in grass-intake predictions (Chaves et al., 2004). The intake prediction model presented by Fuentes-Pila et al. (2003) has also been revised to give reasonably accurate estimation of DMI for dairy cows in confinement (Rim et al., 2008). The computer-based programs were not available for this study but the model presented by Fuentes-Pila et al. (2003) was included for further consideration. The model includes LW, milk yield, milk fat and protein yields, and the month of lactation as animal factors. The dietary factors considered were NDF, ADF, CP and hemicellulose contents of the diet.

In the current study, the Fuentes-Pila et al. (2003) model underestimated the measured grass intake in the tie stall by only 3.8 % in experiment 2 (II) when directly measured grass intake was used as a reference (Table 4). The difference between estimated intake by Fuentes-Pila et al. (2003) and measured grass intake on pasture was markedly larger, showing 29 % greater grass intake, according to the model estimation compared to the sward-cutting method in experiment 4 (III) (Table

4). The difference was even greater when the indirect herbage mass measurement by Stockdale (1984) was used in experiment 3 (III).

The nutritive value of fresh grass offered in the barn (experiment 2, II) was comparable to that of the grass on the pasture (experiment 3, III). This was because the experiments were conducted at the same time and the barn-fed grass came from the same field as used in experiment 3, and the cows were also comparable in terms of milk yield and stage of lactation. Thus, the difference between modelled intake in the barn and that on pasture originates from the grazing situation and from individual differences among the experimental cows.

The indirect herbage-intake measurement method of Stockdale (1984) contains uncertainty. However, if indirect measurement methods have no severe bias, the model of Fuentes-Pila et al. (2003) overestimates DM intake on pasture. This suggests that intake on pasture is lower compared to confinement, despite the grass herbage being of the same quality. The hypothesis of lowered intake on pasture is supported by results from Law and Ferris (2011). Milk production statistics from Finland (Tiina Sirkjärvi, Valio Ltd, personal communication) also show that the amount of milk produced during the summer is lower, compared to the production potential of Finnish herds, which may indicate problems either with pastures or silage-feeding during the summer.

The substitution rate of grass by concentrates was lower when using sward-cutting methods compared to that of the estimated grass DMI by the model of Fuentes-Pila et al. (2003) or nutrient requirements in experiments 3 and 4 (Table 4). Substitution rate over 0.6 kg/kg (DM basis) is possible when the energy content and availability of forage is high and/or high amounts of concentrate are used (Huhtanen et al., 2008; Kuoppala et al., 2008). The reason for a high substitution rate based on feed recommendations is the nonlinear effect of increasing energy content of the diet on milk production. This leads to underestimation of grass intake with increasing amount of concentrate supplementation when the quadratic energy intake correction is not used (MTT 2013).

Restricted forage allowance decreases a cow's energy balance and the substitution rate remains low (Peyraud and Delaby, 2001). Average herbage mass was 2400 kg DM/ha and HA also was quite low (21 kg DM/cow) in experiment 3, which explains both low intakes and low substitution rate at pasture. Marginal response to supplementation was higher in experi-

Table 4. Measured and estimated grass dry matter intake (DMI) for experiments 2 (II), 3 and 4 (III) and substitution rate (decrease in grass DMI per increase in concentrate DMI).

	Experiment 2			Experiment 3			Experiment 4		
Concentrate kg/day	a	b	c	a	c	d	a	c	e
0	16.4	17.2	15.0	19.2	15.6	12.8			
3	15.8	16.5	13.4	18.4	13.6	11.7	18.0	14.6	14.2
6	14.3	14.6	12.7	17.2	12.4	11.3	16.0	12.2	11.4
9				15.4	11.1	9.2	14.5	10.7	11.8
Substitution kg/kg	0.40	0.50	0.44	0.49	0.58	0.46	0.67	0.75	0.46

a, Modelled according to Fuentes-Pila et al. (2003)
b, Measured intake in the stall, experiment 2 (II)
c, According to nutrient requirements (MTT 2013). Live weight change has been taken into account
d, Raising plate measurement, Stockdale (1984)
e, Sward cutting method, experiment 4 (III)

ment 3, compared to experiments 2 and 4, which supports the low substitution rate and consequently limited amount of grass DMI in experiment 3.

Experiment 3 (III) included relatively low HA combined with, on average, a concentrate supplementation of 4.5 kg. This was not a sustainable situation because the cows were not able to eat enough grass and they lost LW (Table 5) despite the fact that some grass was left after grazing. The availability of grass decreases all the time after turn out to pasture when grazing proceeds, and HA probably limits intake during the last part of the day. The utilization of herbage on offer decreases strongly above a HA of 20 kg DM/d, but from the nutritional point of view a HA in the range of 20–25 kg DM/d is still relatively low, at least when used with a low amount of concentrate supplementation. According to Peyraud et al. (1996), the herbage intake increased by 0.25 kg DM/kg DM offered between 19 and 29 kg DM allowance. Thus, the intake would increase more than 2 kg DM/d if the HA increases from 20 to 30 kg DM/d. Above this allowance level, the increase in intake is negligible and utilization is poor (Peyraud et al., 1996). To optimize overall resource utilization it would be more sensible to ensure adequate nutrient intake by using concentrate feeds rather than to use a very high HA.

3.2 Ruminal digestive processes under grazing conditions

Rumen dry matter pool size

The rumen DM pool size provides useful information in order to study rumen fill as a limiting factor for intake. Rumen physical size sets the upper limit for DMI of high-producing cows or cows fed high-forage diets (Allen, 2000; Boudon et al., 2009). The importance of this physical regulation becomes increasingly dominant when milk yield, and consequently nutrient intake-demand, increases (Linton and Allen, 2008).

Pasture-based diets contain high proportions of forage and therefore rumen fill could become a limiting factor for intake under grazing. However, many studies have shown that rumen fill is not a limiting factor for intake when highly digestible forage is used (Bosch et al., 1992; Chilibroste et al., 1997; Rinne et al., 2002; Huhtanen et al., 2007).

This study included rumen pool size measurements in experiments 2 (II) and 9. The rumen DM pool size was on average 2.0 kg/100 kg LW in experiment 2, which was comparable to the average rumen DM pool size of silage diets (Table 6). The effect of forage type (fresh vs ensiled grass) on rumen DM pool was tested with re-

Table 5. Live weight changes (kg/d) of cows fed cut grass or grazed at pasture with different levels of concentrate (II, III and experiment 6 (see appendix 1)).

Concentrate, kg	0	3	6	9	12
Experiment 2	-0.25	-0.36	0.33		
Experiment 3	-0.49	-0.54	-0.31	0.01	
Experiment 4		0.18	0.21	0.31	
Experiment 5			0.24	0.15	0.47
Experiment 6			0.13	0.55	1.13

gression analysis but it was not significant (P=0.54). There also was no significant interaction between forage type and any independent variable. A remarkably low rumen DM pool size was measured in experiment 9, where it was 1.3 kg/100 kg LW at the beginning of the summer despite a higher average NDF content of grass compared with that of experiment 2 (556 vs 509 g/kg DM). This is in contradiction with the increasing grass silage NDF content, which has been reported to increase rumen pool size (Rinne, 2000). The DM pool size increased during the summer, being 1.8 kg DM/100 kg LW at the end of experiment 9. Milk yield (average 19.9 kg) and consequently energy requirement was remarkably lower in experiment 9 than in experiment 2 (average 27.7 kg). If the nutrient requirement is low, there is no need to use full rumen capacity, which partly explains the low rumen fill in experiment 9.

According to the regression analysis, the DMI and concentrate DMI increased rumen DM pool significantly (42–47 g/kg/100 kg LW) when included alone into the model (Table 7). The significance of intake disappeared when forage iNDF was included into the model. However, the R^2 for iNDF increased when concentrate DMI was also included as independent variable.

Table 6. Rumen pool size of dry matter (DM), neutral detergent fibre (NDF), potentially digestible NDF (pdNDF), the digestion rate (k_d) pdNDF and the passage rate (k_p) of indigestible NDF (iNDF) with different forage types. The values are averages within the experiments.

		kg/100 kg live weight				k_d of	k_p of
		DM	NDF	pdNDF	iNDF	pdNDF	iNDF
Bosch et al. (1992)	Fresh cut grass	1.90	1.12	-	-	-	-
Virkajärvi et al. (2002)	Grazed grass	1.86	1.00	0.68	0.32	6.8	1.8
Experiment 2 (II)	Fresh cut grass	2.01	0.97	0.60	0.37	8.9	1.8
Experiment 9 [1]	Grazed grass	1.54	0.80	0.56	0.24	10.6	2.7
	Mean	**1.83**	**0.97**	**0.61**	**0.31**	**8.8**	**2.1**
Rinne et al. (2002)	Grass silage	1.91	1.17	0.81	0.36	5.6	2.5
Kuoppala et al. (2009)	Grass silage	2.12	1.19	0.90	0.29	3.4	2.3
Kuoppala et al. (2010)	Grass silage	1.95	1.17	0.83	0.34	3.8	2.6
	Mean	**1.99**	**1.18**	**0.85**	**0.33**	**4.3**	**2.5**

[1]appendix 2

Table 7. Mixed model regressions for predicting rumen dry matter pool size (kg DM/100 kg live weight, dependent variable) based on the dry matter intake and forage fibre composition.

X1 [1]	X2 [1]	Intercept	SEM [2]	$Slope_1$	SEM	*P*	$Slope_2$	SEM	P	R^2, [3]	RMSE [4]
Rumen DM pool size:											
CDMI [5]		1.73	0.068	0.042	0.0123	0.003				0.49	0.13
DMI [6]		1.07	0.291	0.047	0.0162	0.027				0.42	0.13
NDF [7]		1.37	0.314	0.009	0.0056	0.126				0.09	0.14
iNDF [8]		1.68	0.108	0.036	0.0125	0.024				0.43	0.09
CDMI	iNDF	1.63	0.121	0.014	0.0178	0.448	0.034	0.0131	0.039	0.56	0.09
DMI	iNDF	1.25	0.429	0.023	0.0223	0.350	0.039	0.0132	0.021	0.53	0.09
NDF	iNDF	2.6	0.405	-0.021	0.0089	0.056	0.071	0.0191	0.009	0.66	0.08

[1] Independent variable,[2] Standard error of mean,[3] Coefficient of determination,[4] Root-mean-square error, adjusted for random study effect,[5] Concentrate DM intake, kg/d,[6] Total DM intake, kg/d, [7] Forage neutral detergent fibre content, 10 g/kg DM, [8] Forage indigestible NDF content, 10 g/kg DM

Forage iNDF content alone increased rumen DM pool by 36 g/10 g iNDF/100 kg LW. The average forage iNDF content was higher with ensiled grass compared to fresh cut grass (Table 8), which should lead to pronounced rumen fill when silage was used. However, silage-based diets had 4.5 kg DM higher concentrate supplementation compared to fresh grass diets, which decreased the iNDF content of the total diet.

The increase in grass NDF content had no effect on rumen DM pool size. This is a little confusing because forage NDF and iNDF are positively correlated. With a fresh grass diet the effect of forage NDF content to rumen DM pool size was near to zero, which partly explains the result. Both iNDF and NDF content of the grass increase with advancing grass maturity, but there was a superior effect of iNDF in explaining rumen DM pool size compared to the effect of grass NDF content. This can be seen also in the experiment 9, where low iNDF content of grass (29 g/kg DM) was linked with low rumen DM pool at the beginning of the summer.

The results of this study support the previous findings where rumen fill is not a limiting factor for intake. The iNDF content of the herbage of the pasture sward is low, which enables high DMI despite a low amount of concentrate supplementation.

Rumen NDF pool size and fibre digestion

Forage intrinsic cell-wall properties affect forage intake and digestibility of nutrients. The digestion rate of cell solubles (organic matter–NDF) is high and the digestion is almost complete (Nousiainen et al., 2009). The digestibility of the cell wall fraction is more variable compared to that of the cell soluble fraction and it differs according to plant species, maturity and average temperature during the season. Both the time after harvest and the effect of high ambient temperature decrease grass cell-wall digestibility (Thorvaldsson et al., 2007). The time between harvests is relatively short on pasture swards, and therefore grass digestibility remains high if the grazing rotation intervals are maintained at a fast-enough level.

This study does not contain different grass maturity stages because the herbage of the grazed sward should remain within relatively narrow limits of digestibility. In general, the postponed growth stage decreases the digestion rate of pdNDF (Bosch et al., 1992; Rinne et al., 2002; Kuoppala et al., 2010). This is also shown in Table 6, where the k_d of pdNDF is markedly higher with early-maturity grass diets than with silage diets, resulting in lower rumen NDF pool size with fresh-grass diets compared to silage diets. The clearly higher k_d of pdNDF on grazed-grass diets than on silage diets would allow high DM intake potential at pasture.

Concentrate supplementation tended to decrease the k_d of pdNDF in experiment 2 (II). This agrees with the results presented by Cajarville et al. (2006) where 6 kg maize-wheat grain mixture decreased k_d for NDF compared with sole pasture diet. The decreased k_d of pdNDF occurred at the highest (6 kg/d) concentrate level. Unfortunately, the experiment did not include higher amounts of concentrate such as up to10 kg/d, which are widely used in Finnish farms during the grazing season.

The k_p of iNDF was numerically lowest in experiment 2 (II) and also in that of Virkajärvi et al. (2002) as shown in Table 6. According to Krizsan et al. (2010), the k_p of iNDF was lower for fresh cut grass than for silages made of grass or mixtures of lucerne and maize. Passage rate increases with increasing NDF intake, proportion of concentrate NDF of total NDF, and diet iNDF to NDF ratio (Krizsan et al., 2010). The k_p of iNDF increased slightly with concentrate supplementation in experiment 2, which is in agreement with this. The high k_p of iNDF in experiment 9 may be a consequence of difficulties in DMI measurement.

There was no marked difference in NDF pool size between fresh-cut grass and ensiled grass diets. High iNDF content of the ensiled grass (Rinne et al., 2002) increased the pool size but high k_p of iNDF decreased the NDF pool size. These opposite effects partly counterbalanced each other.

Rinne et al. (2002) suggested that the accumulation of iNDF in the rumen is one possible factor that can limit intake. The content of iNDF is smaller in concentrates compared to pasture grass (experiment 2, II) so that supplementation decreases iNDF intake and consequently iNDF pool size, as seen also in experiment 2. Early-maturity grass also contains low content of iNDF, so it can be assumed that iNDF pool size is not a limiting factor with supplemented high digestibility grazed-grass diets.

Rumen fermentation

Table 8 contains a rumen fermentation summary comparing fresh grass and silage diets. The hypothesis is that grass-maturity stage explains differences between silage and pasture diets. Forage iNDF concentration provides a good estimate of the maturity stage (Rinne et al., 2002). Forage NDF content also increases with advancing maturity but its structure is not uniform (Nousiainen et al., 2003).

The small but significant apparent differences between forage types in volatile fatty acid (VFA) patterns were higher proportion of acetate and lower proportion of butyrate in fresh-grass compared to ensiled-grass diets (Table 8). Cushnahan et al. (1995) also reported higher proportion of acetate for fresh grass or restrictively fermented grass compared with extensively fermented grass. According to experiments 1 (I) and 2 (II), the concentrate supplementation increased slightly the proportion of butyrate and decreased the proportion of acetate, so the differences in concentrate supplementation between the diets probably explain, at least partly, the differences between forage types. Increasing forage maturity, in terms of forage iNDF content, increased rumen acetate and decreased rumen butyrate proportion similarly as in the analysis reported by Rinne (2000).

Water soluble carbohydrates, as sugar in fresh grass, has usually increased both butyrate and propionate and decreased that of acetate (Khalili, 1992). The high sugar content of fresh grass (MTT 2013) did not produce butyrate in the current study (Table 8). An explanation for this could be found from continuous intake of grass sugar which does not decrease rumen pH rapidly. High levels of molasses (Khalili, 1992) or sucrose given twice daily (Syrjälä, 1972; Khalili and Huhtanen, 1991) clearly increased the molar proportion of butyrate whereas continuous infusion of sucrose or a moderate amount of molasses did not have this effect (Khalili and Huhtanen, 1991).

Pasture herbage as a sole-diet is a good substrate for microbial growth, which can be seen as a high VFA concentration in rumen fluid, the high k_d of pdNDF and relatively low rumen pdNDF pool size when fresh-grass diets were fed to cows (Table 6). The high VFA concentration with fresh-grass diet in Table 8 agrees with the results presented by Holden et al. (1994), who reported increased rumen VFA concentration from 118 mmol/l to 132 mmol/l when early-heading stage orchard grass (Dactylis glomerata) silage was replaced by early-growth stage orchard grass grazed at pasture.

The rumen ammonia concentration was unnecessarily high with the fresh-grass diets compared to silage diets. This originated from high CP content of early growth-stage grass. The high rumen-ammonia concentration, together with high rumen

Table 8. Rumen fermentation characteristics for grass and silage.

	In forage, g/kg DM		ECM	Concentrate	VFA		mmol/mol			mmol/l
	CP	iNDF	kg/d	kg DM/d	mM/l	pH	Acet.	Prop.	But.	NH3N
Cut grass and grazed grass diets										
Experiment 1 (I)	209	-	19.3	2.3	129	6.10	647	195	117	16
Experiment 2 (II)	237	49	26.0	2.6	129	6.29	675	180	106	18
Experiment 9	212	51	19.5	0	121	6.34	644	193	132	18
Virkajärvi et al. (2002)[1]	214	49	22.5	0	152	6.22	653	191	115	14
Mean	219	50	21.6	1.6	126	6.24	655	189	118	17
Silage diets										
Kuoppala et al. (2010)	139	75	24.3	7.1	104	6.54	649	194	124	8
Rinne et al. (2002)	141	77	20.5	6.2	128	6.10	647	159	146	11
Vanhatalo et al. (2009)	123	67	26.1	7.7	105	6.43	641	181	139	3
Mean	132	72	23.3	7.0	117	6.27	644	170	143	7
The significance between the forage types, P					0.21	0.84	0.047	0.512	0.041	0.013
The significance for forage iNDF content, P for slope					0.04	0.002	0.006	0.302	0.007	0.012
Slope [2]					-2.1	0.04	4.65	-1.04	-2.61	-0.67

[1] The publication includes the description of the experiment but no rumen fermentation data
[2] Slope for increasing forage iNDF content as 10 g /kg DM.
CP = crude protein, DM = dry matter, iNDF = indigestible neutral detergent fibre, ECM= energy corrected milk yield, VFA = volatile fatty acids, Acet. = acetic acid, Prop. = propionic acid, But. = butyric acid

VFA concentration, could lead to metabolic feedback which limits intake as discussed in experiment 2 (II). It would be possible to produce highly digestible grass with low CP content if N fertilization is maintained at a low level.

Fresh-grass and ensiled-grass diets did not differ in rumen pH whereas increasing iNDF concentration in forage increased rumen pH (Table 8). It is widely reported that concentrate supplementation decreases rumen pH, so the reason for the absence of difference between forage types in pH may be related to smaller amount of concentrate supplementation with fresh-grass diets compared to silage diets. The effects of grass maturity and the amount of concentrate counterbalanced each other. High ammonia concentration with the fresh-grass diets also buffers rumen pH.

The low content of iNDF in early growth-stage grass (Table 8, Rinne (2000)), and the use of high amount of non-fibre carbohydrates in the form of supplemental concentrate feeds, decreases rumen pH, which can lead to accumulation of lactic acid in the rumen if pH decreases below 5.5 (Allen, 1997). Accumulation of lactic acid causes acidosis (Clark et al., 1992; Allen, 1997).

A practical problem with full-time grazed cows is that the daily amount of concentrate has to be distributed in only two portions, which are provided during the two daily milking periods. This can result in a rapid decrease in rumen pH. According to experiments 3-6 it is possible to use as high as 12 kg concentrate mixture/d when it is divided into four daily portions. However, in 2 (II) we did not have data from situations in which high amounts of concentrates are given only twice daily, which is the typical practice for full-time grazing cows milked in parlours.

Increasing the amount of cereal-based concentrate has variable effects on rumen fermentation. In general, concentrate supplementation decreases both rumen pH and the proportion of acetic acid, whereas it increases the proportion of butyric acid in VFA and total VFA concentration, and maintains or increases the proportion of propionate (I, II, Kolver and Veth, 2002; Bargo et al., 2003). Increasing grass digestibility (i.e. decreasing iNDF content, Table 7) changed the rumen fermentation pattern similar to the change caused by increasing the amount of concentrates. From a nutritional point of view, early growth-stage grass affects the rumen physiology in the same way as concentrate supplementation. Forage type (ensiled or fresh) itself has only minor effects on rumen fermentation pattern. This finding is also supported by Cushnahan et al. (1995).

Microbial protein synthesis and nitrogen utilization

Milk production is limited by the supply of energy and protein (Clark et al., 1992). Amino acids absorbed from the small intestine originate from microbial protein synthesis (MPS) in the rumen and from the rumen-undegradable fraction of the dietary protein. Based mainly on Finnish data, Broderick et al. (2010) reported that, on average, 32 % of dietary CP passed from the rumen and 68 % was degraded in the rumen. It is essential to maximize MPS because of the high proportion of degraded protein. However, the undegraded part of true protein also has importance in milk production. Fresh grass contains only 10 %–20 % non-protein nitrogen (Hatfield et al., 2007) whereas 90 % of proteins are broken down into smaller fractions during ensiling (Messman et al., 1994). Thus, fresh grass contains higher amount of bypass protein available for milk production, compared with than in grass silage.

The efficiency of MPS (g microbial N/kg digestible organic matter (DOM) intake) for silage-fed cows may be lower than that of fresh-grass-fed cows (Thomas and Thomas, 1985). Extensive fermentation of silage and poor fermentation quality have been stated as reasons for this (Huhtanen, 1998). The average MPS efficiency in experiment 2 (II) was 27.4 g N/kg DOM intake, showing higher efficiency compared to the average value of 20.1 g N/kg DOM intake with grass silage reported by Ahvenjärvi (2002), or the average efficiency of 24.0 g N/kg DOM intake as reported by Huhtanen (1998).

Increasing silage digestibility has tended to increase MPS (Rinne et al., 2002). Thus, high grass digestibility partly explains the high MPS reported in experiment 2 (II). The intake of DOM is the best predictor for daily microbial protein production (Huhtanen and Nousiainen, 2012). The amount of MPS can be calculated as 152 g MPS/(DOM- RUP) where RUP is the amount (kg) of rumen-undegradable protein (MTT 2013). According to this, the MPS in the rumen should have been 290–322 g N/d but the measured values were 359–384 g N/d in experiment 2 (II). This also supports high MPS when grazed-grass diets are used.

Grass of pasture swards has high concentrations of CP (173–234 g/kg DM, Table 3) and consequently a high amount of rumen-degradable protein (RDP), which results in high RDP to organic matter ratio in a typical grazed-grass diet. This leads to high ammonia concentration in the rumen and low utilization of N (experiment 2, II). Protein supplementation would increase this ratio even more. Ammonia itself is a poisonous compound and the liver metabolizes it to urea. This detoxification process reduces the energy available for milk production (Oetzel, 2007). An unnecessarily high grass CP content (234 g/kg DM) was reported in experiment 2, where the milk-urea concentration was as high as 50 mg/100 ml with sole-grass diet. High surplus of CP can also cause problems in fertility (McCormick et al., 1999) and it may be one possible explanation for limited intake (Choung et al., 1990). In addition, unnecessary high use of N, both as fertilization and in feed supplementation, increases the environmental load.

In practice, it is possible to decrease rumen-ammonia concentration by using low-CP concentrate, which decreases the rumen-RDP to organic matter ratio. Concentrate supplementation increases total intake and microbial-N capture. Colmenero and Broderick (2006) concluded that with lucerne and maize silage diets it is unnecessary to increase the dietary CP concentration above 168 g/kg DM, and that CP contents above this level lead to sharp declines in the efficiency of N utilization without any increase in milk or milk protein yield. It is possible to reduce grass CP content by reducing nitrogen fertilization, though usually at the expense of herbage yield.

The grass CP content and HA that occur in organic farming in Finland have been reported to be at lower levels than the values represented by the results in the present study (Kuusela, 2004). The study of Kuusela (2004) reported relatively high milk response to protein supplementation (1.1 kg/kg rapeseed meal DM). This can be explained by a low amount of concentrate supplementation and HA. In these circumstances MPS was probably insufficient in terms of meeting the cows' requirements. A low protein supplementation response with the grazed-grass diet in experiment 8 (IV) supports the observation of high protein value and availability in conventional pasture. Thus, it seems that protein supplementation of cows grazing on pasture should be limited to a lower level than that of cows on silage diets.

From a nutritional point of view, a grazed pasture sward can provide an ideal diet for a dairy cow except for its unnecessarily high herbage-CP content. Well-managed grass has no preservation problems, rumen distension does not limit intake, fibre digestion rate is high, MPS is high and rumen fermentation stays at a safe level if the amount of concentrates is not above the amounts used in this study. However, DMI is a limiting factor for milk production, as discussed in chapter 3.1. It is impossible to quantify the significance of possible imbalance of nutrient intake and metabolic feedback. However, the most likely reason for limited intake at pasture has to be determined from an examination of grazing-management factors such as herbage mass per hectare, allowance per cow, or environmental conditions such as weather.

3.3 Milk production responses to increasing the amount of concentrate in the diet

Figure 1 describes the MR in experiments 1 (I), 3, 4, 5 (III) and 6. The cows within these studies grazed both day and night, and the amount of concentrate varied between 0 and 12 kg/d. The coefficients used in the model are described in Table 9. Concentrate supplementation increased ECM yield up to a level of 8 kg concentrate DM/d with an average MR of 0.68 kg/kg DM, which is a little less than 1.03 kg milk/kg concentrate DM for high yielding cows reported in the review of Bargo et al. (2003). At concentrate-supplementation levels above 8 kg DM the MR decreased, being 0.27 kg/kg. In the more recent study reported by Randby et al. (2012) there was an average MR of 0.99 kg ECM/kg concentrate when silage D-value was above 667 g/kg DM and the amount of concentrate varied between 0 and 8 kg/day. Above 8 kg concentrate supplementation these authors found no response except in the case of low-digestibility silage with a D-value of 601 g/kg DM.

Relatively weak quadratic effect of concentrate supplementation on ECM yield in the current study, as compared to that of Randby et al (2012), was unexpected. An explanation for this could be found in

Table 9. Mixed model regression relationship between concentrate supplementation (linear and quadratic effects, independent variables) and ECM 1, milk protein, milk fat and milk urea contents (dependent variables) in experiments 1 (I) , 3, 4, 5 (III) and 6 (see appendix 1).

			linear			quadratic			R^2 [2]	RMSE[3]
	int.	SEM[4]	X1[5]	SEM	P	X2[6]	SEM	P		
ECM	22.4	1.20	0.95	0.187	0.004	-0.04	0.015	0.066	0.96	0.39
Milk	22.6	1.29	1.06	0.160	0.001	-0.03	0.012	0.054	0.99	0.27
Milk protein	33	0.4	0.15	0.061	0.057	-0.01	0.005	0.337	0.92	0.09
Milk fat	41	0.6	-0.71	0.137	0.004	0.02	0.012	0.136	0.90	0.51
Milk urea	39	1.9	-0.6	0.37	0.173	-0.03	0.026	0.29	0.98	0.42
ECM, Huhtanen, unpubl.[7]	16.3	1.40	2.24	0.301	<0.001	-0.01	0.020	0.004		

[1] Energy corrected milk
[2] Coefficient of determination
[3] Root-mean-square error
[4] Standard error of mean
[5] Linear coefficient for concentrate intake, kg dry matter
[6] Quadratic coefficient for concentrate intake, kg dry matter
[7] Silage based diets. The experiments included in the equation are described in Huhtanen and Nousiainen (2012)

experiment 6 where the MR was exceptionally weak and linear up to 12 kg of concentrate supplementation. There were only two experiments including the highest amount of concentrate so the results of experiment 6 greatly affect the results. Therefore, the dataset used in the current study may be inadequate to draw reliable conclusions about quadratic MR.

Figure 1 also includes milk-response function based on 87 silage-feeding experiments, which is derived from the study published by Huhtanen and Nousiainen (2012). The MR with silage diets is higher when concentrate supplementation was below 8 kg DM, and it was lower above 8 kg DM, compared to the MR with grazed-grass diets in the current study. These results are logical because the MR correlates negatively with forage digestibility (Kuoppala et al., 2008; Randby et al., 2012) and the silage-diet equation also includes low-digestibility forages. Grazing cows can compensate for low levels of concentrate supplementation with high-digestibility grass, and thereby maintain milk yield because rumen fill does not limit intake, as discussed earlier. When the silage reference data were limited to those diets using silage with a D-value above 680 g/kg DM the linear MR between 0-12 kg concentrate DM was 0.55 kg/kg DM, whereas the average linear MR in the present study was 0.57 kg/kg DM. The data for grazed grass used here were so limited that quantitative quadratic MR comparisons between grazed-grass and silage diets cannot be made with any confidence. However, the average differences in MR between silage diets and the present grazed-grass diets were so small that there is no evidence to reject the hypothesis of equal MR between grazed-grass and silage diets at comparable forage digestibility when the amount of concentrate supplementation is between 6-12 kg, as typically used in practice in Finland.

Experiments 5 (III) and 6 included high amounts of concentrates (up to 12 kg/d). The milk fat decrease was quadratic in experiment 5 but linear in experiment 6. When all experiments were combined, both milk fat and urea contents decreased linearly with concentrate supplementation (Figure 2). Milk protein content increased linearly at the same time. According to the responses shown in Figure 2 the highest amount of concentrate did not cause milk-fat depression, which suggests that cows did not suffer from rumen acidosis. On the other hand, body fat mobilization could have maintained milk fat

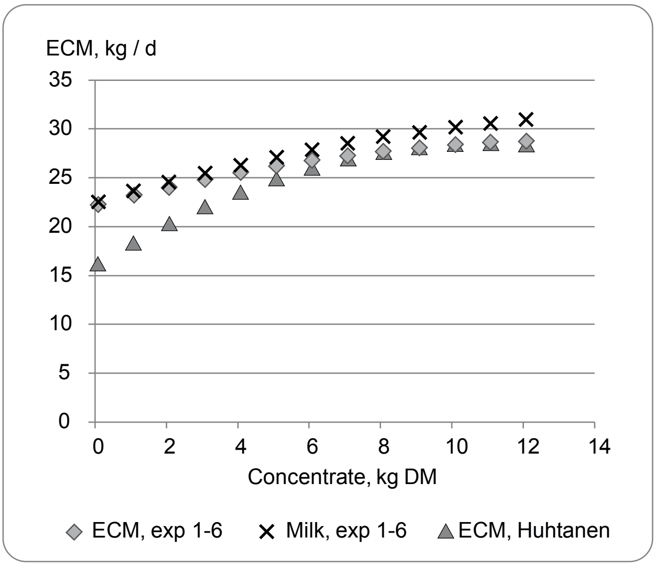

Figure 1. Energy corrected milk yield (ECM) response to concentrate supplementation. Regression analysis included pasture-feeding experiments 1 (I), 3, 4, 5 (III) and 6 (see appendix 1). Silage regression is derived from the study of Huhtanen and Nousiainen (2012) including 87 experiments.

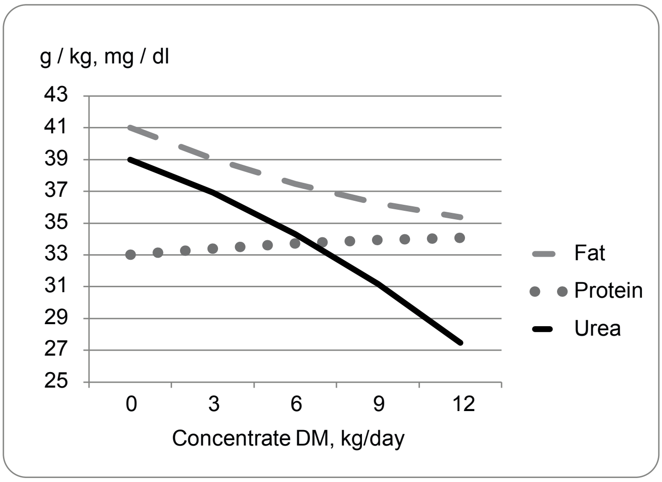

Figure 2. Milk fat, protein and urea contents with increasing amount of concentrate in experiments 1 (I), 3-5 (III), and 6 (see appendix 1).

content during feeding with the low concentrate levels

According to Heuer et al. (1999) the milk fat to protein ratio (FPR) describes a cow's energy balance. If FPR is above 1.5, it indicates abnormally high lipolysis and, consequently, a strongly negative energy balance. Mäntysaari and Mäntysaari (2010) reported FPR above 1.25 during eight weeks after calving when primiparous cows were in a negative energy balance. The highest FPR in this study was 1.2 with a zero-concentrate diet, which suggests that energy balance was not extremely low even with an unsupplemented diet. However, the interpretation of FPR can be different between pasture and silage diets. Buttchereit et al. (2010) concluded that the energy balance stabilizes at the same point as the decrease in FPR stops. In this study, the FPR decreased linearly with supplementation and it is not possible to determine the zero-energy balance based on FPR.

Concentrate supplementation responses at different milk production levels

It is a common practice in Finland to divide cows into different groups according to milk yield and to increase the amount of concentrate with increasing milk production level. This would be reasonable if high-yielding cows were to have higher MR compared to low-yielding cows.

The cows used in this study were divided into three different production-level groups according to the pre-experimental milk yield. The average ECM yield values during the experiments were 32.4 kg for high-yielding, 28.5 kg for medium-yielding and 23.2 kg for low-yielding cows. There was no significant MR × production level interaction between the high- and the low-yielding cows, excluding experiment 6 (Table 10), so that the response was the lowest in the low-yielding group. Numerically, the lowest MR also existed in experiments 1 and 4 within the low-production blocks where the production level was below 25 kg ECM and the cows were late in the lactation (on average 238 d). However, no significant production level × MR interaction was detected, an observation which agrees with the results reported by Stockdale et al. (1987) where initial milk yield before the experiment had no effect on MR. Several other authors have also reported negligible MR × initial milk yield interactions (Kristensen and Aaes, 1999; Delaby et al. 2001; Kennedy et al., 2003).

Pre-experimental blocking of cows leads to a confounded situation between a cow's genetic potential and days in milk. Stockdale et al. (1987) reported decreasing MR when lactation progressed. Coulon and Remond (1991) found the same phenomenon when they compared early lactating cows with mid-lactating cows during an indoor-feeding period. The results reported by Kirkland and Gordon (2001) also support this observation about the effect of lactation stage and these are also seen in Table 10. Kirkland and Gordon (2001) concluded that the stage of lactation had no effect on the net efficiency of metabolizable energy (ME) use, but cows in early lactation partition a greater proportion of their ME intake into milk production, and showed greater milk-yield response

Table 10. The energy corrected milk yields according to initial milk production before experiments. The milk production levels are: less than 28 kg for a low level, 28–35 kg for a moderate level and more than 35 kg for a high level.

		Concentrate, kg/day						P
exp.	production level	0	3	6	9	12	SEM	production level×concentrate[1]
1	low	18.7	19.8				0.66	-
3	low	21.4	24.7	25.7	27.8		1.31	0.45
3	moderate	26.1	28.4	30.2	32.1		1.12	
3	high	27.9	29.2	33.2	34.6		1.10	
4	low		21.6	24.2	24.6		0.83	0.20
4	moderate		26.0	25.6	28.6		1.25	
4	high		29.7	31.0	35.6		1.05	
5	moderate			28.4	31.1	31.1	0.80	0.63
5	high			32.3	34.7	34.2	0.83	
6	low			22.2	24.1	23.6	0.90	0.03
6	moderate			28.0	28.5	29.5	0.85	
6	high			32.3	32.4	33.7	0.93	

[1] production level×concentrate = the overall significance within the experiments for the interaction between initial milk yield and level of concentrate supplementation

to the change in ME intake than cows in mid or late lactation. Cows at the end of lactation are hormonally adjusting their energy to body reserves instead of milk production, so high levels of concentrate are unnecessary during late lactation (Stefanon et al., 2002). An exception to this is the need for weight gain if a cow's body condition is low.

High milk production promotes high DM intake. This leads to a situation where low-yielding cows have a higher proportion of concentrate than high-yielding cows, assuming that both groups have the same amount of concentrate. Milk yield response to concentrate supplementation depends on the energy content of the diet. Therefore, MR should be smaller for low-yielding cows if the same amount of concentrate is used for all cows. In the case of TMR-fed cows, the concentrate proportion of a diet is constant and this problem does not exist. The need for adjusting the amount of concentrate according to milk yield is probably limited to a level where the energy content of the diet would be equal throughout the main part of lactation. The effect of milk-yield level on MR is so small during most of the lactation that it is reasonable to use a flat-rate concentrate-feeding strategy for full-time grazed cows. This strategy is applicable at least below the production level used in this study, where 90 % of observations remained below 34 kg ECM/d. This study cannot give the answer to what the recommended decreasing rate of concentrates should be before drying off.

3.4 Part-time grazing as an alternative strategy to full-time or zero grazing

Seppälä et al. (2006) concluded that grazing at pasture is a more profitable strategy for feeding dairy cows than either part-time grazing or zero grazing. In addition, it is generally accepted that grazing improves cow welfare because 'free foraging' is part of cows' natural behaviour. There are, however, other considerations associated with grazing that have less-advantageous implications for animal welfare. One disadvantage of grazing is that pastures are usually open areas without shading. This potentially increases the risk of summertime heat stress or at least causes discomfort for the cows during the sunny daytime. Legrand et al. (2009) reported an average of 13.0 h/d residence time at pasture when cows had a free choice between pasture and free-stall. Cows spent time outside especially during nights. According to these authors the time that cows spent on the pasture during the day decreased clearly with the increasing humidity-temperature index. The index increases together with both humidity and temperature. Taking into account these factors, part-time grazing could be a recommendable strategy for large herds instead of totally giving up grazing (Chapinal et al., 2010).

Part-time grazing would be a suitable cow-management strategy for large herds because limited grazing time decreases the pasture area needed for the total herd and the distance between the paddocks and the barn remains reasonably short. Night-time grazing is a convenient way to avoid heat stress and it provides optimal circumstances for cows to rest. From the nutritional point of view the mixed diet of silage and grazed-grass makes concentrate feeding as well as ad libitum forage allowance easier to organize compared to sole-pasture feeding. Ensiled grass works as buffer feeding, which helps to maintain a reasonable grazing-rotation interval in variable weather conditions. Full-time grazing and concentrate feeding in the milking parlour is not a very appropriate combination, because of the demand for extra equipment for concentrate feeding. Cows can utilize total mixed ration (TMR) feeding or concentrate feeding stations inside the barn if they are grazing part time.

Night-time grazed (12 h) cows in experiment 7 (IV) produced 3.1 kg/d more ECM compared to silage-fed cows, whereas the milk yield of day-time grazed cows did not differ significantly from that of silage-fed cows in experiment 8 (IV). The energy content of the silage was lower in experiment 8 compared to experiment 7, so the reason for different results originated from differences in the pasture herbage and the grazing time. Experiment 8 had a significant month×grazing treatment interaction, so that at the beginning of the summer the difference in milk yield between the treatments was negligible. The maturity stage of the pasture herbage was relatively advanced at that time, which would have diminished the nutritional value of the diet of the pasture sward. In general, at constant concentrate supplementation the inclusion of grazed grass in silage diets increased milk yield.

In contrast to our results, Bargo et al. (2002) reported decreased DMI and milk yield with grazed-grass diet compared to TMR, despite of the equal energy concentration of pasture and TMR diets. Lower intake with the grazed-grass diet compared to TMR was also reported in the studies of Kolver and Muller (1998) and Bargo et al. (2004). The grazed-grass diet in Kolver and Muller (1998) did not include concentrate, which explains the difference between feeding strategies. Law and Ferris (2011) reported lower milk yield for a ryegrass pasture grazing system compared to total confinement with regrowth grass silage.

These observations support the hypothesis that full-time grazing itself, or conditions on the pasture, high temperature and the work and time required to harvest the grass, possibly limit DMI and the milk yield. According to Kolver and Muller (1998), the reduced intake explained 61 %, increased activity 24 %, urea excretion 12 %, and other reasons 3 % of the 9 kg smaller milk yield that was observed with feeding by grazing at pasture compared to the TMR group. In the study of Bargo et al. (2002), the average temperature was reported to be above 25 °C during three out of five experimental months. During these months the intake of grazed grass was decreased by 2–3 kg DM compared to the first and the last experimental months.

In the study of Chapinal et al. (2010) the overnight grazed cows on an orchard-grass (D. glomerata) sward consumed the same amount of TMR and produced the same amount of milk (38.2 kg ±1.1 kg/d over the 12 weeks after calving) compared to continuous housing in a free-stall barn. The groups also had similar body condition score, LW and milk composition throughout the study. The authors concluded that the pasture was used only as a comfortable resting and walking area, not as a source of nutrients, despite the sward being of good quality (average CP 239 g/kg DM and NDF 543 g/kg DM). Despite the average 40 kg daily milk yield of multiparous cows, it was possible to maintain a sufficient amount of TMR intake during daylight hours (Chapinal et al., 2010). Thus the comparisons between TMR and grazed-grass diets, as reported in the literature, differ markedly from the comparison between silage and grazed-grass diets in our study.

Substitution of grass silage by grazing also resulted in increased milk yield in the experiments described by Dillon et al. (2002). They linked high milk yield with increased DM intake, which is in contrast to the results reported by Bargo et al. (2002). Differences in concentrate supplementation level, silage quality and grass species could explain part of the difference between the studies. Bargo et al. (2002) used a high level of concentrate and maize-lucerne silage-based TMR against an orchard grass-brome grass pasture sward. Dillon (2002) used a moderate amount of concentrate and perennial ryegrass as silage and in the grazed sward. A moderate amount of concentrate leads to a high grass intake; this puts a high demand on forage quality, which occurs naturally in Nordic pastures (Virkajärvi, 2004). High energy content of grazed grass compared to silage, and moderate amount of concentrate would explain the favourable results of grazed-grass diets in the study of Dillon et al. (2002) and experiments 7 and 8 (IV).

One remarkable difference between the contradictory results of TMR diets as reported in the literature and our own results, was the variable milk production level between the studies. In the experiment of Bargo et al. (2002) the cows received bST injections and milk production was high (44.9 kg±7.5, corresponding to 37 kg fat corrected milk) at the beginning of the experiment. High milk yield requires high intake, which is easier to achieve using TMR than grazed-grass diets. The milk yield level was 20–25 kg/d in the study of Dillon (2002), and variation in milk yield within the study reported by Sayers and Mayne (2001) and within our own experiments was 20–35 kg/d.

4 Conclusions and future research needs

1. In this study the herbage of the pasture sward was at an early stage of maturity, and consequently the energy content of the grazed grass was high. This kind of grazed grass resulted in a low rumen fill of cows and the digestion rate of grass pdNDF was high. The differences in rumen fermentation between ensiled and fresh grass could be explained mostly by differences in grass maturity, expressed as grass iNDF content. The ammonia concentration in the rumen was high with the grazed-grass diet, which was due to an unnecessarily high CP content of early-maturity grass with a high level of N fertilization. The high microbial-protein synthesis in the rumen and the high CP content of the herbage of the pasture maintained high protein supply for milk production. This would reduce the need for additional protein supplementation. From the physiological point of view, diets based on grazed grass have no additional limiting factors for intake or milk production compared to silage diets.

2. Despite the absence of physical limitations affecting intake, the measured amount of grass DMI in milk production studies was smaller than that of estimated intake based on feed recommendations or modelled intake. Thus grass-intake reduction due to management factors, such as the availability of grass or heat stress, would seem to be more likely as a limiting factor for milk production from cows grazing at pasture.

3. The milk yield increased quadratically to the highest level of concentrate supplementation, whereas the MR was small with the high amount of concentrates. The results did not differ markedly from the responses obtained from large review based on silage studies. The response to concentrate supplementation was numerically smaller in the case of low-supplemented pasture-based grazing diets when compared to silage. These differences in MR between grazed-grass and silage diets are probably due to the higher energy content of the grazed grass compared with silage.

   Milk production level of the cows did not significantly affect the MR. The interaction between the milk production level and MR was only weak, which supports the use of a flat-rate concentrate feeding strategy for high-quality full-time grazing at pasture during the early and mid lactation. This strategy is applicable, at least below the production level used in this study, where 90 % of observations remained less than 34 kg ECM.

4. Part-time grazing is a suitable feeding strategy under Finnish conditions because it allows a good rotation of grazed pastures to be maintained during the grazing season, and it ensures high milk production level. By the use of part-time grazing it was possible to either maintain or increase milk production level, compared to a silage diet, when the amount of concentrate feeding was moderate. In this way, part-time grazing helps farms to maintain grazed pasture swards as one profitable summer-feeding strategy.

Suggested future research

The work included mainly mid-lactating cows. Thus, it would be useful to study intake and supplementation with high yielding (> 40 kg/d) dairy cows on grazed swards, especially at the beginning of the lactation. The study should include both energy and protein supplementation. The current study suggested that the need for protein supplementation is smaller than the level required for silage diets, but it is not possible to give quantitative recommendations.

The flat-rate concentrate feeding strategy is recommendable on pasture. However, this leads to a situation where high-yielding cows have lower proportions of concentrate in the diet compared to low-yielding cows due to differences in grass intake. Flat-rate feeding also leads to increasing body condition score at the end of the lactation. Thus flat-rate feeding strategy should be studied further so that accurate recommendations can be given.

The grass itself does not limit DMI or milk production from grazed swards. However, grazing contains many management factors that could restrict intake compared to offering a pasture sward as fresh-cut grass. It would be worthwhile to conduct an experiment in which the same grass is fed to cows both as grazed grass and as fresh-cut grass in different conditions. This would clarify the effect that management factors have on intake and milk production.

The proportion of automatic milking systems in Finland is relatively high and it is still increasing. Combining automatic milking with grazing is a challenging task, and this would need more studies to optimize the management strategy.

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The summary of experiment 6.

Effect of high amount of grain based concentrate supplementation on milk production of grazing dairy cows

Material and methods

The objective of the study was to collect additional data for evaluating milk production response to concentrate supplementation, using cows with different stages of lactation. The study was the last of the series of five separate experiments and it was conducted using milled grain as a concentrate. The main concentrate type within earlier experiments was pelleted concentrate including cereal by-products.

The study was conducted using a replicated (n = 11) 3 × 3 Latin square design with Holstein-Friesian multiparous lactating cows (average days in milk 120±80 d; milk yield 32.9 kg; live weight 623±45 kg at the beginning of experiment) at MTT Agrifood Research Finland. The cows were blocked according to pre-experimental milk yield to three blocks; less than 28 kg for a low level, 28–35 kg for a moderate level and more than 35 kg for a high level. Cows grazed timothy-meadow fescue (Phleum pratense–Festuca pratensis) pasture full-time in an intensive rotation, with an estimated herbage allowance of > 25 kg DM/ha. Each of three periods lasted 21 d including the first 14 d of transition and a 7 d data-collection period. Treatments consisted of three levels of concentrate supplementation (6, 9 and 12 kg/d) fed during milkings. The concentrate consisted of barley (300), oats (300), barley fibre feed (300) and rapeseed meal (100), kg/t on air-dry basis. In addition cows received a supplementary mineral mixture. The mean compositions of the experimental feeds are presented in Table 1. Data were analyzed by Analysis of Variance for a Latin Square design according to the MIXED procedure of SAS. The model included the fixed effect of period, treatment, block, and treatment by block interaction. The random effect was a cow. The mean values of dependent variables were further divided into orthogonal contrasts to assess the linear and quadratic effect of concentrate supplementation.

Results and discussion

Grass neutral detergent fibre (NDF) content was the lowest in period two and the highest in period three. It is typical that grass NDF content is lowered in regrowth grass (Kuoppala et al 2010). On the other hand, grass NDF content increases with advancing maturity and with the pronounced growing time. The changes in grass chemical composition are rapid in the beginning of the summer. However, the changes, including grass crude protein content, were within typical variation during the season when an intensive grazing rotation is used.

Both milk and energy corrected milk (ECM) yield responses to increasing concentrate supplementation (MR and MRe for milk and ECM yields, respectively) were linear. There was no significant quadratic effect for MR or MRe. The mean MRe, 0.27 kg/kg concentrate DM, was very low compared with earlier results based on the same herd (Sairanen et al. 2006) or when compared with the review presented by Bargo et al. (2003). The concentrate used in this study was mainly milled grain whereas the earlier experiments were conducted using pelleted commercial concentrate. The energy content of barley fibre feed was 11.5 MJ ME/kg DM which is lower compared to grain, but the difference was not remarkable. The differences between concentrate types do not explain this low MRe in the current experiment compared to earlier results. The standard error in the present study was reasonable low so the experiment itself was conducted properly. The mean crude protein content of the diet was not too high to explain the low MRe either.

There was block by concentrate interaction showing the lowest MRe in low yielding block. This kind of interaction is difficult to prove and there are few reports supporting this result (Stocdale et al., 1987). The milk production level itself is weaker factor to explain differences in MRe compared to days in milk before experiment.

Concentrate supplementation increased both milk protein and lactose content whereas milk fat content decreased. These are typical changes and reported in earlier studies (Bargo et al., 2003). The absence of quadratic effect in milk fat content suggests that there was not lack of effective fiber even with the highest level of concentrate supplementation.

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Table 1. The mean compositions of the experimental feeds.

	grass/period			concentrate
In dry matter	1	2	3	
Crude protein, g/kg	178	171	163	156
Metabolizable energy, MJ/kg	11.23	11.18	10.78	12.24
NDF, g/kg	518	473	566	285

NDF = neutral detergent fibre

Table 2. Milk production of cows supplemented with increasing amount of concentrates.

	concentrate, kg/d			concentrate		block x concentrate	
	6	9	12	SEM	P_{lin}	SEM	P
ECM, kg	27.5	28.3	28.9	0.53	<0.001	0.95	0.026
Milk, kg	29.1	30.2	31.2	0.65	<0.001	1.14	0.057
Fat, g/kg	37.2	36.1	35.2	0.591	<0.001	1.03	0.42
Protein, g/kg	33.5	33.9	34.2	0.34	0.002	0.59	0.88
Lactose, g/kg	45.5	45.5	45.8	0.27	0.025	0.48	0.48

ECM = energy corrected milk

Appendix 2.

The summary of experiment 9.

Effects of season on rumen dry matter pool of pasture-fed dairy cows

Material and methods

Experiment 9 included 5 rumen-fistulated cows fed solely on a grazed pasture diet supplemented with minerals. The experiment was designed to clarify the differences in rumen content and fermentation pattern during the grazing season and the only treatment was a period, four in total. The first data collection period started on 10 June and lasted five days. The time difference between the data collection periods was two weeks. The measurements included milk yield, grass intake, rumen pool size, rumen fermentation and the digestibility of the diet. The dry matter intake (DM) was measured using chromium oxide Cr_2O_3 (as Cr-mortanded straw) as an indigestible fecal marker and indigestible neutral detergent fibre (iNDF) as an internal marker to determine diet digestibility. In addition, alkanes were used as an alternative method (Dove and Mayes, 1991) for grass intake measurement during the periods three and four. Synthetic n-alkane (C_{32}) was dosed twice daily (240 mg/day) using gelatine capsules and the intake was calculated based on alkane dose, the alkane content in the herbage, and the ratio of the dosed and natural alkanes in the feces. The capsules were added to the rumen five days prior to collection period and continued for ten days. The fecal collection was conducted after morning and evening milking, by sampling individual dung patches on the pasture. The statistical analysis was conducted using analysis of variance with repeated measurements. The model included period as repeated fixed variable and cow as random variable. The regression model included a compound symmetry covariance matrix for a dependent variable. Treatment differences were declared at $P < 0.05$ using a multiple comparison test (adjusted Tukey).

Results and discussion

The cows were late in lactation and the average milk yield was 22.4 kg/d (± 5.2 kg) at the beginning of the experiment. The grass-intake measurement using chromium as an indigestible marker seemed to work properly excluding period 4 which presents unrealistic high intake taking account the stage of lactation. The relation between intake and milk production suggest that the intake is slightly underestimated in period one. A possible source of error could be found in overestimated DM digestibility. The amount of faeces remained at the same level in periods two to four, so the overestimation of grass intake in period four arises from an unrealistic high DM digestibility. The alkane method did not work at all in period four. The standard error for intake measurement was relative low, which suggests possible problems in the laboratory analysis. The alkane content in grass was very low, which increases challenges both in sampling and analysis. Sormunen-Cristian et al. (2005) also reported low contents of alkanes in Finnish grass, but they concluded that the alkane method gives reliable estimate for intake in lambs.

The rumen DM pool was the lowest in period one which is supported by high DM digestibility. This is reasonable due to early maturity stage of pasture. The iNDF content of the grass was the lowest in period one, which describes well the grass maturity stage. The rumen DM content was higher in late summer compared to early summer.

Rumen pH was lowest and ammonia concentration was highest in period one. Grass maturity stage explains this because high digestible grass ferments rapidly and early maturity grass contains high amount of nitrogen. The high digestible grass increased the molar proportion of propionate in expense of acetate. The effect of season on the proportion of butyrate was variable. In general, the changes in proportions of volatile fatty acids were inconsistent.

References

Dove, H. and Mayes, R.W. 1991. The use of plant wall alkanes as marker substances in studies of the nutrition of herbivores: A review. Australian Journal of Agricultural Research. 42:913-952.

Sormunen-Cristian, R., Nykänen-Kurki, P. & Jauhiainen, L. 2005. Estimation of grass and white clover grass intake by n-alkanes in lambs. Proceedings of the 2th COST 852 Workshop Held in Grado, Italy 10-12 November 2005. p. 169-173

Table 1. The main results of the experiment including ECM yield, grass intake, rumen pool size and rumen fermentation.

	Period 1	Period 2	Period 3	Period 4	SEM
ECM yield, kg/d	23.5 a	18.9 b	19.3 b	15.9 c	1.33
Grass intake with chromium[1], kg DM/d	13.2 a	14.5 a	14.7 a	18.0 b	0.88
Grass intake with alkanes[2], kg DM/d			11.5 a	4.4 b	0.3
Diet DM digestibility[3], iNDF, g/kg DM	784 a	725 b	728 b	785 a	6.58
Rumen dry matter pool, kg DM	6.8 a	8.0 b	9.2 c	10.2 c	0.45
Rumen iNDF pool, kg	0.8 a	1.6 b	1.5 b	1.5 b	0.11
Rumen NDF pool, kg	3.4 a	4.6 b	4.9 c	4.8 d	0.28
In grass/kg DM					
Neutral detergent fibre, g	556	615	550	553	
Crude protein, g	252	175	203	219	
iNDF, g	3.4	6.0	7.2	6.5	
C33[5], mg	33.9	45.8	21.6	68.3	
C32[4], mg	6.3	7.3	10.5	16	
MJ ME	11.5	10.9	11.0	11.2	
Rumen fermentation					
pH	6.16 a	6.45 b	6.33 ab	6.47 b	0.061
NH3N, mmol/l	25.1 a	15.8 b	14.8 b	18.8 c	0.76
Acetic acid, mmol/mol	610 a	660 b	620 a	640 b	0.007
Propionic acid, mmol/ mol	210 a	180 b	190 b	180 b	0.004
Butyric acid, mmol/mol	130 a	120 b	140 c	130 a	0.003

ECM = energy corrected milk, DM = dry matter, SEM = standard error of mean, NDF = neutral detergent fibre, iNDF = indigestible neutral detergent fibre

The LS means with different letters within a row differ significantly (P<0.05)

1 C2O3 used as external faecal marker

2 The intake measurement using method described in Dove and Mayes (1991)

3 Indigestible NDF as external marker for diet DM digestibility

4 Even chain fatty acid in grass

5 Odd chain fatty acid in grass

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