High quality grass silage to dairy goats- effect on energy balance, milk yield and quality

Grassurfôr av høg kvalitet til mjølkegeit- effekt på energibalanse, mjølkeytelse og mjølkekvalitet

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Ingjerd Dønnem

Department of Animal and Aquacultural Sciences

Norwegian University of Life Sciences

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ABSTRACT

Dønnem, I. 2010. High quality grass silage to dairy goats- effect on energy balance, milk yield and quality. Norwegian University of Life Science, Philosophiae Doctor (PhD) Thesis 2010:48, ISSN 1503-1667, ISBN 978-82-575-0958-3.

The general aim of this thesis has been to investigate the effect of high quality silage, harvested at an early stage of maturity, on energy intake, energy balance, milk production and milk quality of goats. It was also of interest to study the relationship between different levels of concentrate supplementation and the silage harvesting time on these parameters. Two studies were performed and formed the basis of three papers.

In the first study 18 goats were fed grass silage harvested at three different stages of maturity from the primary growth combined with two different levels of concentrate in a cyclic change-over design. The goats were grouped into three blocks according to their body condition just after kidding; poor, medium and high. The experiment was performed during the first 18 weeks of lactation. Paper I evaluated the effect of the nutritive characteristics and digestibility of the silages on the goat's performance. Increasing the digestibility by earlier harvesting time increased the intake of grass silage and the milk production. Increased energy intake due to improved silage digestibility resulted in higher milk production than seen with increased energy intake due to increased concentrate level. However, utilization of nutrients to milk production increased with postponed harvesting time, because less energy was used for body fat deposition. In the same study the energy balance and the milk quality of the goats were evaluated (Paper II). The calculated energy balance decreased and the serum non-esterified fatty acids (NEFA) concentration increased with decreasing energy content (postponed harvesting time and low level of concentrate) in the diet. The concentration of milk FFA was highest when the highest concentrate level was offered. During the first 18 weeks of lactation a high energy balance was correlated to a high milk FFA concentration and a poor sensory milk quality. Goats with initial poor body condition had higher milk FFA concentrations than goats in higher initial body condition during the whole experimental period.

In the second study, the aim was to study the changes of the goats' energy status during early and mid lactation when offered rations with various energy concentrations, and relate this to milk quality (Paper III). The experimental feeds were grass silage harvested in the primary growth at two stages of maturity supplemented with two different levels of concentrate. Energy status was estimated from lactation week 2 to 18 by studying changes in body composition (measured by computer tomography), calculated energy balance and blood parameters. After the indoor feeding experiment there were two measurements of milk parameters on mountain pasture. During the indoor experimental period only the goats fed the lowest energy diet (the latest harvesting time and lowest level of concentrate) mobilized from the adipose tissue. The rest of the goats had an energy intake high enough to deposit body fat throughout these 16 weeks. The concentration of FFA in milk increased with increased energy intake, which suggests that during the present condition, a high plane of nutrition during early and mid lactation increased lipolysis in milk.

The main conclusions from the present studies are that improving the silage digestibility by early harvesting time increases energy intake, energy balance and milk production, and it is possible to feed goats the first 18 weeks of lactation with sufficient energy to avoid fat mobilization. However, feeding to a positive energy balance worsened the milk quality. It appears that when a high nutrient supply supports both high milk production and deposition of adipose tissue, it increases the lipolytic activity in milk.

SAMMENDRAG

Dønnem, I. 2010. Grassurfôr av høg kvalitet til mjølkegeit- effekt på energibalanse, mjølkeytelse og mjølkekvalitet. Universitetet for miljø- og biovitenskap, PhD avhandling 2010:48, ISSN 1503-1667, ISBN 978-82-575-0958-3.

Hovedmålet med denne avhandlingen har vært å undersøke effekten av surfôr av høg kvalitet, høsta på et tidlig utviklingsstadium, på energiopptak, energibalanse, mjølkeytelse og kvalitet. Det var også av interesse å undersøke samspillet mellom to ulike kraftfôrnivå og surfôrets høstetid på disse parametrene. Det ble utført to forsøk som ga grunnlag for tre artikler.

I det første forsøket ble 18 geiter fôra grassurfôr høsta på tre ulike utviklingstrinn i førsteslåtten, kombinert med to ulike kraftfôrnivå i "cyclic change-over" design. Geitene ble gruppert inn i 3 blokker ut fra hvilket hold de var i rett etter kjeing: dårlig, medium eller høgt. Forsøket pågikk de 18 første ukene i laktasjonen. Artikkel I evaluerte effekten av næringsverdien og fordøyeligheten av surfôret på geitenes fôropptak og mjølkeproduksjon. Ved å øke surfôrets fordøyelighet økte fôropptak og mjølkeytelse. Økt energiopptak på grunn av økt fordøyelighet av surfôret ga høgere mjølkeytelse enn økt energiopptak på grunn av økt kraftfôrnivå. Utnytting av næringsstoffene til mjølkeproduksjon økte med utsatt høstetid. I det samme forsøket ble energibalanse og mjølkekvalitet hos geitene evaluert (Artikkel II). Kalkulert energibalanse gikk ned og innholdet av ikke-esterifiserte fettsyrer (NEFA) i serum økte med redusert energiopptak. Konsentrasjonen av frie fettsyrer (FFA) i mjølka var høgest ved den høgeste kraftfôrmengden. I løpet av de første 18 ukene i laktasjonen var en høg energibalanse korrelert med høgt innhold av FFA i mjølka og en dårlig smakskvalitet. Geiter i dårlig hold ved kjeing hadde en høgere FFA konsentrasjon i mjølka utover i forsøket enn geiter i godt hold ved kjeing.

I det andre forsøket var målet å undersøke endringer i geitas energistatus gjennom tidlig- og midtlaktasjon ved fôring av rasjoner med ulik energikonsentrasjon, og se dette i forhold til mjølkekvalitet (Artikkel III). Forsøksfôret var grassurfôr høsta på to ulike utviklingstrinn i førsteslåtten kombinert med to ulike kraftfôrnivå. Energistatus fra laktasjonsuke 2 til 18 ble kartlagt ved å måle endringer i kroppssammensetning, ved hjelp av datatomograf, energibalanse

og blodparametre. Etter innefôringsperioden ble mjølkeytelse og sammensetning målt to ganger på fjellbeite. Gjennom innefôringsperioden var det kun geitene som fikk rasjonen med lavest energikonsentrasjon som mobiliserte av kroppsfettet. De andre geitene hadde høgt nok energiopptak til å avleire kroppsfett gjennom disse 16 ukene. Konsentrasjonen av FFA økte med økt energiopptak.

Konklusjonene fra denne studien er at å forbedre fordøyeligheten av surfôret ved høsting på et tidlig utviklingstrinn øker energiopptak, energibalanse og mjølkeproduksjon. Studien viser også at det er mulig å fôre geiter de første 18 ukene av laktasjonen med nok energi til å unngå fettmobilisering. Likevel, fôring til en positiv energibalanse hadde ingen positiv effekt på mjølkekvaliteten. Resultatene tyder på at når et høgt næringsopptak øker både mjølkeproduksjon og kroppsfettreserver, øker også lipolyseaktiviteten i mjølka.

ABBREVATIONS

BMI – body mass index

BW – body weight

CP – crude protein

CT – computerized X-ray tomography

DM – dry matter

DMI – dry matter intake

D-value – digestible organic matter in dry matter

ECM – energy corrected milk

FFA – free fatty acids

LPL – lipoprotein lipase

MFGM – milk fat globule membrane

MUFA – monunsaturated fatty acids

N – nitrogen

NDF – neutral detergent fibre

NEFA – non-esterified fatty acids

NEL – net energy lactation

NPN – non-protein nitrogen

PBV– protein balance in the rumen

PUFA – polyunsaturated fatty acids

SDMI – silage dry matter intake

WSC – water soluble carbohydrates

LIST OF ORIGINAL PAPERS

This thesis is based on the following original papers referred to by author and their Roman numerals in the text:

I. Dønnem, I., Randby, Å. T. and Eknæs, M. Effects of grass silage harvesting time and level of concentrate supplementation on nutrient digestibility and dairy goat performance. *Animal Feed Science and Technology. In press.*

II. Dønnem, I., Randby, Å. T. and Eknæs, M. Effect of grass silage harvesting time and level of concentrate supplementation on goat milk quality. *Animal Feed Science and Technology. In press.*

III. Dønnem, I., Eknæs, M. and Randby, Å.T. Energy reserves, measured by computer tomography (CT)-scanning, and milk quality of dairy goats fed rations with various energy concentrations. *Livestock Science. Submitted.*

1. INTRODUCTION

Goats in Norway kept for milk production are fed indoors during winter and released to graze on natural mountain or forest pastures in spring or early summer. They produce about 60- 65 % of the milk on indoor feeding (TINE, 2006) with diets based on grass silage and concentrate. Timothy grown with meadow fescue and red clover in a multispecies sward is the main forage crop for silage. Due to variations of climate and topography in Norway there will be variations in goat farming conditions. However, a common trend is that the goats are fed silage of moderate to low energy content, low digestibility and a high content of fiber. It is generally recognized that increasing the amount of fiber depresses digestibility of the dietary components in the feeds (Santini et al., 1992). The level of dry matter intake (DMI) or ingested energy is the main factor influencing milk yield and composition of dairy goats (Morand-Fehr et al., 2007). Improvements of the grass silage quality could potentially increase feed intake. There are a number of factors affecting silage quality. However, a young stage of maturity at harvest and consequently highly digestible feed is a prerequisite for a high energy and protein silage, and thereby high energy intake and production (Sauvant et al., 1987; Rinne et al., 1999; Huhtanen et al., 2007).

Due to high concentrate prices there are substantial economic benefits from production and feeding of high-quality forages in goat production systems in Norway. In addition, the cultural value of Norwegian goat farming (grazing on natural pasture) is a reason to focus on improving grass silage quality instead of to increase the concentrate feeding. There seems to be a growing consumer trend in favor of agricultural products with beneficial effects on human health and with local and environmental friendly trademarks.

The main products of goat farming are cheese products from the milk, and mainly brown whey cheese. However, the production of white cheeses increases both for domestic consumption and export (TINE, 2006). This production requires a better and more stable quality of the raw milk than whey products. In parts of the year there is a prominent problem with high concentrations of free fatty acids (FFA) and off- flavors of the milk, especially a specific rancid and tart flavor. A high concentration of FFA is found to be correlated to the frequency of off- flavor (Collins et al.,

2003) and rancid and tart flavor specifically (Eknæs and Skeie, unpublished). The concentration of FFA in milk is a measure of lipolysis, i.e. the hydrolysis of fat globule triglycerides into FFA (Chilliard et al., 2003). In early lactation, goats often are in negative energy balance and they mobilize considerable amounts of lipids stored in adipose tissue to maintain milk production to their genetic potential (Dunshea et al., 1990). Energy mobilization may subsequently result in high concentrations of FFA and off- flavors of the milk (Eknæs et al., 2006). In addition, the milk fatty acid composition will differ from that of milk synthesized when animals are in energy balance. If animals are fed a high energy diet in early lactation it may reduce the problem with underfeeding (Morand-Fehr et al., 2007), and this can be solved by feeding silage of high quality supplemented with concentrate. Norwegian goats are kept on natural pasture in 3-4 months during summer, and there is a particular problem with high FFA concentration and poor sensory milk quality during this period (Eknæs and Skeie, 2006).

1.1 Silage quality

The nutritive value of grass silage produced in Norway is highly variable (TINE, Statistical reports 2002-2008). This may be challenging when trying to maximize the use of grass silage in diets to productive animals rather than increasing the proportion of concentrate.

The quality of forages manipulated by the stage of grass maturity has been thoroughly studied throughout the history of animal science because of its importance to the performance of ruminant based production systems (Rinne, 2000). The primary goal in silage production is to close the gap between the feeding value of the original crop and that of the resulting silage. The parameters affecting silage quality can be divided into crop related factors and fermentation related factors (Charmley, 2001). Crop related factors are mainly related to maturity stage at harvest, and thereby the chemical and physical changes in plants. Digestibility of grass is to a large extent determined by the stage of plant maturity. Delayed harvest will decrease the content of digestible organic matter per kg dry matter (DM) in silage (D-value) (Thomas et al., 1981). D-value is probably more representative of the maturity stage at harvest than other chemical entities such as neutral detergent fiber (NDF) or crude protein (CP). The average daily decrease in silage D-value is reported to be 4.8 g/kg DM by Rinne et al. (1999), 5.4 g/kg DM by Randby (2003), and 5.0 g/kg DM by Kuoppala et al. (2008). Deinum et al. (1981) observed that D-value declined

the faster the further north the grass was growing when comparing the development of timothy at latitudes from 51 to 69°N. Rinne et al. (1997; 1999) found a curvilinear decrease in D-value when comparing silages harvested at four stages of maturity, and suggested that cumulative temperature explains D-value better than the date of harvest or the chemical composition of the grass. The general changes of chemical composition of the grass at delayed harvest are decreased content of CP and increased content of NDF. Lignification of the cell wall fraction increases with plant maturity and reduces digestibility, since lignin interacts with other cell wall components to provide structural integrity and is resistant to hydrolysis by rumen microorganisms (McDonald et al., 1991).

The proportion of total N as protein in fresh herbage is 75 to 90 %. In preserved silage, however, less than 50 % of total N is present as protein, mainly due to proteolysis by plant enzymes and microbial activity to non-protein N (NPN). The NPN fraction is mainly made up of peptides, free amino acids, amides and nitrates (McDonald et al., 1991). Non-protein N is highly degradable in the rumen and is rapidly converted to ammonia (Givens and Rulquin, 2004). The extent of proteolysis will increase during a long wilting period under humid conditions, and during ensiling if the temperature in the silo is high and the fall of pH during ensiling is slow (McDonald et al., 1991). There will be an increased concentration of fiber in silage compared with the herbage, which may be due to proportionally larger losses of other chemical components during ensilage by respiration, fermentation, and/or effluent losses of soluble nutrients (Rinne et al., 1997). This results in a lower digestibility of the ensilage than the fresh herbage (McDonald et al., 1991).

It is essential to have a good microbial fermentation process to produce high quality silage. A good fermentation process is dependent on the type and quality of the forage crop, and on the harvesting and ensiling technique. Sugars are the main substrates for both respiration and fermentation, whereof the water soluble carbohydrates (WSC) are more important than the structural carbohydrates. However, structural carbohydrates can be degraded during the ensiling period by acid hydrolysis or microbial breakdown (McDonald et al., 1991). Silage additives are used to ensure that the lactic acid bacteria dominate the fermentation and to inhibit microbial growth. The additives will lead to a rapid fermentation (quick lowering of pH), decreased

proteolysis and decreased content of acetic and butyric acid and ethanol (McDonald et al., 1991). A purpose of using silage additives is also to improve the nutritional value of silage and to minimize ensiling losses (McDonald et al., 1991). The extent of fermentation is correlated to the DM content and especially at low DM content (<20-30 %) it is important to use silage additives that inhibits clostridial growth (McDonald et al., 1991). The fermentation quality criteria presented by Saue and Breirem (1969) is used as an assessment of fermentation quality by the commercial feed analysis laboratory (Eurofins AS, Moss, Norway). Table 1 presents this fermentation quality criteria for low DM (<25 %) grass silage and the average DM content, pH, NH_3, lactic acid, acetic acid, and butyric acid for silage samples analyzed from 2002 to 2008 in Norway (TINE, Statistical reports 2002-2008). The concentration of fermentation parameters as a mean for all analyzed silage samples is within the criteria of fermentation quality, except of the content of NH_3 of total N.

Table 1. Criteria defining good fermentation in low DM (<250 g DM/kg) grass silage, and average DM content, pH, NH_3, lactic acid, acetic acid, and butyric acid for silage samples analyzed from 2002 to 2008 in Norway (TINE, Statistical reports 2002-2008).

	DM (%)	pH	NH_3 (% of total N)	Lactic acid (% of DM)	Acetic acid (% of DM)	Butyric acid (% of DM)
Fermentation quality criteria, Saue and Breirem (1969)	<25	<4.2	<8.0	5.0- 8.0	1.0- 3.0	<0.3
2002	25.6	4.15	9.8	6.0	2.1	0.3
2003	24.2	4.06	10.5	6.4	2.2	0.3
2004	23.6	4.19	10.6	6.7	2.1	0.3
2005	24.1	4.08	10.2	6.5	2.7	0.2
2006	24.5	4.06	10.0	5.8	2.2	0.2
2007	25.1	4.10	9.5	7.0	2.1	0.2
2008	27.5	4.13	7.7	7.8	1.9	0.3

Silage fermentation characteristics may influence feed intake, and of the individual fermentation parameters the total acid concentration was found by Huhtanen et al. (2002) to be the best predictor of silage dry mater intake (SDMI). Feed intake and fermentation products can modify the profile of nutrients absorbed from the digestive tract and therefore affect milk yield and composition (Huhtanen, 1993). Both yields of milk, energy corrected milk (ECM), milk fat and protein are found to decrease with increasing extent of fermentation. Reduced milk fat content with increasing lactic acid or total acid in silage may be attributed to the reduced ratio between acetic and butyric (lipogenic) acid and propionic (glucogenic) acid in the rumen, as propionic

acid is the main end-product of ruminal lactate fermentation (Huhtanen, 1993). Milk protein content decreases as microbial protein synthesis in the rumen decreases. The metabolism of silage fermentation products in the rumen provides little or no ATP for microbial synthesis. Therefore restricting fermentation would yield more energy for rumen microbes and support greater rates of microbial synthesis (Chamberlain, 1987).

1.2 Milk fat lipolysis

Milk fat lipolysis is the hydrolysis of triglycerides into FFA, mono- and diglycerides, and in some cases, glycerol (Deeth, 2006). The lipolytic activity is in most cases caused by the lipolytic enzyme, lipoprotein lipase (LPL) (Deeth, 2006). Milk fat lipolysis can also in a small extent result from microbial lipolysis, which are negligible in milk of reasonable microbial quality (Chilliard et al., 1984). "Spontaneous lipolysis" in cold, stored milk is due to the action of LPL, which can be stimulated ("induced lipolysis) by agitation, foaming or temperature changes (Chilliard et al., 2003). Potential maximum milk LPL activity is more than 500 times higher than spontaneous lipolysis in goat milk (Chilliard et al., 2003). Milk LPL originates from either adipose tissue LPL or mammary LPL (Chilliard et al., 1979). Adipose tissue LPL could either be transported actively from the blood, through the mammary secretory cell and into the milk, or enter the milk by paracellular leakage, while mammary synthesized LPL could be secreted into milk with either caseins or fat globules (Chilliard et al., 1979; 2003). A link between milk LPL activity and adipose tissue LPL activity is also suggested by their positive correlation observed by Chillard et al. (1979) and Chilliard (1985).

The lipolytic system differs between the goat and the cow (Chilliard et al., 1984). Spontaneous lipolysis is not correlated to LPL activity in bovine milk, and lipolysis remains generally low despite the high LPL activity of this milk. Contrary, in goats' milk the lipolysis is well correlated to milk LPL activity. This is explained by the fact that milk LPL is largely bound to casein micelles in cows' milk, thus decreasing enzyme-fat substrate interactions, while a large proportion of goat milk LPL are bound to cream (Chilliard et al., 2003). Chilliard et al. (1984) found that cow milk LPL was distributed with 6 % in the cream, 17 % in the serum and 78 % in the caseins, while the corresponding values for goat milk were 46, 46 and 8 %, respectively.

The appearance of the characteristic goat flavor in cold, fresh milk is due to the high content of C6:0, C8:0, C10:0 and branched chained C9 and C10 fatty acids (methyl- and ethyl- C8), which are more abundant in goat's than in cow's milk fat (Ha and Lindsay, 1993; Sanz Sampelayo et al., 2007). The combination of milk LPL characteristics of goats and milk fatty acid composition could explain the relationship between LPL, lipolysis and goat flavor (Chilliard et al., 2003). Goat flavor must be distinguished from tart and rancid flavor, as is seems to appear at lipolysis levels much lower than those responsible for the latter off-flavors (Chilliard et al., 2003). Tart, rancid, goaty and bitter flavors are all categorized as lipolyzed off- flavors (Shipe et al., 1978). Lipolyzed flavor is found to be correlated to a high level of milk FFA, especially short and medium chain FFA (Scanlan et al., 1965; Park, 2001; Collins et al., 2003).

There are large differences between goat breeds in the level of LPL activity and spontaneous lipolysis, which could be related in part to the casein α-s1 genotype (reviewed by Chilliard et al., 2003). Norwegian goats have a high frequency of the α-s1 casein genotype (F or "null") that secrete a milk with lower fat and protein content and higher levels of LPL activity and goat flavor than goats of A or "strong" genotype (Skjevdal, 1979; Delacroix-Bucket and Lamberet, 2000).

Milk fat globule membrane

Triglycerides represent the major component of milk lipids. The remaining components are associated with the milk fat globule membrane (MFGM) surrounding the triglyceride droplets (Evers, 2004). The stability of the MFGM would influence the susceptibility of milk to lipolysis (Deeth, 2006). Phospholipids, glycosphingolipids and cholesterol are important precursors in synthesis and stability of MFGM (Evers, 2004). Phospholipids are synthesized *de novo* in the mammary gland, while cholesterol are both supplied by the blood plasma and synthesized in the mammary gland (Nielsen and Jakobsen, 1994). In experiments with cows a large milk fat globule have been found to be more susceptible to coalescence and lipolysis, and there is a positive relationship between the size of the fat globules and the fat percentage (Wiking et al., 2003) and between diurnal fat production and the average diameter of the milk fat globules (Wiking et al., 2004). This indicates that when the fat synthesis is high, the synthesis of membrane material is limited (Wiking et al., 2004). Eknæs et al. (2009) found a positive correlation between the level

of milk FFA and milk fat content in goats. Eknæs (2009) hypothesized that goat milk with high concentrations of FFA has lower concentration of MFGM components (e.g. cholesterol and phospholipids) than normal goat milk.

1.3 Hypothesis and objectives

The following hypotheses were formulated:

1. Grass silage harvested at a very early state of maturity will have a higher nutrient digestibility than silage harvested at a later stage of maturity.
2. Goats fed the grass silage with highest D-value (g digestible organic matter per kg dry matter) will have a high milk production although supplemented with a low level of concentrate.
3. The milk yield of goats fed grass silage harvested at an early state of maturity will differ less between two concentrate levels than the yield of goats fed grass silage harvested later.
4. Goats fed silage of high quality supplemented with concentrate will obtain a higher energy balance in early lactation than goats fed a ration with lower energy content.
5. By avoiding a high mobilization of energy reserves in early lactation by feeding a high energy ration goats will produce milk of stable quality in early and mid lactation.

The following objectives of this thesis were stated:

1. To study the effect of timing of harvest of grasses (mainly timothy and meadow fescue) in the primary growth on
    - nutrient digestibility of the silage
    - feed intake by dairy goats
    - milk production and milk quality by dairy goats
    - the goats' energy balance.
2. To evaluate the interaction between the silage harvesting time and level of concentrate supplementation on feed intake, milk production and milk quality.
3. To examine how diets with various energy concentration affects the energy status of dairy goats in early lactation and how this is related to milk quality.

2. BRIEF SUMMARY OF PAPERS I-III

Paper I

Effects of grass silage harvesting time and level of concentrate supplementation on nutrient digestibility and dairy goat performance

The objective of this study was to evaluate the effects of grass silage harvesting time (HT) combined with two levels of concentrate on dairy goats' performance during early and mid lactation. The experimental silages were prepared from timothy- dominated primary growth at three stages of maturity: very early (HT 1), early (HT 2) and normal (HT 3). The silages were fed *ad libitum* to 18 goats of the Norwegian Dairy Breed and supplemented with a low (LC; 0.6 kg daily) or normal (NC; 1.2 kg daily) level of concentrate in a cyclic change- over design with 4 periods of 4 weeks. The goats were grouped into three blocks according to their body condition just after kidding; poor, medium and high body condition. Digestibility and feed values of the silages were determined, and feed intake and milk production were recorded.

Main results:

- The D-value of the silages was 771, 696 and 619 for HT 1, 2 and 3, respectively.
- Postponing the harvesting time and increased concentrate allowance decreased silage dry matter intake (DMI).
- Milk yield and yields of milk constituents decreased with postponed harvesting time
- The efficiency of nutrient utilization for milk production was best when LC was fed and increased with postponed harvesting time.
- Marginal ECM production response to increased NEL intake was higher when intake was increased due to higher silage digestibility (0.14 kg ECM/ MJ NEL) compared with increased NEL intake due to increased concentrate level (0.11 kg ECM/ MJ NEL).

Main conclusions

The intake of grass silage increased when improving the quality by earlier harvesting time. Higher intake by early harvest increased milk production. Improving silage quality by earlier harvesting time resulted in higher milk production than seen with increased concentrate level. A decrease in silage quality could not be fully compensated for by increased concentrate feeding.

Due to higher BW gain of the goats, utilization of nutrients to milk production by very early harvesting time was lower than by postponed harvesting time. Practical implications of the results may depend on whether the aim is to maximize the production per goat or per hectare of grass.

Paper II

Effect of grass silage harvesting time and level of concentrate supplementation on goat milk quality

The objective of this work was to evaluate the effect of grass silage harvesting time and two levels of concentrate on goat milk quality during early and mid lactation of the goats. Milk was sampled from the same experiment as performed in Paper I. One-day milk samples for chemical and sensory analyses were taken at the end of each of the four periods. The energy balance of the goats was calculated.

Main results:

- Sensory milk taste quality was not affected by dietary treatment. Milk FFA concentration was higher when NC than LC was fed.
- The proportion of short and medium chain fatty acids in milk fat decreased with postponed harvesting time and LC, while most of the long chain fatty acids (including C18:1c9) increased with postponed harvesting time and LC.
- The calculated energy balance decreased and the serum non-esterified fatty acids (NEFA) concentration increased with decreasing energy content in the diet.
- Goats with initial poor body condition had higher milk FFA concentrations than goats in higher initial body condition.
- High milk FFA was correlated to poor sensory milk taste quality, low serum NEFA concentration, low C18:1c9 proportion and high energy balance.

Main conclusions:

Very early harvested grass silage, combined with the highest concentrate level, increased the milk yield and the energy balance. The goats with the highest energy intake were in a positive energy balance throughout the 18 first weeks of lactation. The experiment supported previous

research by revealing that a high level of milk FFA had a negative effect on sensory milk taste. However, higher energy intake did not reduce lipolysis, as measured by milk FFA concentration, or improve the taste quality of the milk. It rather tended to be opposite, at least as regards the concentrate level. High energy balance was correlated to a high concentration of milk FFA and poor taste quality. This study suggested that increased energy intake and energy balance during the first 4 months of lactation does not improve milk quality.

Paper III

Energy status, measured by computer tomography (CT)-scanning, and milk quality of dairy goats fed rations with various energy concentrations

The objective of this work was to study the changes in the energy status of dairy goats during early and mid lactation when fed rations with various energy concentrations, and relate this to milk quality. The experimental feeds were grass silage harvested in primary growth at two stages of maturity: very early or normal (HT 1 and HT 3, respectively), supplemented with either low (LC; 0.6 kg daily) or normal (NC; 1.2 kg daily) level of concentrate. The rations were fed to 12 goats from lactation week 3 to 18, which were grouped into three blocks according to their body condition; poor, medium and high body condition. Energy status was estimated by changes in body composition measured by computer tomography (CT), calculated energy balance and blood parameters. Feed intake and milk yield were recorded every week and milk samples for chemical and sensory analysis were collected every fourth week. After the indoor feeding experiment there were two measurements of milk parameters on mountain pasture.

Main results:

- Calculated adipose tissue mass of the goats was highest when fed HT 1 and NC.
- During the first 18 weeks of lactation only the goats fed the lowest energy diet (HT 3, LC) mobilized from the adipose tissue, and the majority of the mobilization was from lactation week 2 to 8 (74 g fat per day).
- Calculated energy balance was positive for goats fed HT 1 throughout the indoor experiment. The goats offered HT 3, LC was primarily in negative energy balance, while the goats offered HT 3, NC reached energy balance at lactation week 8.

- Milk quality, measured by milk FFA content and sensory quality, was not significantly affected by dietary treatment. There was, however, a tendency ($P = 0.09$) to higher concentration of FFA for goats offered HT 1 than HT 3.
- Milk FFA content increased when the goats were let out to mountain pasture.

Main conclusion:

The mass of adipose tissue of the goats increased with increasing energy content of the diet. During the first 18 weeks of lactation only the goats fed the lowest energy diet mobilized from the adipose tissue. The rest of the goats had an energy intake high enough to deposit body fat throughout these 18 weeks. Consequently, it was possible to feed goats in early lactation with sufficient energy to avoid fat mobilization. The results of this study indicate that a high plane of nutrition during early and mid lactation increase milk lipolysis. A high energy ration indoors during the first 18 weeks of lactation did not improve milk quality neither simultaneously nor later on mountain pasture

3. GENERAL DISCUSSION

3.1 Intake of grass silage

The energy supply to an animal is controlled by the amount of feed offered to the animal, the amount of feed the animal consumes and the concentration of available energy in a unit of feed, i.e. the digestibility (Rinne, 2000). The level of dry matter intake or ingested energy is the main factor influencing animal performance (Morand-Fehr et al., 2007). Intake of forage is generally closely related to digestibility and cell wall content (Van Soest, 1994). The effect attributed to the cell wall is due to an interaction among fill, rumen stretch, time available for eating and energy density (Van Soest, 1994). For ensiled forages this intake relationship is somewhat weaker due to formation of fermentation products during the fermentation process (Huhtanen et al., 2002). The experimental silages (Paper I-III) were preserved with a relatively high amount of an acid-based additive to ensure good fermentation quality and minimize differences caused by variable preservation conditions. The fermentation quality was good but there were, however, differences in the amount of total acids in the silages showing that the fermentation had been more extensive during the first harvesting time. Still we could not detect any depressed feed intake.

The comparison of how different silage qualities have affected the intake have in the literature in many cases been confounded by variation in D-value, DM concentration and fermentation characteristics. Huhtanen et al. (2002; 2007) estimated the relationship between silage parameters and intake and found that silage D-value explain silage intake better than fermentation quality, DM and NDF concentration. Huhtanen et al. (2002; 2007) reported the following relationship between the silage parameters and intake: SDMI increases linearly with increased D-value; fermentation products depress SDMI, and of these the total acids is the best SDMI predictor; increased DM concentration increases SDMI, but the mechanism behind it may be confounded with fermentation quality and digestibility.

In our study (Paper I) the most digestible silages contributed to highest SDMI. The effect of a 100 g/kg DM increase in silage D-value on silage DMI was 0.27 kg. A relative silage dry-matter intake index was prepared by Huthanen et al. (2002) and updated by Huhtanen et al.

(2007) to use in practical ration formulation system using silage quality parameters. A D-value of 680 g/kg DM, total fermentation acids of 80 g/kg DM and DM concentration of 250 g/kg is used as standard for a well-preserved silage, which has an index of 100. According to the SDMI index the experimental silages (Paper I) had indexes of 115, 105 and 95 for HT 1, 2 and 3, respectively, which fits fairly well to the relative recorded DM intakes. Intake of NDF was numerically highest when HT 2 was fed. This coincide with what was reported by Huhtanen et al. (2007); there is a curvilinear relationship between NDF intake and D-value, the maximum intake being reached at a D-value of 640 g/kg DM.

The DMI of goats rises just after parturition and reaches a maximum between 6 and 10 weeks of lactation (Sauvant et al., 1991). This is in line with the present thesis (Paper III), where the goats, with continuous feeding, reached their peak energy intake at lactation week 8 to 10. After reaching its maximum the energy intake decreased with about 0.16 MJ NEL/week as an average of all dietary treatments.

3.2 Factors affecting milk production and composition

3.2.1 Responses to grass silage quality

Improving the silage quality and digestibility by harvesting the crop at an early stage of maturity has consistently increased milk yield and milk protein concentration (Huhtanen, 1993). In the present thesis (Paper I) the milk production increased by 0.5 kg per 100 g/kg DM increase in silage D-value. Improving silage digestibility resulted in higher milk yield than seen with increased concentrate level, as also observed by Rinne et al. (1999). Higher milk protein concentration is probably related to increased intestinal supply of amino acids to the animals. Calculated over several experiments with cows, the response to increased digestibility of silage in milk protein concentration was 0.16 g/kg per 10 g/kg increase in D-value (Huhtanen, 1993). Rinne et al. (1999) found a curvilinear change in protein concentration when feeding cows grass silage of four different stages of grass maturity, and the increase was only 0.07 g/kg. This is similar as found in the present thesis (Paper I), where the increase was 0.05 g/kg per 10 g/kg increase in D-value.

Effects of silage digestibility on milk fat concentration have been variable (Huhtanen, 1993). Some work has found that higher fiber content of late cut silages increase milk fat content (e.g. Santini et al. (1992) in goats and Sutton and Morant (1989) in cows). However, high fiber silage can limit feed intake, resulting in reduced availability of metabolites for milk production and reduction in milk solid production (Sutton, 1989). This was probably the case in our experiment (Paper I), where the fat concentration was lowest when silage from HT 3 was fed. However, NDF intake per kg body weight (BW) tended to be highest when silage from HT 2 was fed, which also provided the highest milk fat concentration. Effects of date of harvest on milk fat concentration will also depend on the effects of grass maturity on rumen fermentation. Generally, diets low in fiber causes a decreased ruminal production of acetic and butyric acid, and increased production of propionic acid, the former being the principal precursors of fat synthesis in the mammary gland, and induce a decrease in the milk fat content (Sanz Sampelayo et al., 1998). However, there may be an inconsistent and unpredictable effect of grass maturity on rumen fermentation, as discussed by Rinne et al. (1997). In some work there has been found a decreased proportion of butyric acid with increased maturity of the grass ensiled, which may explain the higher milk fat content sometimes observed with early-cut silages as compared with late-cut silages (Huhtanen, 1993).

The silage harvesting time may also influence fatty acid composition in milk. Harvesting at an early stage of plant development will increase the concentration of polyunsaturated fatty (PUFA) acids in silage (Boufaied et al., 2003). This is concurrent with the fatty acid profile of our experimental silage, where the proportion of C18:3-c9c12c15 decreased with postponed harvesting time. Increased content of not protected PUFA in the diet, will mainly increase the concentration of milk C18:0 and C18:1 due to hydrogenation in the rumen, at the expense of the short and medium- chain fatty acids (Chilliard et al., 2003). In the present study (Paper II) the milk C18:0 was not affected by harvesting time and both monounsaturated fatty acids (MUFA) and PUFA in milk were more abundant at postponed harvesting time. The milk fatty acid composition was most probably more reflected by energy intake and energy balance of the animals than the diet composition.

3.2.2 Responses to concentrate level

The milk production response to concentrate supplementation depends on the quality of forage offered and is expected to be high if forage quality is low and minimal if forage quality is high (Min et al., 2005). In our study (Paper I and II) we observed that a decrease in silage quality could not be fully compensated for by increased concentrate feeding; there was no significant interaction between harvesting time and concentrate level on milk yield and composition. However, there was an increased milk yield when feeding higher level of concentrate (Paper I and II), and an increased milk protein concentration, as often seen with increased concentrate allowance (e.g. Min et al., 2005; Lefrileux et al., 2008).

Of all milk components, fat is most variable in the milk of ruminants. Milk fat content and composition can readily be modified by changing the feeding regimen (Palmquist et al., 1993). Intake of highly fermentable carbohydrates and the amount and composition of dietary fat are among the major factors affecting the milk fat content (Palmquist et al., 1993). High intakes of highly fermentable carbohydrates (usually > 50% of feed DM) may depress milk fat percentage (Palmquist et al., 1993). Biohydrogenation of polyunsaturated fatty acids in the rumen is reduced when high concentrate diets are fed. This response is associated with shifts in bacterial populations, causing a reduction in the conversion of C18:1-trans isomers to C18:0 (a shift of C18:1- t11 to C18:1- t10) in the rumen (Loor et al., 2004). When milk fat depression occurs, changes in milk fatty acid composition also take place, with a decrease in proportion of short chain fatty acids and an increase in proportion of C18 fatty acids (Palmquist et al., 1993). Goats are less sensitive than cows to milk fat depression. This is likely due to a lower ruminal yield of C18:1- t10 of goats, combined with the fact that the mammary lipogenesis seems much less responsive to post-ruminally infused C18:2-t10c12-CLA (Chilliard et al., 2007). There was no indication of any milk fat depression in the present study (Paper I - III).

In Paper II the content of short and medium chain fatty acids in milk decreased with low concentrate allowance. When energy availability is reduced, along with fat mobilization, the intermediary supply of acetate and glucogenic compounds decrease, causing less synthesis of short and medium chain fatty acids through mammary *de novo* synthesis (Palmquist et al., 1993).

3.2.3 Responses to lactation stage and energy balance

It is reported by Sanz Sampelayo et al. (1998) that goat milk production and composition are more dependent on animal energy balance than on the diet composition. The energy balance in lactating animals can be estimated by the difference between ingested energy and requested energy for body maintenance and for milk secretion. This balance is variable, according to animal milk genetic potential and lactation stage (Chilliard et al., 2003). In early lactation, goats are normally in negative energy balance and are able to mobilize efficiently from their body fat stores in order to maintain milk production (Dunshea et al., 1990; Eknæs et al., 2006; Ngwa et al., 2009). During negative energy balance milk fat synthesis is partly based on mobilized fat (NEFA) and the fat composition of milk will therefore differ from that of milk synthesized when animals are in positive energy balance. The major fatty acids in body fat stores of goats are C18:1-c9, C16:0 and C18:0 (Banskalieva et al., 2000). A study performed by Chilliard et al. (1977) revealed that there is preferentially a release of C18:1-c9 from adipose tissue when goats experience a negative energy balance. The mobilized fatty acids will be incorporated into milk fat (Palmquist et al., 1993). Chilliard et al. (2003) reported that 59% of the variability of milk C18:0 + C18:1 fatty acids was linked to changes in energy balance of the goats, while the present thesis (Paper II) estimated this relationship to be 50%. Paper II showed that milk C18:1-c9 was high when energy balance was low ($r = -0.56$, $P<0.001$) and milk C18:1-c9 was highly positively correlated ($r = 0.56$, $P<0.001$) to serum NEFA, as also found by Eknæs et al. (2006).

Both milk protein and milk fat concentration is high after parturition and then decreases during the major part of lactation in the goat (Brendehaug and Abrahamsen, 1986). This is related to a dilution effect due to the increase in milk volume until the lactation peak, and the decreased fat concentration may also be related to a decrease in fat mobilization that decreases the availability of plasma NEFA for mammary lipid synthesis (Chilliard et al., 2003). In our study (Paper III) the goats fed the highest energy diet maintained a steady milk fat concentration and fat secretion throughout the first 18 weeks of lactation, probably due to a continuous high supply of substrates from ruminal digestion.

3.3 Nutrient utilization

A high DMI may enhance milk production by increased supply of energy and nutrients available for synthesis of milk components. However, feeding strategies that increase milk yield do most often not improve nutrient efficiency for milk production. By improving the utilization of nutrients of silage-based diets the overall efficiency of milk production will increase, and thereby reduce feed costs per kg milk produced.

Low nitrogen (N) efficiency of some silage-based diets is believed to be a major cause of large N losses to the environment, and are mainly a reflection of the low efficiency of N capture in the rumen (Givens and Rulquin, 2004). Utilization of silage N for milk production is often seen to decrease as dietary CP concentration and protein balance in the rumen (PBV) increase (Huhtanen et al., 2008), as also seen in the present thesis (Paper I). The content of soluble N in grass silage and the degradability of N in the rumen is high, and increases with earlier harvesting time (Givens and Rulquin, 2004), which is in accordance with Paper I. Other factors that can influence the rumen degradation of silage N is the use of silage additives and the extent of wilting (Givens and Rulquin, 2004). The ensiling process uses a substantial proportion of the energy normally available for microbial growth, and a poor silage preservation will increase the proportion of ammonia, which, if not captured as microbial protein, will be excreted as urea (Givens and Rulquin, 2004). According to Huhtanen et al. (2008) the milk production level is less important to determine the N efficiency. The most effective strategy to improve N efficiency for milk production and to decrease N losses in manure, is to avoid feeding diets with excessively high CP concentration and especially excess ruminally degradable N.

At lower energy intake, cows are found to be more efficient in utilizing energy for milk production (Schei et al., 2005), which is also found in our experiments with goats (Paper I). If a great part of the energy intake is canalized to the body (body fat deposition), the calculated feed efficiency for milk production is lower, compared to feeding to maintain zero or negative energy balance. Conversion of energy to fat deposition is highly energy-demanding, and thus a less efficient process than milk production (Van Soest, 1994). Opposite, the energy derived from body fat mobilization is efficiently used for milk production, and will thus improve the energy

efficiency. To maintain a high energy efficiency for milk production, the best way is to avoid overfeeding and fat deposition.

3.4 Energy status of the lactating goat

Possible fat mobilization during early lactation will be a result of a strongly depressed body fat synthesis combined with an increased lipolysis (Madsen, 1988). However, body fat mobilization will to a great degree vary according to the severity of undernutrition (feeding level and duration) and to initial body fatness (Chilliard et al., 2000). When the undernutrition is moderate, initial body fatness has only a limited influence on fat mobilization in lactating ewes (Cowan et al., 1982). Dunshea et al. (1989) estimated a body fat loss of 64 g/day on primiparous goats from lactation week 2 to 5 of lactation. Eknæs et al. (2006) noted a decrease in mass of adipose tissue of Norwegian dairy goats from lactation week 2 to 18 (i.e., 7.35 to 3.87 kg). In contrast to these previous results, the goats in our study (Paper III) mainly deposited adipose tissue from lactation week 3 to 18. Only the goats fed the lowest energy diet (HT 3, LC), mobilized from their body fat store (in average 29.7 g per day), whereof the majority of the mobilization was between lactation week 2 and 8 (74 g per day). The high energy intake in this study, caused by either highly digestible silage or normal level of concentrate or both, prevented fat mobilization during the 18 first weeks of lactation. Madsen (1988) evaluated fat turnover rate in dairy goats in relation to lactation stage and feeding level, and found that lactation stage was the dominant determinant of the fat turnover rate while feeding level had insignificant effects. Our results (Paper I-III) indicated that when offering highly digestible grass silage high yielding goats were able to eat to positive energy balance even in early lactation.

Ngwa et el. (2009) compared the effect of two dietary forage levels and stage of lactation on body composition of Alpine dairy goats. Body composition was determined by slaughter measures. A low forage (40 %) diet, which had highest energy concentration, resulted in greater body fat mass than a high forage (60 %) diet. This is in line with the present thesis (Paper III), where higher energy intake increased the body fat mass. In the study by Ngwa et al. (2009) the daily change in body fat mass was not significantly affected by the dietary level, in contrast to the present study.

Energy balance is closely linked to blood concentrations of NEFA. If goats are in negative energy balance there is a high rate of lipolysis in adipose tissue which elevates the concentration of blood NEFA (Dunshea et al., 1989). NEFA contributes to milk fat secretion, and thus spare glucose and amino acids for the mammary gland (Chilliard et al., 2000). The NEFA concentration at zero energy balance was by Dunshea et al. (1989) calculated to be 0.217 mmol/l, which corresponded well with our results; 0.244 mmol/l in Paper II and 0.249 mmol/l in Paper III. In Paper III NEFA concentrations were above this respective level from kidding until lactation week 5 as a mean for all dietary treatments. Eknæs et al. (2006) reported NEFA concentration above this levels from kidding until the 7th month of lactation. Here the goats grazed on mountain pasture from the 2nd to 4th month of lactation, thus having a presumed lower energy intake than the goats in the present experiments (Paper I-III). Dunshea et al. (1989) observed that when goats moved into positive energy balance after lactation week 5, the increased fat deposition was not necessarily associated with reduced NEFA concentration, indicating that NEFA concentrations are of limited value for quantifying energy surplus.

3.5 Body condition

The goats in the present experiments were allocated to 3 blocks according to their body condition (poor, medium or high) before the start of the experiments. This blocking was done because initial body condition could affect both intake-, milk- and energy status parameters. As goats deposit most of their body fat as visceral fat (Colomber-Rocher et al., 1992; Marinova et al., 2001), scoring of body condition may be difficult in goats. In Paper I and II the body mass index (BMI) (BW/neck height^2), the same as used for humans, was used as a measure of body condition. A goat body mass index has previously been applied also by Tanaka et al. (2002). In Paper III CT scanning and a visual determination of the goats' body condition from the scans were used to assign the goats into the blocks.

There is an inverse relationship between fatness and food intake in ruminants, and a part of the reason is the reduction in abdominal capacity to accommodate the digestive tract with the increase in volume of abdominal fat (Forbes, 1993). In Paper I we observed that intake of DM

per kg BW tended to be higher for the goats with poor body condition compared with goats with medium or high body condition. A low body condition at the time of kidding would restrict the pool of nutrients available for use in support of milk production (Ngwa et al., 2009). In our study (not published) we observed that goats with initial poor or medium body condition tended to produce less ECM and milk fat per day than goats in high body condition.

Due to possible mobilization in early lactation it is important that body fat at the time of parturition is adequate to maintain milk production to their genetic potential (Ngwa et al., 2009). Adequate visceral fat at the time of parturition would also minimize mobilization of protein tissue (Ngwa et al., 2009). In our study, goats with a high body condition at kidding maintained a low level of milk FFA during the first 18 weeks of lactation (Paper II). High body condition at kidding was also related to a high sensory milk quality. The present study suggested that goats in high condition at kidding, with body fat available for mobilization, are most likely to produce high quality milk throughout early and mid lactation. Eknæs et al. (2006) suggested that goats produce milk with a low FFA concentration as long as they have body fat mass to mobilize.

3.6 Factors affecting milk quality

The measures of milk quality in this discussion are considered to be milk FFA concentration and sensory milk taste.

3.6.1 Nutrient supply

One objective of the present thesis was to study whether a high energy intake provided by improved grass silage quality could improve milk quality. No other published results have been found on feeding forages to goats and their effects on milk lipolysis. Chazal et al. (1987) studied the level of FFA in milk from cows in late lactation. First the cows were on pasture, and then they were fed hay or grass silage indoors, both of high nutritive value. Feeding grass silage enhanced FFA compared with pasture or hay. Increased level of lipolysis occurring with grass silage was presumed to result from the method of forage conservation. In the present thesis the earliest harvested silage caused numerically highest level of milk FFA in the change-over study (Paper I and II), and a tendency ($P = 0.09$) to highest FFA in the continuous study (Paper III).

The extent of lactic, acetic and propionic acid fermentation was somewhat lower with delayed harvesting time (Paper I), and there was a tendency of a positive correlation between milk FFA and total acids in silage ($r = 0.21$, $P = 0.07$). Further studies are required to establish whether there is a relationship between silage fermentation quality and milk FFA concentration.

Forages may give milk an off-flavor (predominantly feed flavor) when fed both fresh and preserved, the latter by substances produced during silage fermentation (Randby et al., 1999). There is identified a large number of fermentation products that are able to impart off-flavors to milk (Morgan and Pereira, 1962). Feed flavor was observed to a small extent in the present study (Paper II). The most dominant type of off-flavor was tart flavor, which is characterized as a lipolyzed flavor, which has got its term from the lipase-catalyzed hydrolysis of milk fat triglyceride. Other flavors in this category are rancid, goat and bitter flavors (Shipe et al., 1978). Goat flavor seems to appear at lipolysis levels much lower than those responsible for the tart and rancid off-flavors (Chilliard et al., 2003). Even-numbered fatty acids, C4:0 and C6:0-C10:0 are the major contributors to lipolyzed flavors (Scanlan et al., 1965).

Generally, energy supplementation, by increased concentrate allowance, will improve the energy balance for goats in early and mid lactation. In our study (Paper II) the goats fed the highest concentrate level produced milk with highest FFA concentration. Eik et al. (1991) observed no change in milk FFA content when different levels of concentrate were fed to dairy goats in different stages of lactation. Eknæs and Skeie (2006) found that milk FFA in grazing goats were not affected by concentrate level, but goats given the highest concentrate level produced milk with a lower frequency of off-flavor in mid lactation.

Supplementing diets with sources of polyunsaturated fatty acids has unanimously decreased the milk LPL activity. Chilliard et al. (2003) found that LPL activity and spontaneous lipolysis decreased in goats fed hay- or corn silage-based diets when fat was added (unprotected C18:1-, C18:2- and C18:3-rich oils). Bernard et al. (2005) also reported that supplementing the same fatty acids sharply decreased milk LPL activity. Chilliard et al. (2003) hypothesized that milk LPL activity decreased when supplemental lipids were fed because more mammary LPL was directed towards the basal membrane of the secretory cells, where it is needed to allow the

uptake of blood triglycerides, and less LPL enzyme was transported in the mammary alveolar cells towards the milk. A study by Eknæs et al. (2009) revealed that feeding concentrate with a high fat supplement, consisting mainly of the saturated long chain fatty acids C16:0 and C18:0, increased the C16:0 proportion in milk and reduced the frequency of rancid and tart taste of milk, but did not affect the milk FFA concentration. However, Astrup et al. (1985) showed that feeding concentrate added C16:0 and C18:0 fatty acids increased the respective fatty acid in milk and tended to reduce the level of milk FFA and the goat flavor in milk. In the present study (Paper II) a high proportion of C16:0 fatty acid in milk was rather related to poor milk quality, both as regards FFA concentration and milk taste quality. While a high C16:0 proportion in milk may have been supplied to the udder mainly from feed supplements and body fat stores in the referred studies (Astrup et al., 1985; Eknæs et al., 2009), it was mainly a product of *de novo* synthesis in the present study.

Plasma cholesterol is found to increase with increased level of fat intake (Palmquist and Conrad, 1978), which is in accordance with the present results (Paper II); cholesterol in serum was higher with earlier harvesting time. Astrup et al. (1985) observed an increased level of cholesterol in blood when feeding concentrate added C16:0 and C18:0 fatty acids. Cholesterol is one of the important precursors in synthesis and stability of the MFGM (Nielsen and Jakobsen, 1994; Evers, 2004). Eknæs (2009) hypothesized that an increased intake of C16:0 increases the cholesterol level in blood, and thus improves the stability of the MFGM. When mammary *de novo* synthesis of fatty acids is high, mammary cholesterol synthesis is found to be low (Smith et al., 1986). This indicates that when *de novo* fatty acid synthesis is high plasma supply of cholesterol may be most important for the maintenance of MFGM synthesis (Nielsen and Jakobsen, 1994). We observed (Paper II and III) that goats fed the highest energy diet had highest proportion of short and medium chain fatty acids in milk, which suggests that a high supply of substrates from ruminal digestion which supported both high milk production and deposition of adipose tissue, could give a lack of precursors for MFGM through suppression of mammary cholesterol synthesis and therefore caused high FFA and poor milk quality.

3.6.2 Responses to lactation stage and energy balance

According to Chilliard et al. (2003) the goat milk lipolysis and LPL activity are at their highest after the lactation peak, and are low before week 4 and after week 30 of lactation. In the present study (Paper III) milk FFA was clearly lowest at lactation week 2, and the highest concentrations were found after the lactation peak, in accordance with Chilliard et al. (2003). Further, the level increased when the goats were let out to mountain pasture. Eknæs et al. (2006) showed that substantial energy mobilization in early lactation did not elevate milk FFA content. However, in lactation week 11-18, when the goats were let out on pasture, the goats started to produce milk of inferior quality. The effect of energy intake and stage of lactation will be confounded in these situations.

In a study with early lactating goats reviewed by Chilliard et al. (2003), milk LPL activity and lipolysis decreased during a 2-days fasting period, and rebounded at the beginning of the refeeding period. Similarly, Eknæs and Skeie (2006) observed a decrease in milk FFA concentration and a higher sensory quality during starvation (2 days with no pasture and restrictive hay supplementation). These short time fasting experiments had similar outcome as 16 weeks of low energy feeding (HT 3 with LC) in the present thesis (Paper II and III). The high quality milk seemed to have been produced by goats in negative or low energy balance that received fatty acids for milk fat secretion from mobilized body fat. This is supported by the correlations between high milk FFA and both low serum NEFA and high energy balance found in Paper II.

The LPL in adipose tissue hydrolyzes plasma triglycerides at the surface of capillary endothelial cells and supplies adipocytes with fatty acids (Borensztajn et al., 1972). Its activity is dependent on the nutritional status of the animals. At negative energy balance, when fat are mobilized, LPL activity in adipose tissue is low (Borensztajn et al., 1972). Chilliard et al. (1977) observed a low LPL activity in adipose tissue of goats in negative energy balance in early lactation. When the animals return to a positive energy balance the adipose tissue LPL activity will increase (Borensztajn et al., 1972; Chilliard, 1985). A high level of adipose tissue LPL activity is accompanied by high blood glucose content (Chilliard et al., 1977), which indicate that adipose tissue LPL activity is regulated by blood glucose and insulin (Borensztajn et al., 1972). Related

to our experiment, the goats with highest energy supply were never in negative energy balance and deposited adipose tissue during the first part of lactation. We could suggest that these goats had a high adipose tissue LPL activity from the start of their lactation, and as the activity of adipose tissue LPL is positively correlated to milk LPL activity (Chilliard et al., 1979), it can partly explain the tendency of highest milk FFA when the highest energy ration were fed. The first study (Paper II) also showed that blood glucose is positively correlated to milk FFA.

CONCLUDING REMARKS

Silage production will remain a key part in Norwegian goat production as well as in ruminant production systems in general. By increasing the digestibility of the grass silage, by harvesting at an early stage of maturity, the feed and energy intake increased notably. The intake was mainly dependent on the silage digestibility. The higher energy and protein intake, obtained when the earliest harvested silage was offered, increased daily yields of milk, ECM and milk constituents of fat, protein and lactose. Improving silage quality by earlier harvesting resulted in higher feed intake and milk yield than seen with increased concentrate level. A decrease in silage quality could not be fully compensated for by increased concentrate feeding. Utilization of nutrients to milk production by very early harvesting time was lower than by postponed harvesting time.

All experiments in this thesis showed that the goats were in positive energy balance when they were offered the earliest harvested silage. This study indicates that it is possible to feed goats in early lactation with sufficient energy to give a high milk yield and at the same time avoid fat mobilization. During the first 18 weeks of lactation, only the goats fed the lowest energy diet mobilized from the adipose tissue. The rest of the goats deposited body fat throughout this period. This study suggests that a high energy balance during early and mid lactation increase lipolysis in milk, maybe caused by increased milk LPL activity and reduced stability of the MFGM.

FUTURE PERSPECTIVES

The problem with poor milk quality has a very high focus in the Norwegian goat milk production. This thesis aimed amongst other to study whether a high quality silage can improve the goats energy balance in early lactation and hence lighten the problem with poor milk quality. It was clearly possible to improve their energy balance, but there were, however, some implications of poorer milk quality at increased energy balance. It must be stated that this was evaluated during the first 4 months of lactation, whereas the problem with the milk quality is most prominent during mid-lactation at mountain pasture. To understand the effect of different energy status and the variation in milk quality during different stages of lactation, there is need of more basic knowledge about the lipolytic system of the goat. The relationship between the metabolism at low energy intake and the correlation between adipose tissue LPL and milk LPL activity require more research. A high body condition at kidding seemed to have a positive effect on the milk quality throughout the first part of the lactation. It is however of interest to examine whether this effect is caused by a hereditary correlation between high body condition and low milk lipolysis, or if milk lipolysis can be reduced by increasing the body condition of the goats in the dry period. It is also of interest to do an additional thorough evaluation of how the energy supply and energy status of the goats in the indoor feeding period affect the milk quality later at pasture.

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Paper I

Effects of grass silage harvesting time and level of concentrate supplementation on nutrient digestibility and dairy goat performance

Ingjerd Dønnem, Åshild T. Randby, Margrete Eknæs

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Effects of grass silage harvesting time and level of concentrate supplementation on nutrient digestibility and dairy goat performance

I. Dønnem, Å.T. Randby, M. Eknæs

Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway.

Corresponding author:

Ingjerd Dønnem

ingjerd.donnem@umb.no

Phone: +47 64966037

Fax: +47 64965101

Abstract

The effect of harvesting time (HT) of timothy-dominated grass silage and level of concentrate on the chemical composition of silage, and on feed intake and milk production by Norwegian dairy goats, were evaluated. The silages were prepared from the primary growth at three stages of maturity: very early (HT 1), early (HT 2) and normal (HT 3). The silages were fed *ad libitum* to 18 goats of the Norwegian Dairy Goat breed in early lactation and supplemented with a low (LC; 0.6 kg per goat daily) or normal (NC; 1.2 kg per goat daily) level of concentrate. The experiment was conducted as a cyclic changeover design with four periods of 28 days using three blocks of goats according to their initial body condition (poor, medium or high body condition). Silages contained 771, 696 and 619 g digestible organic matter per kg dry matter in silage (D-value) for HT 1, 2 and 3, respectively. Postponing the harvesting time decreased ($P < 0.001$) silage dry matter intake (DMI) and silage DMI per kg body weight (BW). Increased concentrate allowance decreased silage DMI, with substitution rates (decrease in silage DMI when concentrate dry matter intake is increased, kg/kg) of 0.43, 0.21 and 0.27 at HT 1, HT 2 and HT 3, respectively. Milk yield and yields of milk constituents decreased ($P < 0.001$) with delayed harvesting time and thus reflected the changes in silage D-value. Milk free fatty acids (FFA) concentration was not affected by dietary treatments. The efficiency of nutrient utilization was best when LC was fed and increased with postponed harvesting time. The higher energy efficiency of the HT 3 LC fed goats indicates that these goats canalized a higher proportion of energy intake to milk production, compared to goats fed NC and earlier harvested silage. Marginal ECM production response to increased net energy lactation (NEL) intake were higher when intake was increased due to higher silage digestibility (0.14 kg ECM/ MJ NEL) compared with increased NEL intake due to increased concentrate level (0.12 kg ECM/ MJ NEL). Improving silage quality by earlier harvesting time resulted in higher feed intake and milk yield than obtained by the same increase in NEL intake by concentrate supplementation.

Keywords: Dairy goats, grass silage harvesting time, concentrate, feed intake, milk production.
Abbreviations: AAT, amino acids absorbed from the small intestine; aNDFom, NDF assayed with alpha amylase and expressed exclusive of residual ash; BMI, body mass index; BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; D-value, digestible organic matter in dry matter; ECM, energy corrected milk; FFA, free fatty acids; ME, metabolizable

energy; N, nitrogen; NDF, neutral detergent fiber; NEL, net energy lactation; PBV, protein balance in the rumen, SDMI, silage dry matter intake;

1. Introduction

There are about 40 000 dairy goats in Norway and cheese products from goat milk are the main products of goat farming. The level of dry matter intake or ingested energy is the main factor influencing yield and composition of the milk (Morand-Fehr et al., 2007). Due to the cold climate in Norway, the grazing period is restricted and the goats are fed indoors for 7-9 months per year. This makes grass silage the most important feed resource. Timothy grown with meadow fescue and red clover in a multispecies sward is the main forage crop for silage. The silage fed has traditionally low digestibility and high content of fiber. It is generally recognized that increasing the amount of fiber depresses digestibility of the dietary components in the feeds (Santini et al., 1992). Underfeeding of the goats appears to be an extensive problem, especially in early and mid lactation (Hadjipanayiotou and Morand-Fehr, 1991), resulting in energy mobilization (Eknæs et al., 2006). Improvements of the grass silage quality could potentially increase feed intake and milk yield. There are a number of factors affecting silage quality (Charmley, 2001). However, young stage of maturity at harvest and consequently highly digestible feed is a prerequisite for high intake and better performance (Sauvant et al., 1987; Rinne et al., 1999; Huhtanen et al., 2007).

In the dairy goat winter diet, grass silage is supplemented with concentrate feeds of varying amount. Increased energy and protein intake from concentrate could to some extent solve the underfeeding problem. However, due to the large land area covered by grass, and the cultural value of Norwegian goat farming (grazing on natural pasture), improved grass silage quality should be the main focus on farm practices. The marginal response of dairy cows to increased energy intake (kg ECM per additional MJ NEL) has been found to be higher from improved silage digestibility than from increased concentrate level (Rinne et al., 1999), another argument for putting effort in correct timing of the silage harvest.

The impact of harvesting time of the grass silage on intake by cattle has been described in several studies (e.g. Rinne et al., 1999; Dawson, 2002; Kuoppala et al., 2008; Randby et al., 2010). However, as cattle and goats are reported to have different digestive capacities for silage (Tolkamp and Brouwer, 1993), it may be difficult to draw conclusions about goats from these

reports. As far as known, no experimentation investigating the effect of grass silage harvesting time on goats' performance exists. Therefore, the objective of this work was to evaluate the effects of grass silage harvesting time and two levels of concentrate on dairy goats' performance.

2. Materials and methods

2.1. Silage preparation

The silages were made at the experimental farm at Ås, Norway (60°N, 11°E) in 2007 from two fields, one established in 2004 and the second in 2005. The crop was harvested from the primary growth at three stages of maturity (harvesting times (HT)): (1) Very early (HT 1), 23-24 May, (2) Early (HT 2), 4 June and (3) Normal (HT 3), 13 June. The sward consisted of timothy (*Phleum pretense*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pretense*) in proportions of 0.47, 0.37 and 0.15, respectively. Other species and weeds made up 0.01. Phenological development stage of timothy at harvest was determined as mean stage by weight (MSW) (Moore et al., 1991). The MSW values of 2.26, 2.93 and 3.19 for the three harvesting times, respectively indicated that stage 1 and 2 were dominated by tillers in stem elongation with 2 and 3 visible nodes, respectively, and stage 3 was dominated by tillers with visible head, but without head stems (early heading). After mowing, the grass was wilted for 1 to 10 h during daytime or 16 to 20 h over night to achieve a target dry matter (DM) content of 250 g/kg DM. Weather conditions during harvest were mainly sunny with only a light rainfall during the first harvesting time. The grass was baled using an Orkel GP 1260 (Orkel AS, Fannrem, Norway) roundbaler with 20 fixed knives, and preserved with the acid-based additive GrasAAT N-Lacto (730 g formic acid and 15 g lactose per kg; Addcon Nordic AS, Porsgrunn, Norway) applied at a rate of 4.4 L/ton. The bales were wrapped in 6 layers of 0.025 mm thick and 750 mm wide white plastic film (Trio Wrap, Trioplast AB, Sweden). Each bale was weighed (720 to 894 kg on fresh weight basis) and sampled to measure DM yield. Before feeding, the silage was processed through a mixer wagon (Euromix I 1070, Kuhn, Saverne, France) to reduce the possibilities for feed selection and enhance the intake, and then fed within four days. The median particle length of the silages was measured by hand sorting.

2.2. Experimental design, animals and diets

The experiment was carried out with 18 goats of the Norwegian Dairy Goat breed in 2nd to 8th lactation which kidded between 8th and 21st of January 2008. Average body weight (BW) 2 days after kidding was 63±10.5 kg. The experiment started on February 6th after a preparation period for nearly two weeks. It was conducted as a cyclic changeover design (Davis and Hall, 1969) with 4 periods of 4 weeks using three blocks each of six goats. The goats were assigned to blocks according to their body condition, where block 1: poor body condition; block 2: medium body condition and block 3: high body condition. As goats deposit most of their body fat as visceral fat (Colomber-Rocher et al., 1992; Marinova et al., 2001) scoring of body condition of goats may be difficult. Body mass index (BMI) (BW/neck height^2), the same as used for humans, was therefore used as a measure of body condition. A goat body mass index has previously been applied also by Tanaka et al. (2002). The goats within blocks were allocated randomly to one of six treatments. Thus 3 goats (one from each block) were fed the same diet in each period. The six treatments consisted of three silage qualities and two concentrate levels. Animals were offered silage from HT 1, 2 or 3 *ad libitum* and either low (LC; 0.6 kg per goat daily) or normal (NC; 1.2 kg per goat daily) level of concentrate. The concentrate was a mixture made with the following composition (per kg): 120 g extracted soybean meal, 50 g heat-treated, extracted rapeseed meal (ExPro 00E), 523 g barley meal, 175 g oats, 50 g wheat bran, 45 g molasses, 10 g Nutrifeed, 5.7 g limestone powder and 21 g minerals and vitamins. A mineral and vitamin mixture (Felleskjøpet, Norway) was supplied (25 g) to the goats fed low concentrate level. The mixture consisted of (per kg) 170 g Ca, 35 g P, 45 g Mg, 65 g Na. The first week of each period was regarded as an adaptation week and was not included in the statistical analysis to reduce the possible carry-over effects.

2.3. Animal management

The goats were housed in individual stalls and were milked in a milking stable twice a day at 06:30 and 16:00 h. Milk yield was measured evening and morning for three consecutive days per week, and individual milk samples were collected at the same time. Grass silage was offered for *ad libitum* intake twice daily at 06:00 and 15:00 h such that silage residues averaged 10%. Concentrate was distributed four times per day, at each milking and two hours after each milking. Individual feed intake was recorded four consecutive days per week. The goats were

weighed in the beginning of week 2 and end of week 4 of each period at 12.30 pm for three consecutive days.

2.4 Digestibility and rumen in sacco studies

In vivo apparent digestibility of the silages and concentrate was measured with sheep by total faecal collection. The study was conducted as a Latin square design for silages, with 3 sheep, 3 periods and 3 silages. The silages were given at maintenance level; 900 g silage-DM per sheep daily. Concentrate digestibility was also measured using 3 sheep fed 400 g silage-DM (HT 3) plus 500 g concentrate-DM per day. 10 g mineral mixture and 10 g NaCl was supplemented per day. Sheep were fed twice daily and had free access to drinking water. In sacco degradability of feeds was studied according to the NorFor standard 070910 (http://www.norfor.info/Files/pdf-dokumenter/pdf_lab/Analyses/NorFor_in_sacco_standard_070910.pdf).

True digestibility of rumen undigested N was measured in duodenally fistulated cows using the mobile bag technique (Hvelplund et al., 1992), incubating feed residues after 24 (silage) and 16 h (concentrate) rumen incubation. The concentration of amino acids absorbed from the small intestine (AAT) and protein balance in the rumen (PBV) were calculated according to Madsen et al. (1995). Silage metabolizable energy (ME) and NEL concentration from the in vivo (sheep) digestibility trial were calculated according to the Dutch NEL- system (Van Es, 1975; 1978).

2.5. Estimation of feed efficiency and requirements

Utilization of NEL was calculated both as milk energy output/ (NEL intake-NEL for maintenance) and milk energy output/ NEL intake (included maintenance requirement), expressed as NEL and NEL_{IMR}. AAT utilization was calculated as milk protein output/ (AAT intake-AAT for maintenance). The effects of BW gain were ignored. NEL requirements for maintenance were assumed to be 0.2560 MJ per kg $BW^{0.75}$, according to Van Es (1975; 1978). AAT requirements for maintenance were calculated as 3.25 g AAT per kg $BW^{0.75}$, according to Verite and Peyrand (1989). Energy balance was calculated as NEL intake less NEL for maintenance and milk production. Energy requirements for milk production (NEL, MJ/day) was calculated as 3.036 x ECM + 0.00503 x ECM^2 (ECM in kg/day), according to Van der Honing and Alderman (1988) and AAT (g/day) requirement for milk production was calculated as 40 x ECM + 2.0 x ECM^2, according to Madsen et al. (1995).

2.6. Sampling and chemical analyses of feeds

A core sample of fresh crop was taken from each bale during harvesting and composited to samples of approximately 15 bales. Silage was sampled once a week during feed out, and stored for -20°C until analysis. The crop samples and fresh silage material were dried at <60°C to constant weight and weighed warm to obtain DM concentration. Subsequently, samples were adjusted to room temperature and humidity. The samples (composited to one silage sample per period) were milled on a Retsch Impeller-Type Cutting Mill SM 1 (Retsch GmbH & Co. KG, Haan, Germany) to pass a 1.0 mm screen prior to analyzes of DM, neutral detergent fiber (NDF) and total- nitrogen (TN) for the fresh crop samples, and DM, NDF, NDF-N, acid detergent fiber (ADF), lignin, TN, ash, and crude fat for the silage. Content of DM of the dried samples was determined by further oven-drying for 4 h at 103°C. The concentration of NDF was analyzed according to Mertens et al. (2002) using Na-sulphite, alpha amylase and ash correction (aNDFom), and ADF was analyzed according to Method 973.18 (AOAC, 2000) with the modification that the samples were not washed with acetone, and were corrected for ash (ADFom). The concentration of lignin was analyzed according to Robertson and van Soest (1981) with permanganate (lignin (pm)). Acid detergent lignin was determined according to Van Soest et al. (1991). Total-N was analyzed by the Kjeldahl method on a Kjeltech Auto 1035/38 (Tecator AB, Höganäs, Sweden) using a Cu catalyst. Ash content was determined by ignition of the dried sample at 550°C for 4 h. Crude fat was determined after hydrolysis of the samples with 3 M HCl, extraction with petroleum ether, and then distillation of the eluent followed by drying and weighing the residues. The N content of NDF residues (NDF-N) was determined as for total N.

Fresh samples of the silage from each period were analyzed for NH_3-N, pH, water- soluble carbohydrates (WSC), organic acids, and ethanol. Content of NH_3-N was analyzed with an electrode on a PC-titrate (Man-Tech, Ontario, Canada). Samples were diluted with distilled water and stored at 4^{o}C for at least 12 hours before pH was measured with Termo Orion 420A+ pH-meter with Orion 9107BN electrode. Carbohydrates were extracted in 0.05 M Na-acetate buffer for the determination of WSC contents. Sucrose and fructans were hydrolysed with 0.074 *M* H_2SO_4 in 90 ^{o}C for 70 min. Monosaccharides were further converted to glucose-6-phosphate and fructose-6-phospate by an enzymatic method using kit K-FRUGL (Megazyme, Wicklow,

Ireland). The concentrations were determined spectrophotometrically by the increase in absorbance of NADPH at 340 nm. Organic acids and ethanol were analyzed by HPLC using a VA 300/7.8 Nucleogel Ion 300 OA column (Machery-Nagel) at 50^{o}C (mobile phase, 0.010 M H_2SO_4 at 0.6 ml/min) with a UV spectrophotometric detector for lactic acid (LA) and a refractive index detector for other acids and ethanol. Oven DM contents of the silages were corrected for volatile loss according to Norfôr DM determination 070921 (http://www.norfor.info/Files/pdf-dokumenter/pdf_lab/Analyses/NorFor_DM_Determination_070921.pdf).

Samples of silage from each harvesting time was taken out to the digestibility study and *in sacco* study and analyzed as described for silages. In addition they were analyzed for crude fiber according to method 962.09 (AOAC, 2000). Silage residues were analyzed in order to study possible selection. Residues from all animals fed the same silage were sampled twice a week, composited to one sample per week and analyzed for content of DM and aNDFom according to procedures described for silages. Concentrate was sampled from the whole batch in period 1, 2, and 3 and analyzed for DM, NDF, TN, starch, ash, crude fat and WSC. Content of DM was determined by drying for 4 h at 103°C and starch was determined by an enzymatic method (α-amylase and amyloglucosidase) (Megazyme, Wicklow, Ireland). A sample of concentrate was taken out to the digestibility study, and in addition to analyses described above, analyzed for crude fiber as for the silages.

2.7. Chemical analyses of milk

Milk was preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4°C. Weekly milk samples from each goat were analyzed for fat, protein, lactose, urea and free fatty acids (FFA) content with an infrared milk analyzer (Milkoscan 6000, Foss- Electric, Hillerød, Denmark) within 4-7 days after sampling. Three sampling points for FFA were left out of calculations because of long storage time before analysis (11-13 days). Energy corrected milk (ECM) yield was calculated according to Sjaunja et al (1990). Content of milk solids was calculated by adding up fat, protein and lactose content.

2.8. Statistical analysis

2.8.1. Silage composition

Statistical analysis of the silage composition was conducted using the GLM procedures of SAS (SAS, 2003) according to the model $Y_{ij}= \mu + D_i + E_{ij}$, where Y_{ijk}= the dependent variable; μ= the overall mean; D_k= effect of silage k, k=1,…3; and E_{ij} = random residual.

2.8.2. Animal Performance

Statistical analysis from the animal performance experiment was conducted using results from the last 21 days of each experimental period. The data was analyzed using the MIXED procedures of SAS according to the model $Y_{ijklmno}= \mu + B_i + G_{j(i)} + P_k + H_l + L_m + C^{(H)}_n + C^{(L)}_o + C^{(HL)}_{no} + HL_{lm} + BP_{ik} + BH_{il} + BL_{im} + PH_{kl} + E_{ijklm}$, where $Y_{ijklmno}$ = the dependent variable; μ= the overall mean; B_i= effect of block i, i=1,…3; $G_{j(i)}$ = effect of goat j within block i, j = 1,…6; P_k = effect of period k, k = 1,…4; H_l = effect of harvesting time l, l = 1,…3; L_m = effect of concentrate level m, m = 1, 2; $C^{(H)}_n$ = carry-over effect of harvesting time l, l = 1,…3; $C^{(L)}_o$ = carry-over effect of concentrate level m, m = 1, 2; $C^{(HL)}_{no}$ = carry-over effect of interaction between harvesting time and concentrate level; HL_{lm} = interaction between harvesting time l and concentrate level m; BP_{ik} = interaction between block i and period k; BH_{il} = interaction between block i and harvesting time l; BL_{im} = interaction between block i and concentrate level m; PH_{kl} = interaction between period k and harvesting time l; and $E_{ijklmno}$ = random residual. All terms were considered fixed, except for $G_{j(i)}$, which was considered random.

2.8.3. Digestibility trial and in sacco incubation

Digestibility trial with sheep (silage) was analyzed according to the model $Y_{ijk}= \mu + A_i + P_j + D_k + E_{ijk}$, where Y_{ijk}= the dependent variable; μ= the overall mean; A_i= effect of animal i, i=1,…3; P_j= effect of period j, j=1,…3; D_k= effect of diet k, k=1,…3; and E_{ijk} = random residual. In sacco incubation was analyzed with the same model with the exception that the effect of period was omitted. Both analyses were done using the GLM procedures of SAS.

For all models applied, means are reported, mean separation was done by least significant difference and treatment effects were declared significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3. Results

3.1. Herbage, silage and concentrate nutritive characteristics

The composition of the original herbage before the ensiling process of HT 1, 2 and 3 were: DM, 223, 227, 225 g/kg; CP, 158, 130, 104 g/kg DM; aNDFom, 408, 493, 546 g/kg DM; WSC, 169, 95, 91 g/kg DM. The calculated DM yield was 3350, 5210 and 6250 kg per hectare for HT 1, 2 and 3, respectively. Table 1 presents the chemical composition of the silages and the concentrate. The concentration of protein in the DM decreased and aNDFom concentration increased with postponed harvesting time. All the silages were of good fermentation quality. However, the extent of lactic, acetic and propionic acid fermentation was reduced with delayed harvesting time. The concentration of WSC in silage was low compared to the concentration in the herbage at all harvesting times.

The median particle length of the offered silages was 20, 22 and 21 mm for HT 1, 2, and 3, respectively. The silage residues had aNDFom contents of 439, 538 and 597 g/kg DM of HT 1, 2 an 3, respectively, which were close to silage aNDFom contents, showing no extent of feed selection.

3.2. Digestibility and rumen in sacco

Results from the digestibility trial in sheep of apparent digestibility, in-sacco degradation parameters of total N, aNDFom and starch, and estimated values for ME, NEL, AAT and PBV are presented in Table 2. Postponing the harvesting time decreased ($P \leq 0.01$) the apparent digestibility of all the nutrients and the digestible organic matter per kg DM (D- value) in silage. Postponing the harvesting time also decreased ($P \leq 0.01$) soluble protein, degradable protein and estimated values for ME, NEL, AAT and PBV, and increased indigestible NDF ($P = 0.001$).

3.3. Feed and nutrient intake and body weight change

Silage and total intakes expressed in kg DM/day and in g per kg BW increased ($P < 0.001$) with earlier harvesting time (Table 3). Compared to normal harvesting time (HT 3) the silage intake increased by 11 % at early harvesting time (HT 2) and 32 % at very early harvesting time (HT 1). Postponing the harvesting time decreased ($P < 0.001$) the estimated intakes of NEL, AAT and PBV. aNDFom intake per kg BW tended ($P = 0.08$) to be higher when HT 2 compared

to HT 1 was fed. Increase in concentrate allowance decreased ($P < 0.001$) silage intake by 0.21, 0.10 and 0.14 kg, which corresponds to substitution rates (decrease in silage DMI when concentrate DMI is increased, kg/kg) of 0.43, 0.21 and 0.27 at HT 1, HT 2 and HT 3, respectively. Goats fed 1.2 kg concentrate per day (NC) had 16% higher ($P < 0.001$) total DMI per kg BW than those on 0.6 kg concentrate per day (LC). BW gain (g/day) was almost 4-fold higher ($P = 0.001$) for goats on NC than on LC. The energy balance decreased ($P < 0.001$) with postponed harvesting time and low level of concentrate. Only HT 1 provided a positive energy balance.

3.4. Milk production and composition

There was a positive effect ($P < 0.001$) of earlier harvesting time on the daily yield of milk, ECM and the milk constituents fat, protein and lactose (Table 4). Compared to normal harvesting time (HT 3) milk yield increased by 0.27 kg at early harvesting time (HT 2) and 0.69 kg at very early harvesting time (HT 1). The milk protein and fat concentrations were lower ($P \leq 0.05$) at normal harvesting time than at HT 1 and HT 2. Concentration of urea in milk was only affected ($P < 0.001$) by level of concentrate where NC showed 8% higher values than LC.

Increase in concentrate allowance stimulated to higher daily yields of milk, ECM, and all the milk constituents and also protein concentration (Table 4). Increased concentrate level increased daily milk yield by 0.26, 0.41 and 0.42 kg for goats fed silage from HT 1, 2 and 3, respectively, but the interaction HT × C was not significant ($P = 0.51$). Milk FFA was not affected by harvesting time ($P = 0.39$) but increased with increased concentrate level ($P = 0.03$).

3.5. Effects of body condition

Intake and weight parameters that were, or tended to be affected by body condition (block) are presented in Table 5. Goats with poor body condition had a lower ($P < 0.04$) silage and total intake of both DM and aNDFom than goats with medium or high body condition. However, poor body condition tended ($P = 0.06$) to increase total DMI per kg BW compared with medium or high body condition. Goats with poor and medium body condition gained more ($P < 0.02$) body weight than goats with high body condition.

3.6. Efficiency of nutrient utilization

The effects of experimental treatments on the efficiency of nutrient utilization are presented in Table 6. Nitrogen utilization (calculated as N output in milk/N intake) was lower ($P < 0.001$) at HT 1 than of later harvested silage. Postponing the harvesting time increased ($P < 0.001$) the apparent efficiency of utilization of AAT, NEL and NEL_{IMR}. Increase in concentrate allowance decreased ($P \leq 0.02$) utilization of N, AAT, NEL_{IMR}, and feed efficiency (ECM produced per kg DMI). There was an interaction between HT and concentrate level for NEL utilization, where NC decreased the utilization by 0.02, 0.08 and 0.16 for HT 1, 2 and 3.

4. Discussion

4.1. Characterization of the silages

The experimental silages were preserved with the addition of a relatively high amount of an acid-based additive to ensure good fermentation quality and minimize differences caused by variable preservation conditions. The total content of fermentation products decreased with postponed harvesting time, in line with decreased consumption of WSC during silage fermentation. Although energy concentration was indeed high in silage from HT 1, the protein concentration was at a moderate level, and lower than often observed in such very early harvested silages (Rinne et al., 1999). This was probably due to a moderate spring fertilization of the sward (70 kg N/ha).

The depression of digestibility of aNDFom was higher between HT 2 and HT 3 than between HT 1 and HT 2. Also indigestible NDF and indigestible N, as found by in-sacco studies, indicated greater differences in digestibility between HT 2 and HT 3 than between HT 1 and HT 2.

The average daily decrease in silage D-value was 7.2 g/kg DM (5.8 g/kg between HT 1 and HT 2, and 9.6 g/kg between HT 2 and HT 3). This mean daily decrease was somewhat higher than earlier reported (i.e. 4.8 g/kg DM in Rinne et al. (1999) and 5.0 g/kg DM in Kuoppala et al. (2008)). Rinne et al. (1999) suggested that cumulative temperature explains D-value better than the date of harvest or the chemical composition of the grass. In the current experiment the mean day temperature was 12°C between HT 1 and HT 2, and increased to 20°C between HT 2 and HT 3, which may have contributed to the large decrease in digestibility between HT 2 and HT 3.

HT 1 represented a very early harvesting time with energy content of 7.18 MJ NEL/kg DM. No published results have been found on feed intake and performance by dairy goats fed grass

silage harvested as early as HT 1 presented in this study. Several studies have been performed on cows (e.g. Rinne et al. (1999), Dawson et al. (2002), and Kuoppala et al. (2008)), which coincide with present result benefits in silage intake and milk yield.

4.2. Effects on feed intake and live weight gain

The most digestible silages contributed to highest silage DMI. Positive effects on DMI by increasing organic matter digestibility are reported by e.g. Sauvant et al. (1991) for goats and Forbes (1993) for ruminants in general. In the present study the effect of a 100 g/kg DM increase in silage D-value on silage DMI was 0.27 kg. Rook et al. (1991) reported silage DMI by cows to be at top at D-value 750 g, which coincide with the present results. Contrary to this Cerrillo et al. (1999) found no effect of hay maturity on DMI by goats, but digestibility or energy content of the hays were not reported. D-value and total fermentation acids in silage are two important factors in the silage DMI (SDMI) index used to predict the relative silage intake of dairy cows (Huhtanen et al., 2002; Huhtanen et al., 2007), which may be used for dairy goats as well. A D-value of 680 g/kg DM is used as standard for a well- preserved silage, which has an index of 100. According to the SDMI index the experimental silages had indexes of 115, 105 and 95 for HT 1, 2 and 3, respectively, which fits fairly well to the relative recorded DM intakes. Intake of aNDFom was numerically highest when HT 2 was fed. This coincide with what reported by Huhtanen et al. (2007); there is a curvilinear relationship between aNDFom intake and D-value, the maximum intake being reached at a D-value of 640 g/kg DM.

The intake of DM per kg BW tended to be higher for the goats with poor body condition compared with goats with medium or high body condition. It has been observed an inverse relationship between fatness and food intake in ruminants (Forbes, 1993), which may explain the above trend.

Goats are known to select their feed (Morand-Fehr, 2003), but in this study the grass silage was chopped to a short particle length in order to reduce selection. This appeared to be successful, because the analysis of the silage residues showed no selection of the feed.

In early lactation goats are normally in negative energy balance, and mobilize extensively from their body fat stores (Dunshea et al., 1990). In line with this, the goats had an average negative energy balance in all periods in the present experiment. However, apart from a few observations of BW loss, the goats gained BW in all periods. There is often a lack of agreement

between actual BW gain and body energy gain (Dunshea et al., 1990). However, the observation that the goats gained weight could probably result from the fact that some of the goats were in their second to fourth lactation and still growing. Additionally, mobilized body fat during lactation may be replaced by increased body water concentration, giving a poor relation between BW gain and energy balance (Bondi, 1987). The calculated energy balance in the present experiment was positive only for the goats fed HT 1 and decreased with decreasing energy content in the diet (postponed harvesting time and low level of concentrate).

4.3. Effects on milk production and composition

Milk production increased by 0.5 kg per 100 g/kg DM increase in silage D-value, in accordance with previous found effects on milk production from improved diet organic matter digestibility (Sauvant et al., 1987; Hussain et al., 1996). Increased fiber content of the feed had a negative effect on milk yield, in accordance to Santini et al. (1992). The higher milk yield at HT 1 and HT 2 may have been caused both by higher intake of DM and by higher concentration of NEL pr kg DM than HT 3.

Like other ruminants goats may respond to increased dietary NEL or protein levels with higher milk production. Goats fed HT 1 with both concentrate levels, or HT 2 with NC had NEL intakes at 1.00 to 1.11 of the calculated daily energy requirements while the other dietary treatments had NEL intakes at 0.81 to 0.93 of the requirements. AAT intakes were at approximately 0.93 of the daily requirements for all dietary treatments. The present dietary treatments were not balanced for CP content, which could have caused a confounding between the harvesting time and dietary CP concentration on milk production. The PBV was negative for HT 3 which may have limited ruminal microbial activity, which together with rumen undegraded protein (RUP) form the amount of amino acids available for absorption in the small intestine (Volden and Harstad, 1995). Of the experimental feeds the concentrate had a higher amount of protein in g/kg DM than the silage. High- protein ingredients as soybean meal and rapeseed meal in the concentrate are also known to have a better amino acid profile than grass silage protein (Korhonen et al., 2002). The main source of AAT from silage, however, is microbial protein, which has good amino acid profile. The increased milk yield due to higher level of concentrate fed may have been a combined effect of increased energy and protein intake and improved protein quality. Feeding a high AAT supply in early lactation when the energy content in rations

is low will be positive for the milk yield and milk protein production, but may increase the energy mobilization (Schei et al. 2005). In the present experiment the moderate supply of AAT may have limited milk production for all diets. Especially for the HT3 diets which were below requirements also for energy and PBV, milk yield may have been severely affected by lack of available nutrients. For the goats on HT1 with NC it may be suggested that with higher AAT supply, milk yield could have been higher on the expense of body fat deposition. On the other hand goats fed HT1 with LC had a fairly high milk yield and a moderate BW gain in spite of the moderate AAT supply, and with 0.77 of the DM intake provided by grass silage. Further, the observed high milk urea concentrations were more related to the highest concentrate level than to the maturity stage of the grass silage, which does not support that higher AAT supply provided by concentrate would give higher milk protein yield and a better balanced ration. However, milk yield responses observed in 4-week periods in the present study might have been smaller than if the same six diets were fed continuously for 18 weeks. Yet, significant carry over effects were not detected.

Higher milk protein concentration in cows has been associated with high digestibility of silages and high DMI and is probably related to increased intestinal supply of amino acids to the animals (Huhtanen, 1993). In the present experiment milk protein concentration was similar for the two first harvesting times, but lower for HT 3. By this, it appears to be no profit in milk protein concentration with a very early harvest compared to an early harvest, as also found by Rinne et al. (1999). Increase in concentrate allowance led to higher milk protein concentration, as often seen in earlier results (Huhtanen, 1993; Min et al., 2005; Lefrileux et al., 2008). An increase in daily concentrate DM intake of 0.5 kg improved milk protein concentration by 0.70 g/kg. Milk urea content also increased when more concentrate was fed, suggesting that only a portion of the increased supply of protein from NC was incorporated in milk protein.

Dairy goats need an adequate amount of fiber to maintain normal milk fat content. Santini et al. (1992) presented a positive linear relationship between fiber intake and milk fat concentration. In the present study, aNDFom intake per kg BW tended to be highest when silage from HT 2 was fed, which provided the highest milk fat concentration. Effects of date of harvesting on milk fat concentration will also depend on the effects of grass maturity on rumen fermentation. In general, low dietary fiber will lead to a lower ruminal acetate to propionate ratio and a lower milk fat concentration (Santini et al., 1992).

4.4. Marginal responses to increased energy intake and feed utilization efficiency

There was no significant interaction between silage digestibility and level of concentrate supplementation on feed intake, as also found by Rinne et al. (1999) with dairy cows, indicating that the positive response in silage DMI to D-value was not significantly reduced with higher concentrate level within the studied range. Positive effects on milk production have been observed consistently from increased level of concentrate supplementation (e.g. Min et al., 2005; Lefrileux et al., 2008). Across harvesting times, increase in milk yield was 61 g per 100 g additional concentrate eaten. Lefrieleux et al. (2008), observed an increase of 87 g milk per 100 g additional concentrate when Alpine goats grazing on cultivated pasture increased their daily intake of concentrate from 0.65 to 1.3 kg.

Marginal response to additional NEL intake provides useful information about the biological efficiency of milk production. The average marginal ECM response to increased concentrate allowance was 0.12 kg ECM per 1 MJ increase in NEL. When the estimated NEL intake was increased by earlier HT, 0.14 kg ECM was produced per 1 MJ increase in NEL. Thus, additional NEL derived from improved silage digestibility yielded more milk than additional NEL derived from increased concentrate level in accordance with studies with cows (Rinne et al., 1999).

The diets with highest CP concentration also gave the highest milk yields. In spite of this, utilization of N was reduced by higher CP intake. Milk N efficiency is often seen to decrease as dietary CP concentration and PBV increase, and milk production level is less important to determine the efficiency (Huhtanen et al., 2008). At lower energy intake, goats were more efficient in utilizing energy for milk production, which is in accordance with the findings on dairy cows reported by Schei et al. (2005). The higher energy balance for the HT 1 fed goats indicates body fat deposition which may be the main reason for the calculated low energy utilization for these goats when the BW gain was ignored. Conversion of energy to fat deposition is highly energy-demanding (Van Soest, 1994). Opposite, the energy derived from body fat mobilization for the goats fed HT 2 and HT 3 was efficiently used for milk production, and thus improved their energy efficiency, compared to the goats fed HT 1. When calculating energy efficiency including the maintenance requirements, the differences between the harvesting times were much smaller than when subtracting the maintenance requirements. As energy intake

increases a lower portion of the intake is used for maintenance compared to milk production, and thus gives a more effective production.

5. Conclusions

The intake of very early harvested grass silage was high compared to the normal harvested grass silage, and depended mainly on the digestibility of the silages. Higher energy and protein intakes by early harvest increased milk production. Improving silage quality by earlier harvesting time resulted in higher feed intake and milk yield than seen with increased concentrate level. A decrease in silage quality could not be fully compensated for by increased concentrate feeding. Due to higher BW gain of the goats, utilization of nutrients to milk production by very early harvesting time was lower than by postponed harvesting time. Practical implications of the results may depend on whether the aim is to maximize the production per goat or per hectare of grass.

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Table 1. Chemical composition and fermentation quality of silages and concentrate

	Harvesting time					
	1	2	3	SEM	*P*-value[1]	Concentrate
Dry matter, g/kg	236	239	238	6.30	NS	881
Chemical composition, g/kg DM						
Organic matter	928[b]	931[b]	937[a]	1.97	0.02	942
Crude protein (CP)	156[a]	125[b]	105[c]	3.82	<0.001	177
Fat	34.3	30.4	28.7	2.60	NS	43.5
Starch						427
Neutral detergent fibre (aNDFom[2])	433[a]	539[b]	584[b]	15.3	<0.001	181
Acid detergent fibre	265[a]	335[b]	382[c]	7.51	<0.001	
Acid detergent lignin	46.8[a]	57.3[b]	75.9[c]	2.26	<0.001	
Water soluble carbohydrates	36.6	14.8	18.5	14.0	NS	53.6
Lactic acid	93.2[a]	81.0[a]	58.8[b]	6.54	0.01	
Formic acid	8.5[b]	10.7[ab]	15.5[a]	1.58	0.03	
Acetic acid	18.6[a]	12.3[b]	10.8[c]	1.71	0.02	
Propionic acid	5.9[a]	4.3[ab]	3.8[b]	0.52	0.04	
Butyric acid	0.3	0.4	0.2	0.066	NS	
Ethanol	14.8	19.3	18.9	1.45	NS	
NH_3-N g/kg of Total N	64.3	86.5	73.4	8.02	NS	
pH	4.19	4.26	4.26	0.039	NS	

[a,b,c] Means with different subscripts within a row differ (P<0.05)
[1]Effect of harvesting time
[2]NDF assayed with alpha amylase and expressed exclusive of residual ash

Table 2. Apparent digestibility[1] and in sacco degradation characteristics of silages from different harvesting times and concentrate

	Harvesting time					
	1	2	3	SEM	*P*-value[2]	Concentrate
Apparent digestibility						
Dry matter	0.813[a]	0.735[b]	0.655[c]	0.002	<0.001	0.784
Organic matter	0.832[a]	0.751[b]	0.663[c]	0.002	0.001	0.810
Neutral detergent fibre	0.820[a]	0.749[b]	0.644[c]	0.004	0.002	0.382
Crude protein	0.805[a]	0.721[b]	0.641[c]	0.010	0.010	0.819
Fat	0.678[a]	0.641[b]	0.588[c]	0.005	0.010	0.868
D-value[3], g/kg DM	771[a]	696[b]	619[c]	2.054	0.001	763
In sacco degradation of						
Total N						
Soluble N	0.736[a]	0.683[b]	0.681[c]	0.0004	<0.001	0.297
Degradable N	0.229[b]	0.243[a]	0.154[c]	0.001	0.01	0.677
N k_d /t[4]	0.096[a]	0.065[c]	0.066[bc]	0.001	0.001	0.087
EPD_8[5]	0.860[a]	0.792[b]	0.751[c]	0.0006	<0.001	0.642
Digestibility of RUP[6]	0.866[a]	0.825[b]	0.728[c]	0.006	<0.001	0.913
Indigestible N[7]	0.018[c]	0.036[b]	0.068[a]	0.002	<0.001	0.031
NDF						
Degradable NDF	0.913[a]	0.876[b]	0.752[c]	0.002	0.001	
NDF k_d /t[8]	0.076[a]	0.054[b]	0.046[c]	0.0006	<0.001	
iNDF[9]	0.087[c]	0.124[b]	0.248[a]	0.0002	0.001	
$ENDFD_3$[10]	0.643[a]	0.542[b]	0.424[c]	0.001	<0.001	
Starch						
Soluble starch						0.161
Degradable starch						0.820
ESD_8[11]						0.903
ME[12], MJ/kg DM	11.9[a]	10.6[b]	9.2[c]	0.033	0.001	11.8
NEL[13], MJ/kg DM	7.18[a]	6.17[b]	5.26[c]	0.021	0.001	7.10
AAT[14], g/kg DM	78.0[a]	76.5[b]	69.5[c]	0.099	0.001	109
PBV[15], g/kg DM	46.8[a]	4.00[b]	-20.4[c]	0.167	<0.001	2.59

[a,b,c] Means with different subscripts within a row differ ($P<0.05$)
[1]Estimated *in vivo* using sheep
[2]Effect of harvesting time
[3]Digestible organic matter in DM determined *in vivo* using sheep.
[4]Fractional rate of N degradation, per h
[5]Effective rumen protein degradability, calculated with rumen passage rate of 0.08 per h.
[6]Intestinal digestibility of rumen undegraded protein
[7]Rumen and intestinal indigestible N
[8]Fractional rate of NDF degradation, per h
[9]Rumen undegradable NDF
[10]Effective rumen NDF degradability, calculated with rumen passage rate of 0.03 per h
[11]Effective rumen starch degradability, calculated with rumen passage rate of 0.08 per h
[12]Metabolizable energy determined *in vivo* using sheep.

[13]Net energy lactation determined *in vivo* using sheep.
[14]Amino acids absorbed in the small intestine.
[15]Protein balance in the rumen

Table 3. The effect of harvesting time and concentrate level on goats daily feed, nutrient and energy intake and body weight gain

Harvesting time	1		2		3			*P*-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT × C
Intake, kg DM										
Silage	1.76^{a}	1.55^{b}	1.44^{c}	1.34^{d}	1.32^{d}	1.18^{e}	0.051	<0.001	<0.001	NS
Concentrate	0.53	1.02	0.53	1.01	0.53	1.04	0.019			
Total ration	2.29^{c}	2.58^{a}	1.97^{d}	2.34^{bc}	1.85^{e}	2.20^{c}	0.056	<0.001	<0.001	NS
Nutrient intake, g										
Silage aNDFom	763^{ab}	668^{c}	778^{a}	720^{bc}	768^{a}	681^{c}	26.21	NS	<0.001	NS
Total aNDFom	860	852	872	901	863	867	26.56	NS	NS	NS
Crude protein	367^{b}	425^{a}	274^{d}	346^{c}	232^{f}	306^{e}	7.880	<0.001	<0.001	NS
AAT[3]	195^{c}	233^{a}	168^{d}	212^{b}	150^{e}	194^{c}	4.470	<0.001	<0.001	NS
PBV[4]	82.2^{a}	76.1^{b}	7.26^{c}	7.93^{c}	-25.5^{e}	-20.5^{d}	1.819	<0.001	NS	0.02
per kg BW, g										
Silage DM	28.0^{a}	24.8^{b}	23.5^{b}	21.2^{c}	21.4^{c}	18.8^{d}	0.869	<0.001	<0.001	NS
Total DM	36.7^{bc}	41.2^{a}	32.2^{d}	37.7^{b}	29.9^{e}	35.4^{c}	1.028	<0.001	<0.001	NS
Total aNDFom	13.7	13.6	14.2	14.5	14.0	13.8	0.439	0.08	NS	NS
ME, MJ[5]	27.2^{b}	30.7^{a}	21.4^{d}	25.9^{b}	18.4^{e}	23.0^{c}	0.609	<0.001	<0.001	NS
NEL, MJ[6]	16.4^{b}	18.5^{a}	12.6^{e}	15.4^{c}	10.7^{f}	13.5^{d}	0.361	<0.001	<0.001	NS
Body weight gain, g	32^{bc}	127^{a}	30^{bc}	86^{ab}	13^{c}	70^{abc}	21.42	NS	0.001	NS
Body weight, kg[7]	63.5^{ab}	63.7^{a}	61.7^{c}	63.0^{a}	62.4^{bc}	62.7^{abc}	1.638	0.009	0.01	NS
Energy balance, MJ NEL	0.43^{b}	1.78^{a}	-1.92^{d}	-0.11^{b}	-2.66^{d}	-1.02^{c}	0.366	<0.001	<0.001	NS

^{a,b,c} Means with different subscripts within the same row differ (P<0.05)
[1]LC= low concentrate level, NC= normal concentrate level
[2]Effect of harvesting time (HT), concentrate level (C), interaction (HT x C)
[3]Amino acids absorbed in the small intestine
[4]Protein balance in the rumen
[5]Metabolizable energy
[6]Net energy lactation
[7]Mean of initial and final weight in each period

Table 4. The effects of harvesting time and concentrate level on daily milk yield and composition

Harvesting time	1		2		3			*P*-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT × C
Yields										
Milk, kg	3.66^{b}	3.92^{a}	3.16^{d}	3.57^{b}	2.89^{e}	3.31^{d}	0.155	<0.001	<0.001	NS
ECM[3], kg	3.35^{b}	3.58^{a}	2.93^{c}	3.20^{b}	2.50^{d}	2.90^{c}	0.119	<0.001	<0.001	NS
Fat, g	135^{a}	143^{a}	120^{bc}	125^{b}	98^{d}	111^{c}	5.467	<0.001	<0.001	NS
Protein, g	103^{b}	113^{a}	89^{c}	101^{b}	79^{d}	94^{c}	3.436	<0.001	<0.001	NS
Lactose, g	164^{b}	176^{a}	141^{c}	161^{b}	128^{d}	148^{c}	7.094	<0.001	<0.001	NS
Milk solids, g	403^{b}	431^{a}	350^{c}	387^{b}	304^{d}	353^{c}	14.48	<0.001	<0.001	NS
Composition, g/kg										
Fat	36.7^{ab}	36.5^{abc}	38.7^{a}	35.6^{bc}	34.1^{c}	34.3^{bc}	1.354	0.006	NS	NS
Protein	28.4^{a}	29.1^{a}	28.5^{a}	28.6^{a}	27.3^{b}	28.6^{a}	0.489	0.05	0.001	NS
Lactose	44.7	44.8	44.9	45.1	44.5	44.8	0.474	NS	NS	NS
Milk solids	110^{ab}	110^{ab}	112^{a}	109^{ab}	106^{c}	108^{bc}	1.813	0.003	NS	0.09
Milk urea, mmol/l	8.56^{b}	9.23^{a}	8.61^{b}	8.94^{ab}	8.07^{c}	8.98^{ab}	0.223	NS	<0.001	NS
Milk FFA[4], mEq/l	1.96	2.29	2.04	1.73	1.58	2.34	0.346	NS	0.06	NS

[a,b,c] Means with different subscripts within the same row differ (P<0.05)
[1,2] See Table 3.
[3]Energy- corrected milk
[4]Milk free fatty acids

Table 5. Effects of initial body condition (block) on intake and body weight gain

Body condition[1]	Poor	Medium	High	SEM	*P*-value[2]
Initial body weight, BW, kg	52.4±8.77	65.5±4.02	71.3±7.85		
Neck height, m	0.66±0.04	0.68±0.02	0.65±0.06		
BMI	120.9±8.61	141.7±7.39	172.0±13.5		
Silage, kg DM/d	1.27[b]	1.50[a]	1.53[a]	0.071	0.04
Total ration, kg DM/d	2.03[b]	2.29[a]	2.30[a]	0.073	0.03
Silage aNDFom, g/d	645[b]	761[a]	782[a]	37.3	0.04
Total aNDFom, g/d	783[b]	904[a]	921[a]	37.8	0.04
Total DM, g/kg BW	38.6[a]	33.5[b]	34.4[ab]	1.48	0.06
Crude protein, g/d	302[b]	337[a]	336[a]	6.77	0.03
NEL[3], MJ/d	13.4[b]	15.0[a]	15.1[a]	0.45	0.03
BW gain g/d	72.5[a]	88.1[a]	19.6[b]	15.0	0.01

[a,b]Means with different subscripts within a row differ ($P<0.05$)
[1]Body mass index (BMI)= BW/neck height[2] was used as a measure of body condition
[2]Effect of body condition
[3]Net energy lactation

Table 6. Effect of harvesting time and concentrate level on efficiency of nutrient utilization for milk production

Harvesting time	1		2		2			*P*-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT × C
N [3]	0.28^{de}	0.26^{e}	0.32^{ab}	0.29^{cd}	0.34^{a}	0.30^{bc}	0.009	<0.001	<0.001	NS
AAT[4]	0.84^{bc}	0.71^{d}	0.93^{b}	0.73^{d}	1.08^{a}	0.79^{cd}	0.035	<0.001	<0.001	0.07
NEL[5]	0.31^{d}	0.29^{d}	0.42^{b}	0.34^{cd}	0.54^{a}	0.38^{bc}	0.020	<0.001	<0.001	0.005
NEL_{IMR} [6]	0.20^{bc}	0.19^{c}	0.23^{a}	0.21^{bc}	0.24^{a}	0.22^{b}	0.008	<0.001	<0.001	NS
ECM/DMI intake[7]	1.46^{a}	1.39^{ab}	1.50^{a}	1.39^{ab}	1.40^{ab}	1.32^{b}	0.052	NS	0.02	NS

[a,b,c] Means with different subscripts within a row differ (P<0.05)
[1,2] See Table 3.
[3] Calculated as N output in milk/N intake.
[4]Calculated as [milk protein yield/(AAT intake- AAT maintenance requirements)] ignoring the effect of live weight change.
[5]Calculated as [milk energy/(NEL intake- NEL maintenance requirements)] ignoring the effect of live weight change.
[6]Calculated as [milk energy/NEL intake] ignoring the effect of live weight change. $Subscript_{IMR}$= included maintenance requirements.
[7]Calculated as kg ECM/kg DM intake. ECM=energy-corrected milk

Paper II

Effects of grass silage harvesting time and level of concentrate supplementation on goat milk quality

Ingjerd Dønnem, Åshild T. Randby, Margrete Eknæs

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Effect of grass silage harvesting time and level of concentrate supplementation on goat milk quality

I. Dønnem, Å.T. Randby, M. Eknæs

Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway.

Corresponding author:

Ingjerd Dønnem

ingjerd.donnem@umb.no

Phone: +47 64966037

Fax: +47 64965101

Abstract

Milk fat lipolysis giving high concentrations of free fatty acids (FFA) and off-flavor in the goat`s milk is a challenge for the dairy industry in Norway. This has been considered to be caused by underfeeding of the goats and thereby energy mobilization in early and mid lactation. Energy intake can be improved by feeding silage of early harvesting time (HT) and supplementation with concentrate. In the present experiment, 18 goats in early lactation were fed grass silages prepared from the primary growth at a very early, early or normal stage of maturity (HT 1, HT 2 and HT 3 respectively), supplemented with a low (LC; 0.6 kg per goat daily) or normal (NC; 1.2 kg per goat daily) level of concentrate. The experiment was conducted as a cyclic change-over design with four periods of 28 days using three blocks of goats according to their initial body condition (poor, medium or high). Milk and blood samples were collected at the end of each period. Milk yield and yields of milk constituents decreased with delayed harvesting time and with LC. Sensory milk taste quality was not affected by dietary treatment, and milk FFA was highest when NC was fed. The proportion of short and medium chain fatty acids in milk fat decreased with postponed harvesting time and LC, while most of the long chain fatty acids (including C18:1c9) increased with postponed harvesting time and LC. The calculated energy balance decreased and the serum concentration of non-esterified fatty acids (NEFA) increased with decreasing energy content in the diet (postponed harvesting time and low level of concentrate). Goats with initial poor body condition had higher milk FFA concentrations than goats in higher initial body condition. High milk FFA concentration was correlated to poor milk taste quality, low serum NEFA concentration, low C18:1c9 proportion and high energy balance. Our findings suggest that increasing energy intake and energy balance during the first 4 months of lactation does not reduce FFA concentration in goats' milk.

Keywords: Dairy goats, grass silage harvesting time, concentrate, energy balance, milk quality

Abbreviations: BMI, body mass index; BHBA, β-hydroxybutyric acid; BW, body weight; DM, dry matter; ECM, energy corrected milk; FA, fatty acids; FFA, free fatty acids; LPL, lipoprotein lipase; MFGM, milk fat globule membrane; MUFA, monounsaturated fatty acids; NDF, neutral detergent fiber; NEFA, non-esterified fatty acids; NEL, net energy lactation; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

1. Introduction

Milk quality is of specific concern in goat milk production. Rancid and tart flavor is a prominent problem in Norwegian goat milk, and is a challenge for the dairy industry (Eknæs and Skeie, 2006). Recent studies have shown that underfeeding appears to be an extensive problem, especially in early and mid lactation. This results in energy mobilization and subsequently high concentrations of free fatty acids (FFA) and off- flavors in the milk (Eknæs et al., 2006). The concentration of FFA in milk is a measure of lipolysis, i.e. the hydrolysis of fat globule triglycerides into FFA (Chilliard et al., 2003), and the total concentration of FFA is found to be correlated to the frequency of off- flavor (Collins et al., 2003) and rancid and tart flavor specifically (Eknæs and Skeie, unpublished). The level of dry matter (DM) intake or ingested energy is the main factor influencing milk yield and composition of dairy goats (Morand-Fehr et al., 2007). The problem with underfeeding was expected to be solved by feeding silage of high quality supplemented with concentrate.

The fatty acid composition of milk may influence its nutritive and health value for consumers (Mensink et al., 2003). In addition, it may influence sensory quality (Chilliard and Ferlay, 2004). The fatty acid composition of the milk will partly be reflected by the physiological state of the goats. During periods of negative energy balance, animals mobilize lipids stored in adipose tissue (Chilliard et al., 2003), and the fatty acid composition will therefore differ from that of milk synthesized when animals are in energy balance. The composition of the diet will also influence fatty acid composition in milk. Harvesting at an early stage of plant development will increase the concentration of polyunsaturated fatty (PUFA) acids in silage (Boufaied et al., 2003). Increased content of not protected PUFA in the diet will mainly increase the concentration of milk C18:0 and C18:1 due to hydrogenation in the rumen, at the expense of the short and medium- chain fatty acids (Chilliard et al., 2003). Increasing the concentrate proportion in the diet will mainly decrease C18:3, and increase C18:2 in milk (Chilliard and Ferlay, 2004). The antioxidant α- tocopherol (vitamin E) is an important nutrient which contributes to stabilize the unsaturated fatty acids in milk (Focant et al., 1998).

The main objective of this work was to investigate whether increased energy balance of goats during early lactation provided by improved grass silage quality could improve milk quality. The main measures of milk quality were considered to be milk FFA concentration and sensory milk taste, but other quality parameters were included as well.

2. Materials and methods

2.1. Experimental design, animals and diets

The study involved 18 goats of the Norwegian Dairy Goat breed in 2nd to 8th lactation (mean 4th) which kidded between 8th and 21st of January 2008. Their average body weight (BW) 2 days after kidding was 63.0 ± 10.5 kg. The experiment started about 2 weeks after kidding. The goats were assigned to three blocks according to their body condition 1 week before the beginning of the experiment, where block 1= poor body condition; block 2= medium body condition and block 3= high body condition. As goats deposit most of their body fat as visceral fat (Colomber-Rocher et al., 1992; Marinova et al., 2001) scoring of body condition of goats may be difficult. Body mass index (BMI) (BW/neck height^2), the same as used for humans, was used as a measure of body condition. A goat body mass index has previously been applied also by Tanaka et al. (2002). The experiment was conducted according to a cyclic change-over design (Davis and Hall, 1969) with 6 goats in each block and four 28-days experimental periods. Dietary treatment was changed from period to period. Dietary treatment in a 3 × 2 factorial arrangement consisted of three silage qualities and two concentrate levels. Animals were offered silage from harvesting time (HT) 1, 2 or 3 and either low (LC; 0.6 kg per goat daily) or normal (NC; 1.2 kg per goat daily) level of concentrate. The crop was harvested from the primary growth at three stages of maturity: (1) Very early (HT 1), (2) Early (HT 2), (3) Normal (HT 3). More detailed information about the preparation of the silages and the concentrate has been given by Dønnem et al. (submitted).

2.2. Animal management

The goats were housed in individual stalls and were milked twice a day at 06:30 and 16:00 h. Grass silage was given *ad libitum* twice daily at 06:00 and 15:00 h such that silage residues averaged 10%. Concentrate was distributed four times per day, at each milking and two hours after each milking. The goats were weighed 2 days after kidding and thereafter at 12.30 h for three consecutive days in the beginning of week 2 and end of week 4 in each period.

2.3. Feed sampling and analysis

Feed intake was recorded four days per week. Representative samples of silage and silage residues from all harvesting times were collected once and twice a week, respectively, and stored for -20°C until analysis. Concentrate was sampled from the whole batch in period 1, 2 and 3. Chemical analyses of silage and concentrate were done as reported previously by Dønnem et al. (submitted). One representative sample of each silage, and a concentrate sample, were analyzed in duplicate for determination of fatty acid composition. The samples were freeze dried and milled through a 0.5 mm screen (Retsch hammer mill, Haan, Germany). The milled feed samples were directly methylated according to O'Fallon et al (2007) and analyzed with a Thermo Finnigan Focus GC with a split/splitless Focus GC+ injector, and flame ionization detection (ThermoFinnigan, Milan, Italy). Separation was performed with a Restek RT-2560 (100 m × 0.25 mm internal diameter × 0.2 µm film thickness) column (Restek U.S., 110 Benner Circle, Bellefonte, PA). Temperature program, initial: 70ºC with 2 min hold, increased at 20ºC/min to 160ºC with 40 min hold, and further increased at 2ºC/min to 230ºC with 10 min hold. Carrier gas was He with a pressure of 270 kPa. Fatty acid analysis was performed by auto injection of 2 µL of each sample at a split ratio of 20:75, constant flow mode, average velocity 16.8 cm/s. The flame ionization detector temperature was 230ºC. The run time for a single sample was 91.5 min.

2.4. Milk sampling and analysis

Individual milk samples for chemical and sensory analyses were collected evening and morning for one day during the last week of each period. Milk yield of each goat was measured during these one-day milk samplings and presented in this paper, while means of milk yield measured for three consecutive days at week 2, 3 and 4 per period was presented by Dønnem et al. (submitted). A portion of the milk was preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4°C for 2.5 days before analyzing for fat, protein, lactose, urea and FFA content with an infrared milk analyzer (Milkoscan 6000, Foss- Electric, Hillerød, Denmark). Non-preserved milk, also stored for 2.5 days, was analyzed for FFA chemically by Autoanalyzer 3 (Bran + Luebbe GmbH, Norderstedt, Germany), as described by Bråthen (1984). Samples used for analysis of fatty acid composition were frozen at -20°C and stored until analysis. Milk fat was separated using the rapid method described by Feng et al. (2004). Of the separated lipid, 40 mg was transesterified by the method of Christie (1982), as modified by Chouinard et al. (1999) by use of sodium methoxide. Fatty acid methyl esters (FAME) were quantified using GLC (6890N,

Agilent Technologies, Palo Alto, CA, USA) with a split/splitless injector, a (7683B) automatic liquid sampler, and flame ionization detection. Separation was performed with a CP-select CB for FAME (200 m × 0.25 mm i.d. × 0.25 µm film thickness) fused silica capillary column (Varian Inc., Palo Alto, CA). Initially, the oven temperature was 70ºC with 4 min hold, increased at 20ºC/min to 160ºC, and held for 80 min. The oven temperature was then increased to 220ºC at 3ºC/min and held for 28 min. Carrier gas was H_2 with a pressure of 314 kPa. Fatty acid analysis was performed by auto-injection of 1 µL of each sample at a split ratio of 70:1, a H_2 flow of 151 mL/min, and an injector temperature of 280ºC. The flame ionization detector temperature was 290ºC with H_2, air, and N_2 make-up gas flow rates of 40, 450, and 45 mL/min, respectively. The sampling frequency was 10 Hz. The run time for a single sample was 136 min. Fatty acid peaks were identified using 37 component FAME mix and *trans*-11 18:1 from Supelco (Bellefonte, PA).

α- tocopherol was analyzed by diluting one ml of milk with 3 ml 2-propanol containing the internal standard tocol and butylated hydroxytoluene as an antioxidant. After mixing (15 min) and centrifugation (10 min, 4000 x *g* at 10 °C), an aliquot of 20 µl was injected from the supernatant into the HPLC system. HPLC was performed with a HP 1100 liquid chromatograph (Agilent Technologies, Palo Alta, CA, USA) with a HP1100 fluorescence detector, em: 295 nm ex: 330 nm. Tocopherol isomers were separated on a 4.6 mm x 50 mm reversed phase column. A calibration curve was made from analysis of an ethanol solution enriched with known concentration of α-tocopherol.

Sensory analysis was performed by a panel of three trained goat-milk assessors (Norsk Matanalyse, Oslo, Norway), on non-preserved milk, stored for 2.5 days at 4ºC. The panel's average score for each sample was used in calculations. Scores for taste and aroma were evaluated on a five-point scale (where 1 = poor quality milk with serious deviations from normal aroma or taste to 5 = high quality milk with no deviations from normal aroma or taste). For samples scored at 3.7 or lower, the type of off- flavor (i.e. goat flavor, rancid, tart, bitter, microbial, transmitted) was indicated. Some milk samples received more than one type of off-flavor remark.

2.5. Blood sampling and analysis

Blood samples were collected before morning feeding at the end of each period. About 30 min after sampling the samples were centrifuged at 3000 x *g*, and serum was frozen at -80°C until analysis. An Advia 1650 (Bayer Diagnostics, Tarrytown, NY, USA) was used to measure glucose, β-hydroxybutyric acid (BHBA), non- esterified fatty acids (NEFA), cholesterol, triglycerides and lipase in serum.

2.6. Energy calculations

The energy concentration in silages and concentrate and the goats energy requirements for maintenance were calculated according to the Dutch net energy lactation (NEL) system (Van Es, 1975; 1978). The goats energy requirements for milk production was calculated according to Van Der Honing and Alderman (1988). Energy balance was calculated as NEL intake less NEL for maintenance and milk production. Energy corrected milk (ECM) yield was calculated according to Sjaunja et al. (1990). The ECM content measured for three consecutive days at week 2, 3 and 4 per period as presented by Dønnem et al. (submitted) was used in energy balance calculations.

2.7. Statistical analyses

The data was analyzed using the MIXED procedures of SAS (SAS, 2003) according to the model $Y_{ijklmno} = \mu + B_i + g_{j(i)} + P_k + H_l + L_m + C^{(H)}_n + C^{(L)}_o + C^{(HL)}_{no} + HL_{lm} + BP_{ik} + BH_{il} + BL_{im} + PH_{kl} + E_{ijklm}$, where $Y_{ijklmno}$ = the dependent variable; μ= the overall mean; B_i= effect of block i, i=1,…3; $G_{j(i)}$ = effect of goat j within block i, j = 1,…6; P_k = effect of period k, k = 1,…4; H_l = effect of harvesting time l, l = 1,…3; L_m = effect of concentrate level m, m = 1, 2; $C^{(H)}_n$ = carry-over effect of harvesting time l, l = 1,…3; $C^{(L)}_o$ = carry-over effect of concentrate level m, m = 1, 2; $C^{(HL)}_{no}$ = carry-over effect of interaction between harvesting time and concentrate level; HL_{lm} = interaction between harvesting time l and concentrate level m; BP_{ik} = interaction between block i and period k; BH_{il} = interaction between block i and harvesting time l; BL_{im} = interaction between block i and concentrate level m; PH_{kl} = interaction between period k and harvesting time l; and $E_{ijklmno}$ = random residual. All terms were considered fixed, except for $g_{j(i)}$, which was considered random. Pearson correlation coefficients were calculated between some parameters in milk, blood and intake data using SAS (SAS, 2003). Means are reported and mean separation was done by least significant difference. Chi-square tests were used to evaluate the frequencies

of the different types of off-flavors of the milk. Treatment effects were declared significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3. Results

3.1. Diets

The silages and the concentrate fed are characterized in Table 1. Delayed harvesting time decreased the concentrations of crude protein, and increased the concentrations of NDF in the silages. All silages were of good fermentation quality. The most abundant fatty acid in silages was C18:3-c9c12c15 (α-linolenic acid) (Table 2), and its proportion in fat decreased with postponed harvesting time. Almost all the other fatty acids increased with postponed harvesting time. C18:1-c9 was the most dominant fatty acid in the concentrate. C18:2-c9c12 (linoleic acid) and C16:0 were major fatty acids in both silage and concentrate. Postponed harvesting time decreased ($P<0.001$) intake of silage DM and NEL (Table 3).

3.2. Milk production

Daily yield of milk and energy-corrected milk (ECM) decreased ($P < 0.001$) with postponed harvesting time (Table 3). Milk fat concentration was highest ($P < 0.001$) at HT 2. The highest concentrate level stimulated to the highest ($P \leq 0.001$) yields of milk, ECM and protein concentration, and highest ($P < 0.001$) milk urea concentration. The level of α- tocopherol was higher ($P = 0.04$) at HT 1 and 2 than HT 3.

3.3. Free fatty acid content and sensory quality

The content of FFA in milk analyzed with infrared milk analyzer was neither affected ($P > 0.1$) by harvesting time nor concentrate level (Table 3). However, chemically analyzed milk FFA was highest ($P = 0.03$) when NC was fed. Milk taste quality was not affected by dietary treatment. Tart flavor was the most frequent type of off-flavor (Table 4), but no single off- flavor category was affected by dietary treatment.

3.4. Fatty acid composition

The fatty acid composition of goat's milk is presented in Table 5. Twenty-one fatty acids were separated and quantified, of them 11 saturated (SFA), 7 monounsaturated (MUFA) and 3

polyunsaturated (PUFA). The most abundant fatty acid was C16:0 followed by C18:1-c9, C14:0, C18:0 and C10:0. The milk proportion of the short and medium chain fatty acids, C6:0- C14:0, decreased ($P \leq 0.005$) with postponed harvesting time and LC. Further, some of the long chain fatty acids, C16:1-c9, C17, C18:1-c9, C18:1-t9, C18:1-c11, linoleic acid, C20:0 and C20:4 increased ($P \leq 0.003$) with postponed harvesting time. The proportion of α-linolenic acid was highest ($P = 0.001$) in the milk when HT 2 was fed, and a similar tendency was apparent for C16:0. The proportion of C16:0 and all saturated C6:0- C14:0 was highest ($P \leq 0.005$) when NC was fed, while the odd-chain C15:0 and C17:0 and most of the long chain fatty acids, C17:0, C18:1-c9, C18:1-t11, C18:2-c9t11-CLA, α-linolenic and C20:5 (EPA) acid decreased ($P \leq 0.01$) when NC was fed. The n6/n3 ratio, when C18, C20 and C22 fatty acids were included, increased ($P \leq 0.001$) with postponed harvesting time and with increased concentrate level.

3.5. Body weight gain, energy balance and blood parameters

In average for the 4 periods the goats gained BW (g/day) at all dietary treatments (Table 6). BW gain was higher ($P = 0.001$) for goats on NC than on LC. Energy balance was positive for goats fed HT 1, while it was negative for HT 2 and HT 3. Energy balance increased ($P < 0.001$) when NC was fed.

Postponing the harvesting time decreased serum BHBA ($P = 0.02$) and cholesterol ($P = 0.04$) concentration (Table 6). Serum NEFA increased moderately with postponed harvesting time when NC was fed, and dramatically when LC was fed. An increase in concentrate allowance increased glucose ($P<0.001$), and decreased NEFA and BHBA ($P \leq 0.006$) concentration.

In period 1 the goats were in lower ($P < 0.001$) energy balance (-1.53 MJ NEL) than period 2, 3 and 4 (-0.34, -0.17 and -0.31 MJ NEL respectively). The serum NEFA concentration was higher ($P \leq 0.002$) in period 1 (0.55 mmol/l) than in the subsequent periods (0.23, 0.29 and 0.14 in period 2, 3 and 4 respectively). The BW gain was in average positive in all periods, but the goats fed HT 3 and LC in period 1 lost 69 g BW per day. Figure 1 shows the NEFA concentration and energy balance for each dietary treatment in each period.

3.6. Effect of body condition

Goats with poor initial body condition (block 1) had higher milk urea and FFA ($P \leq 0.01$) and poorer milk taste score ($P = 0.02$) than goats with medium (block 2) or high (block 3) body condition (Table 7). Figure 2 presents the milk FFA content for each group of body condition in each period. Goats with poor or medium body condition gained more ($P = 0.01$) BW than goats with high body condition. The proportion of the milk fatty acid C16:0 was highest ($P = 0.05$) for goats with poor initial body condition, while C18:2-c9c12 was highest ($P = 0.04$) in milk from goats with poor or medium body condition.

3.7. Correlations between milk, blood and intake parameters

A high daily yield of milk fat, and also a high milk fat concentration, was correlated to low milk FFA concentration (Table 8), and both high fat yield and -concentration were correlated to high milk taste quality. High milk urea concentration was strongly correlated to high FFA concentration and poor milk taste. Whereas high BW and BMI were correlated to low milk FFA concentration and high milk taste quality (a tendency only for BW and milk taste quality), energy balance and BW gain were correlated to high milk FFA, and energy balance tended to be correlated to poor milk taste quality. High serum NEFA was correlated to low milk FFA. High serum glucose and lipase were correlated to high milk FFA, and both tended to be correlated to poor milk taste quality. High serum cholesterol tended to be correlated to high milk FFA (P=0.07. R^2=0.05). A high proportion of C16:0 in milk fat was strongly correlated to high milk FFA and to poor milk taste quality, whereas a high proportion of C18:0 and C18:1c9 were correlated to low milk FFA. A high proportion of α-linolenic acid in milk fat was correlated to poor milk taste quality (P=0.002. R^2=0.14).

4. Discussion

Milk lipolysis and milk lipoprotein lipase (LPL) activity are highly correlated in goat milk, because the milk LPL are bound to the cream (Chilliard et al., 1984). Milk LPL originates from either adipose tissue LPL or mammary LPL which have leaked into the milk. Its activity is lower during early lactation (up to 3 months after kidding) than in mid lactation and is decreased during short term fasting (Chilliard et al., 2003). The appearance of the characteristic goat flavor in cold, fresh milk is due to the high content of C6:0, C8:0, C10:0 and branched chained C9 and C10 fatty acids (methyl- and ethyl- C8), which are more abundant in goat's than in cow's milk

fat (Ha and Lindsay, 1993; Sanz Sampelayo et al., 2007). The combination of milk LPL characteristics of goats and milk fatty acids composition could explain the relationship between LPL, lipolysis and goat flavor (Chilliard et al., 2003). Goat flavor must be distinguished from tart and rancid flavor, as it seems to appear at lipolysis levels much lower than those responsible for the latter off-flavors (Chilliard et al., 2003). The sensory panel in the present experiment characterized many milk samples as both goaty and tart, indicating that those flavor terms are hard to distinguish from each other. Tart, rancid, goaty and bitter flavors are all categorized as lipolyzed off- flavors (Shipe et al., 1978). Lipolyzed flavor is found to be correlated to a high level of milk FFA, especially short and medium chain FFA (Scanlan et al., 1965; Park, 2001; Collins et al., 2003). The present experiment supports these findings because milk taste score (3.27 in average for all milk samples) was negatively correlated to the level of milk FFA. Milk taste quality was not affected by dietary treatment. Skjevdal (1979) also summarized that different dietary treatments had little influence on the intensity of the flavor of goat's milk. The negative correlation between milk taste quality and the proportion of α-linolenic acid in milk fat were not reflected in any correlation between α-linolenic acid proportion and milk FFA concentration, suggesting that high α-linolenic acid proportion may increase the likelihood of giving other off flavors than goaty, tart or rancid flavor.

In this study milk FFA was determined both chemically and by IR- analysis, where the chemical analysis of FFA is known to be most accurate. A paired t-test showed no significant difference between the two methods. In the discussion of results the values from the chemical analysis are used. When the concentration of milk FFA exceeds 2.0 mmol/l, goat milk is considered as unsatisfactory for cheese production (Skeie, personal communication). In the present experiment none of the dietary treatments resulted in concentrations above this limit, as means over all periods, but the goats fed HT 1 silage and NC had higher values in period 2 and 3.

Milk fatty acids have a dual origin; they are either taken up from plasma lipoproteins (composited of mobilized fat and dietary fat), or they are synthesized de novo in the mammary gland (Chilliard and Ferlay, 2004). In early lactation, goats are normally in negative energy balance, and mobilize extensively from their body fat stores, mainly in the form of NEFA (Dunshea et al., 1989; Dunshea et al., 1990). The major fatty acids in body fat stores of goats are C18:1-c9, C16:0 and C18:0 (Banskalieva et al., 2000), which are incorporated into milk fat during mobilization (Palmquist et al., 1993). In the present study this seemed to be true

especially for C18:1-c9, which was found in highest proportion in milk from goats fed the poorest diet (HT 3, LC) and was highly positively correlated to serum NEFA. Also milk C18:0 was positively correlated to NEFA, but C16:0 was negatively correlated to NEFA and positively correlated to BW gain, suggesting that its major origin was not body fat stores, but *de novo* fat synthesis. This is supported by the C16:0's positive correlation with other short and medium chain fatty acids, and negative correlation with C18:0 and C18:1c9. Chilliard et al. (2003) reported that 59% of the variability of milk C18:0 + C18:1 fatty acids was linked to changes in energy balance of the goats. In the present study the corresponding linkage of milk fatty acids C18:0 + C18:1 to energy balance was 50%. The calculated energy balance in the present experiment was positive only for the goats fed HT 1 and decreased with decreasing energy content in the diet. The fact that NEFA concentration in serum and the proportion of C18:1-c9 in milk increased with decreasing energy content in the diet, indicated that fat mobilization had occurred, especially for the goats fed LC at HT 2 and 3. The NEFA concentration at zero energy balance was calculated to be 0.244 mmol/l ($y= -0.1027x+0.244$, $P < 0.001$, $R^2 = 0.36$, where y= NEFA mmol/l, x= calculated energy balance in MJ NEL, n=72). This corresponds well with the results of Dunshea et al. (1989), where NEFA concentration was 0.217 mmol/l at zero energy balance.

As milk C18:0 was unaffected by harvesting time and both MUFA and PUFA in milk were more abundant at postponed harvesting time, it is apparent that diet composition had a small effect on milk fatty acid composition compared to energy intake and energy balance of the animals. Also Sanz Sampelayo (1998) referred to research indicating that milk composition is more dependent on the energy balance of the animal than on the composition of the diet. The content of short and medium chain fatty acids in milk decreased with decreasing energy content in the diet. When energy availability is reduced, along with fat mobilization, the intermediary supply of acetate and glucogenic compounds decrease, causing less synthesis of short and medium chain fatty acids through mammary de novo synthesis (Palmquist et al., 1993). Long chain (C18) fatty acids have a negative effect on de novo synthesis of especially medium chain fatty acids (Barber et al., 1997), as also found in the present experiment.

C18:2-c9t11-CLA is found to have a beneficial effect on the consumer, mainly related to its anticarcinogenic properties (Griinari et al., 2000). The content of C18:2-c9t11-CLA in milk was not affected by harvesting time, although its precursor C18:1-t11 was clearly reduced with

postponed harvesting time. C18:2-c9t11-CLA is formed during ruminal biohydrogenation of C18:2-c9c12, linoleic acid, but is mainly produced from C18:1-t11 by the action of Δ- 9 desaturase in the mammary gland or in other tissues (Bauman and Griinari, 2001). The concentrate had higher content of linoleic acid than the silage, thus feeding normal level of concentrate led to higher intake of linoleic acid. Despite this, CLA was more abundant in milk from goats fed low level of concentrate. This may be explained by the dominance of the latter route of producing CLA. The n-6/n-3 ratio in milk increased when NC was fed, which is in agreement with Chilliard and Ferlay (2004). The n-6/n-3 ratio also increased with postponed HT, so the lowest, most beneficial n-6/n-3 ratio in milk was obtained by goats offered LC with HT 1 or HT 2.

A study by Eknæs et al. (2009) revealed that feeding concentrate with a high fat supplement, consisting mainly of the saturated long chain fatty acids C16:0 and C18:0, increased the C16:0 proportion in milk and reduced the frequency of rancid and tart taste of milk, but without affecting the milk FFA concentration. Astrup et al. (1985) showed that feeding concentrate added C16:0 and C18:0 fatty acids to dairy goats increased the respective fatty acid in milk and tended to reduce the level of milk FFA and the goat flavor in milk. In the present experiment a low fat concentrate was used (44 g/kg DM) because fat additions could mask the effect of the studied dietary treatments on milk taste and FFA concentrations. Chilliard et al. (2003) reported a negative correlation ($r = -0.70$) between milk LPL activity and milk C16:0 proportion. In the present study a high proportion of C16:0 fatty acid in milk was related to poor milk quality, both as regard to FFA concentration and milk taste quality. While a high C16:0 proportion in milk may have been supplied to the udder mainly from feed addition and body fat stores in the referred studies (Astrup et al., 1985; Chilliard et al., 2003; Eknæs et al., 2009), it seemed mainly to be a product of de novo synthesis in the present study.

High BMI at kidding was related to a high milk quality, both as regards FFA and milk taste quality. The goats with low BMI at kidding had a notable increase in milk FFA from about 6 weeks after kidding and tended to have higher proportion of C16:0 in milk fat than goats in medium or high body condition. The negative correlation between both BMI and BW with FFA suggests that heavy goats in high condition, with body fat available for mobilization, were most likely to produce high quality milk.

The present study, which has generated the presented correlations between milk, blood and intake parameters (Table 8), included a substantial variation in feed composition, energy intake and milk yield. The stage of lactation varied from early (week in milk = 2) to mid lactation (week in milk = 18). The correlations were in general consistent over periods, indicating that they were not results of interactions between the stage of lactation and milk quality.

There was a striking difference between NEL intake and energy balance as regards their correlations with the two major measures of milk quality: milk FFA concentration and milk taste quality. Whereas NEL intake was not correlated to these quality measures ($P>0.5$), high energy balance was correlated to high FFA and poor milk taste quality. This suggests that NEL intake *per se* was not important for milk quality, but how the energy was partitioned into milk or body fat stores, or whether energy was mobilized. High milk quality was obtained both on high and low NEL intakes, but most frequently during negative energy balance. Serum NEFA and high proportions of C18:0 and C18:1 fatty acids in milk fat (associated with negative energy balance) were negatively correlated to milk FFA concentration whereas BW gain, serum glucose and lipase (associated with positive energy balance) were positively correlated to milk FFA. The parameters that were correlated with a high FFA were also correlated with poor milk taste quality and vice versa. The positive correlation between a high daily yield of milk fat, and a high milk taste score, with low FFA concentration, and the tendency of a negative correlation between milk yield and milk FFA, suggested that high quality milk was frequently obtained by high yielding goats although this didn't seem to be an outcome of high NEL intake. The high quality milk seemed rather to have been produced by goats in negative or low energy balance that received fatty acids for milk fat secretion from mobilized body fat through NEFA. This result can be linked to previous research. Chilliard et al. (2003) reviewed the observation that milk and adipose tissue LPL activity decrease during fasting, which will reduce lipolysis in milk. Eknæs and Skeie (2006) observed that goats fed restricted amounts of roughage in a 2 day period had higher sensory milk quality than goats offered hay *ad libitum.*

Milk FFA concentration was positively correlated to milk urea concentration throughout the experiment. However, it is difficult to explain their possible relationship. Milk urea will typically increase when feeding a surplus of protein compared to energy, which is associated with increased mobilization of body fat in early lactation (Schei et al., 2005). In the present experiment a high milk urea concentration was, by contrast, correlated with a positive energy

balance. Milk urea content increased when more concentrate was fed, suggesting that only a portion of the increased supply of protein from NC was incorporated in milk protein.

Besides the amount of milk LPL activity, the stability of the milk fat globule membrane (MFGM) would influence the susceptibility of milk to lipolysis (Deeth, 2006). Phospholipids, glycosphingolipids and cholesterol are important precursors in synthesis and stability of MFGM (Evers, 2004). Phospholipids are synthesized *de novo* in the mammary gland, while cholesterol is both supplied by the blood plasma and synthesized in the mammary gland (Nielsen and Jakobsen, 1994). Plasma cholesterol is found to increase with increased level of fat intake (Palmquist and Conrad, 1978), which is in accordance with the present results; cholesterol in serum was higher with earlier harvesting time. However, serum cholesterol tended to be positively correlated ($P = 0.07$. $R^2 = 0.05$) to milk FFA. This suggests that cholesterol synthesized in the mammary gland, at levels not known in this experiment, was more important for the maintenance of normal MFGM synthesis than circulating serum cholesterol. Another aspect is that fatty acid and cholesterol synthesis are found to be inversely regulated. This means that when *de novo* fatty acid synthesis is high, cholesterol synthesis will be low, and plasma supply of cholesterol may be most important for the maintenance of MFGM synthesis (Nielsen and Jakobsen, 1994). The C4-C10 fatty acids in the present experiment were positively correlated to serum cholesterol ($P = 0.008$. $R^2 = 0.10$) and energy balance ($P = <0.001$. $R^2 = 0.30$) but not correlated to milk FFA. As energy balance and milk FFA were positively correlated, it can be hypothesized that a positive energy balance, giving a high supply of VFA from ruminal digestion which supports both high milk production and BW gain, could give a lack of precursors for MFGM through suppression of mammary cholesterol synthesis and therefore cause high FFA and poor milk quality.

5. Conclusion

Very early harvested grass silage, and also the highest concentrate level, notably increased energy intake and milk yield. Furthermore, the diet fed also influenced the fatty acid composition of the milk. However, higher energy intake did not improve the taste quality of the milk or reduce milk fat lipolysis, as measured by milk FFA concentration. It rather tended to be opposite, at least as regards concentrate level. High energy balance was correlated to high concentration of milk FFA and poor milk taste quality. This study suggests that attempts to improve milk quality

by minimizing the period of negative energy balance not likely will succeed. A high body condition at kidding seemed to be more promising for production of high- quality milk in early and mid lactation.

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Table 1. Chemical composition, fermentation quality and energy value of silage and concentrate

	Harvesting time					
	1	2	3	SEM	*P*-value[1]	Concentrate
Date of harvest in 2007	22-24 May	4 June	13 June			
Dry matter, g/kg	236	239	238	6.30	NS	881
Chemical composition, g/kg DM						
Crude protein (CP)	156[a]	125[b]	105[c]	3.82	<0.001	177
Fat	34.3	30.4	28.7	2.60	NS	43.5
Starch						427
Neutral detergent fibre (aNDFom[2])	433[a]	539[b]	584[b]	15.3	<0.001	181
Water soluble carbohydrates	36.6	14.8	18.5	14.0	NS	53.6
Lactic acid	93.2[a]	81.0[a]	58.8[b]	6.54	0.01	
Acetic acid	18.6[a]	12.3[b]	10.8[c]	1.71	0.02	
Butyric acid	0.3	0.4	0.2	0.066	NS	
Ethanol	14.8	19.3	18.9	1.45	NS	
NH_3-N g/kg of total N	64.3	86.5	73.4	8.02	NS	
pH	4.19	4.26	4.26	0.039	NS	
Net energy lactation, MJ/kg DM	7.18[a]	6.17[b]	5.26[c]	0.021	0.001	7.10

[a,b,c] Means without a common letter within the same row differ ($P<0.05$)

[1]Effect of harvesting time

[2]NDF assayed with alpha amylase and expressed exclusive of residual ash

Table 2. Fatty acid composition (g/ 100 g of total fatty acids) of silages and concentrate

	Harvesting time			
	1	2	3	Concentrate
C8:0	0.28	0.30	0.31	0.14
C10:0	0.25	0.28	0.25	0.06
C12:0	0.17	0.17	0.24	0.40
C14:0	0.49	0.50	0.65	0.80
C16:0	13.8	13.7	16.2	30.0
C16:1-c9	0.10	0.19	0.46	0.23
C17:0	0.09	0.11	0.18	0.07
C18:0	1.09	1.08	1.46	2.42
C18:1-c9	2.37	2.57	3.49	27.21
C18:2-c9c12	14.8	14.5	14.8	27.7
C18:3-c9c12c15	50.5	41.6	36.2	0.07
C20:0	0.28	0.40	0.62	0.21
C22:0	0.56	0.69	0.88	0.13
C22:2	0.16	0.16	0.46	0
C:23	3.24	3.47	4.63	1.28
C24	0.35	0.54	0.72	0.26
C24:1	0.14	0.23	0.45	0.06

Table 3. Effect of harvesting time and concentrate level on daily feed intake, milk yield and milk composition.

Harvesting time	HT1		HT2		HT3			P-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT*C
Intake										
Silage, kg DM	1.76^{a}	1.55^{b}	1.44^{c}	1.34^{d}	1.32^{d}	1.18^{e}	0.051	<0.001	<0.001	NS
Concentrate, kg DM	0.53	1.02	0.53	1.01	0.53	1.04	0.019			
NEL, MJ	16.4^{b}	18.5^{a}	12.6^{e}	15.4^{c}	10.7^{f}	13.5^{d}	0.361	<0.001	<0.001	NS
Milk production										
Milk, kg	3.72^{b}	4.09^{a}	3.29^{c}	3.86^{ab}	2.89^{d}	3.22^{c}	0.185	<0.001	<0.001	NS
ECM[3], kg	3.32^{b}	3.66^{a}	3.04^{c}	3.53^{ab}	2.43^{e}	2.83^{c}	0.142	<0.001	<0.001	NS
Fat, g/kg	36.0^{bc}	35.9^{bc}	39.3^{a}	37.7^{ab}	34.8^{c}	35.1^{bc}	1.476	0.004	NS	NS
Protein, g/kg	27.4^{ab}	28.3^{a}	28.0^{a}	28.0^{a}	26.6^{b}	28.2^{a}	0.535	NS	0.001	NS
Lactose, g/kg	44.4	44.6	44.3	44.2	44.5	44.6	0.480	NS	NS	NS
Milk solids, g/kg[4]	108^{bc}	109^{bc}	112^{a}	110^{ab}	106^{c}	108^{bc}	1.932	0.006	NS	NS
Urea, mmol/l	7.93^{bc}	8.61^{a}	8.21^{abc}	8.58^{a}	7.67^{c}	8.63^{a}	0.248	NS	<0.001	NS
Milk taste[5]	3.03	3.34	3.35	3.34	3.29	3.24	0.252	NS	NS	NS
FFA_{IR}[6], mEq/l	1.20	1.41	1.30	1.09	1.04	1.32	0.206	NS	NS	NS
FFA_{Ch}[7], mEq/l	1.47^{a}	1.68^{a}	1.11^{ab}	1.23^{ab}	0.83^{b}	1.43^{ab}	0.279	NS	0.03	NS
α- tocopherol	0.48^{ab}	0.59^{a}	0.56^{a}	0.54^{ab}	0.47^{ab}	0.43^{b}	0.045	0.04	NS	NS

$^{a.b.c}$ Means without a common letter within the same row differ (P<0.05)

[1]LC= low concentrate level (0.6 kg/day), NC= normal concentrate level (1.2 kg/day)

[2]Effect of harvesting time (HT), concentrate level (C), interaction (HT x C)

[3]Energy- corrected milk

[4]Calculated by adding up fat, protein and lactose concentration

[5]Five point scale where 1= poor quality milk to 5= high quality milk with no deviation from normal aroma or taste

[6]Free fatty acids determined by infrared analysis

[7]Free fatty acids determined by chemical analysis

Table 4. Percentage of samples assigned to the different categories of off-flavor in milk. Number of samples is presented in parentheses, n= 12.

Harvesting time	HT1		HT2		HT3			
Concentrate level[1]	LC	NC	LC	NC	LC	NC	χ^2	P-value[2]
Tart	41.7 (5)	33.3 (4)	41.7 (5)	50.0 (6)	25.0 (3)	41.7 (5)	1.87	NS
Rancid	0.0 (0)	8.3 (1)	16.7 (2)	8.3 (1)	8.3 (1)	16.7 (2)	2.69	NS
Bitter	16.7 (2)	25.0 (3)	16.7 (2)	8.3 (1)	25.0 (3)	16.7 (2)	1.60	NS
Goat flavor	16.7 (2)	8.3 (1)	16.7 (2)	16.7 (2)	0.0 (0)	8.3 (1)	2.81	NS
Other[3]	8.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	33.3 (4)[4]	0.0 (0)	16.5	0.01
All categories	58.3 (7)	41.7 (5)	41.7 (5)	58.3 (7)	58.3 (7)	50.0 (6)	1.61	NS

[1, 2] See Table 3

[3]Fruity and feed flavor

[4]2 of these off-flavor scores were fruity and 2 were feed flavor

Table 5. Effect of harvesting time and concentrate level on fatty acid composition (g/ 100 g of total fatty acids) in milk

Harvesting time	HT1		HT2		HT3			P-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT*C
C4:0	2.90	3.03	2.91	2.88	2.82	2.92	0.091	NS	NS	NS
C6:0	2.44^{ab}	2.52^{a}	2.30^{b}	2.44^{ab}	2.12^{c}	2.29^{b}	0.064	<0.001	0.005	NS
C8:0	2.50^{a}	2.58^{a}	2.22^{b}	2.42^{ab}	1.97^{c}	2.22^{b}	0.094	<0.001	0.005	NS
C10:0	8.59^{a}	8.94^{a}	7.56^{b}	8.27^{ab}	6.32^{c}	7.47^{b}	0.285	<0.001	0.001	NS
C12:0	3.55^{ab}	3.79^{a}	3.05^{d}	3.42^{b}	2.61^{e}	3.17^{cd}	0.155	<0.001	<0.001	NS
C14:0	10.4^{a}	10.6^{a}	10.1^{a}	10.6^{a}	8.97^{b}	10.1^{a}	0.232	<0.001	0.002	NS
C14:1-c9	0.14^{b}	0.13^{b}	0.15^{ab}	0.15^{ab}	0.15^{ab}	0.17^{a}	0.009	0.02	NS	NS
C15:0	0.82^{c}	0.75^{d}	0.93^{a}	0.85^{bc}	0.90^{ab}	0.83^{c}	0.031	<0.001	<0.001	NS
C16:0	28.4^{bc}	29.5^{ab}	29.6^{ab}	30.0^{a}	28.0^{c}	29.7^{ab}	0.532	NS	0.003	NS
C16:1-c9	0.54^{c}	0.54^{c}	0.59^{bc}	0.57^{c}	0.75^{a}	0.66^{b}	0.028	<0.001	0.05	NS
C17:0	0.51^{c}	0.45^{e}	0.59^{b}	0.49^{cd}	0.71^{a}	0.55^{bc}	0.021	<0.001	<0.001	0.07
C18:0	9.85	9.35	9.44	8.97	9.22	9.07	0.421	NS	NS	NS
C18:1-c9	19.7^{c}	19.5^{c}	21.3^{bc}	19.5^{c}	26.3^{a}	22.1^{b}	0.761	<0.001	0.002	0.06
C18:1-c11	0.41^{b}	0.39^{b}	0.40^{b}	0.40^{b}	0.52^{a}	0.47^{a}	0.020	<0.001	NS	NS
C18:1-t9	0.18^{b}	0.17^{b}	0.17^{b}	0.18^{b}	0.19^{a}	0.19^{a}	0.005	<0.001	NS	NS
C18:1-t10	0.20^{bc}	0.21^{ab}	0.17^{d}	0.21^{ab}	0.18^{cd}	0.22^{a}	0.009	NS	<0.001	NS
C18:1-t11	1.04^{a}	0.79^{c}	0.97^{ab}	0.79^{c}	0.90^{b}	0.70^{c}	0.043	0.004	<0.001	NS
C18:2-c9c12	1.27^{e}	1.47^{c}	1.35^{e}	1.54^{c}	1.65^{a}	1.74^{a}	0.048	<0.001	<0.001	NS
C18:3-c9c12c15	0.57^{ab}	0.46^{c}	0.60^{a}	0.50^{c}	0.55^{b}	0.42^{d}	0.019	0.001	<0.001	NS
C18:2-c9t11 (CLA)	0.63^{a}	0.51^{b}	0.61^{a}	0.54^{b}	0.65^{a}	0.49^{b}	0.028	NS	<0.001	0.08
C20:0	0.19^{b}	0.19^{b}	0.20^{a}	0.20^{ab}	0.21^{a}	0.22^{a}	0.007	0.001	NS	NS
C20:4 (ARA)	0.076^{bc}	0.075^{c}	0.083^{a}	0.079^{bc}	0.093^{a}	0.086^{ab}	0.004	0.003	0.06	NS
C20:5 (EPA)	0.062^{a}	0.054^{ab}	0.059^{ab}	0.055^{ab}	0.061^{ab}	0.052^{b}	0.003	NS	0.01	NS
C22:6 (DHA)	0.018^{b}	0.016^{b}	0.021^{a}	0.021^{a}	0.021^{a}	0.021^{a}	0.002	0.03	NS	NS
SFA[3]	70.4^{abc}	71.6^{a}	68.8^{bc}	70.8^{ab}	63.7^{d}	68.4^{cd}	0.881	<0.001	0.001	NS
MUFA[4]	22.2^{bc}	21.7^{c}	23.8^{bc}	21.8^{c}	28.9^{a}	24.5^{b}	0.802	<0.001	<0.001	0.07
PUFA[5]	2.47^{cd}	2.45^{d}	2.54^{bcd}	2.59^{bc}	2.85^{a}	2.65^{b}	0.076	<0.001	NS	0.4
n6/n3[6]	2.02^{c}	2.88^{b}	2.16^{c}	2.95^{b}	2.85^{b}	3.73^{a}	0.081	<0.001	<0.001	NS
Δ^9 desaturation index[7]	0.67^{c}	0.68^{c}	0.69^{bc}	0.69^{bc}	0.74^{a}	0.71^{b}	0.009	<0.001	0.06	0.05

[a.b.c] Means without a common letter within the same row differ (P<0.05)
[1,2] See Table 3
[3]Saturated fatty acids
[4]Monounsaturated fatty acids
[5]Polyunsaturated fatty acids
[6]Including C18, C20 and C22 fatty acids
[7]C18:1-c9/(C18:0+ C18:1-c9)

Table 6. Effect of harvesting time and concentrate level on body weight gain, energy balance and blood metabolites

Harvesting time	HT1		HT2		HT3			P-value[2]		
Concentrate level[1]	LC	NC	LC	NC	LC	NC	SEM	HT	C	HT*C
Body weight, kg	63.5^{ab}	63.7^{a}	61.7^{c}	63.0^{a}	62.4^{bc}	62.7^{abc}	1.638	0.009	0.01	NS
Body weight gain, g/day	32^{bc}	127^{a}	30^{bc}	86^{ab}	13^{c}	70^{abc}	21.42	NS	0.001	NS
Energy balance, MJ NEL	0.43^{b}	1.78^{a}	-1.92^{d}	-0.11^{b}	-2.66^{d}	-1.02^{c}	0.366	<0.001	<0.001	NS
Blood metabolites										
Glucose, mmol/l	3.21^{bc}	3.40^{ab}	3.05^{c}	3.47^{a}	3.05^{c}	3.29^{ab}	0.091	NS	<0.001	NS
NEFA[3], mmol/l	0.20^{b}	0.15^{b}	0.35^{b}	0.17^{b}	0.70^{a}	0.26^{b}	0.080	0.003	0.002	0.06
BHBA[4], mmol/l	1.07^{a}	0.63^{b}	0.70^{b}	0.43^{b}	0.63^{b}	0.52^{b}	0.132	0.02	0.006	NS
Cholesterol, mmol/l	2.45^{b}	2.72^{a}	2.49^{ab}	2.52^{ab}	2.36^{b}	2.28^{b}	0.184	0.04	NS	NS
Triglyceride, mmol/l	0.17	0.21	0.11	0.16	0.18	0.19	0.027	0.08	NS	NS
Lipase, units/l	64.6	71.7	68.3	69.6	53.4	56.1	10.31	NS	NS	NS

[a.b.c] Means without a common letter within the same row differ (P<0.05)
[1.2] See Table 3
[3]Non- esterified fatty acids
[4] β-hydroxybutyric acid

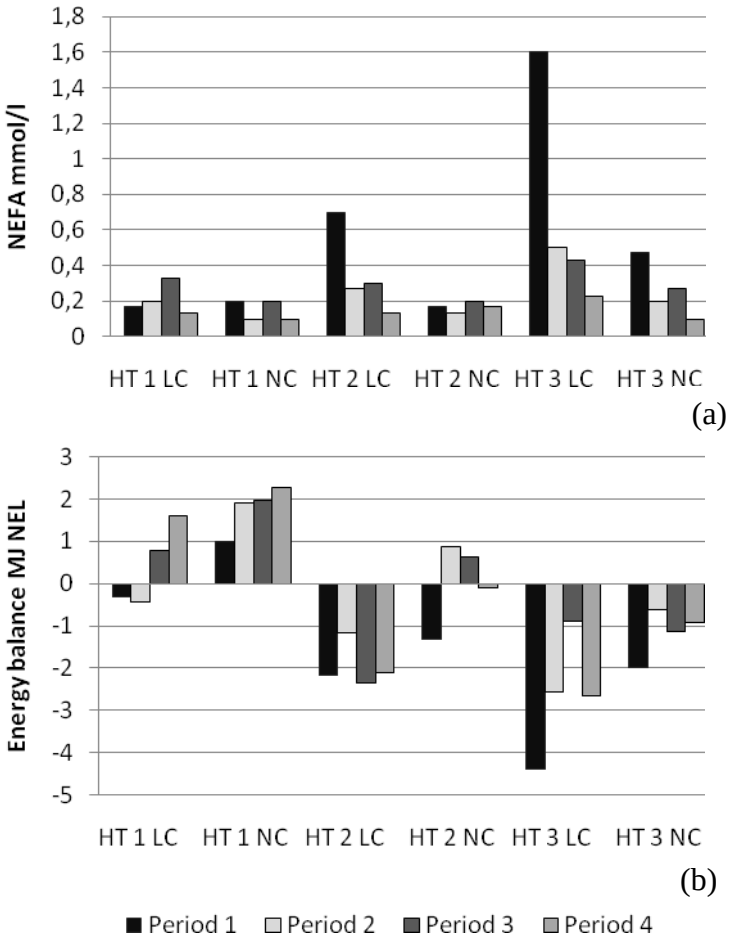

Figure 1. Effects of period and dietary treatment on (a) NEFA concentration and (b) energy balance (HT= harvesting time, LC= low concentrate level (0.6 kg/day), NC= normal concentrate level (0.6 kg/day)).

Table 7. Effects of initial body condition (block) on some milk production and body weight parameters

Body condition[1]	Poor	Medium	High	SEM	*P*-value[2]
Milk urea, mmol/l	9.02[a]	7.83[b]	7.97[b]	0.255	0.009
Milk taste	2.35[b]	3.58[a]	3.87[a]	0.355	0.02
FFA_{IR}, mEq/l	1.94[a]	1.09[b]	0.65[b]	0.256	0.009
FFA_{Ch}, mEq/l	2.27[a]	1.03[b]	0.58[b]	0.351	0.01
C16:0	30.2	28.5	28.9	0.457	0.05
C18:2-c9c12	1.62[a]	1.51[ab]	1.38[b]	0.062	0.04
Body weight gain, g/day	72.5[a]	88.1[a]	19.6[b]	15.11	0.01

[a.b] Means without a common letter within the same row differ ($P<0.05$)
[1]Body mass index (BMI)= Body weight/neck height[2] was used as a measure of body condition
[2]Effect of body condition

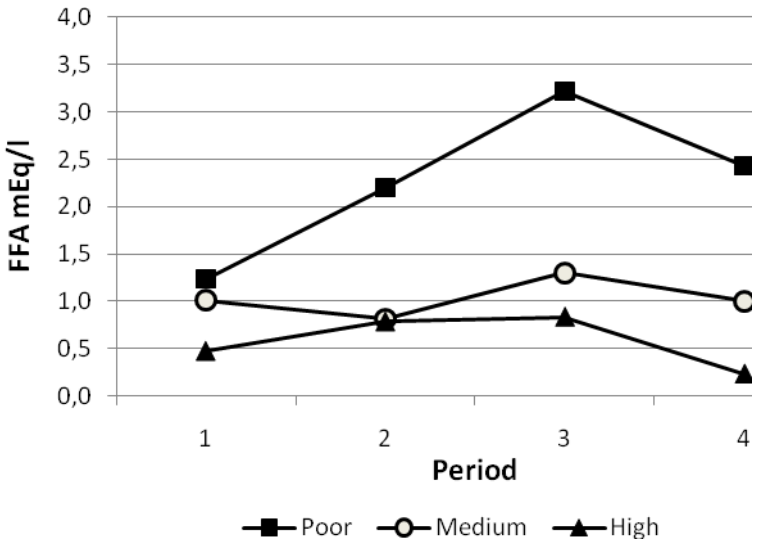

Figure 2. Effects of body condition (poor, medium and high) and period on content of milk free fatty acids (FFA).

Table 8. Pearson correlation coefficients of some milk, blood and intake parameters that are correlated to milk quality (FFA concentration and milk taste quality)

	Milk							Serum			Energy balance/BW			
	Fat yield	Urea	Taste	FFA_{Ch}	C16:0	C18:0	C18:1-c9	NEFA	Glucose	Lipase	Energy balance	BMI	BWgain	BW
Fat g/kg	0.40***	-0.12	0.52***	-0.25*	-0.25*	0.12	-0.19	-0.01	0.04	-0.07	-0.01	0.08	-0.09	0.07
Fat yield	-	0.05	0.36**	-0.31**	-0.15	0.36**	-0.30*	-0.10	0.02	-0.17	-0.01	0.29*	0.05	0.07
Urea	0.05	-	-0.42***	0.39***	0.52***	-0.17	-0.33**	-0.44***	0.43***	0.19	0.25*	-0.20	0.15	-0.39***
Taste	0.36**	-0.42***	-	-0.79***	-0.53***	0.20	0.11	0.16	-0.21	-0.22	-0.23	0.37**	-0.15	0.20
FFA_{Ch}	-0.31**	0.39***	-0.79***	-	0.49***	-0.31**	-0.32**	-0.25*	0.33**	0.37**	0.40***	-0.40***	0.29*	-0.25*
C16:0	-0.15	0.52***	-0.53***	0.49***	-	-0.62***	-0.52***	-0.44***	0.48***	0.38**	0.30*	-0.07	0.23*	-0.17
C18:0	0.36**	-0.17	0.20	-0.31**	-0.62***	-	0.22	0.39***	-0.49***	-0.46***	-0.31**	-0.03	-0.26*	-0.06
C18:1-c9	-0.30*	-0.33**	0.11	-0.32**	-0.52***	0.22	-	0.56***	-0.43***	-0.19	-0.56***	0.04	-0.49***	0.05
NEFA	-0.10	-0.44***	0.16	-0.25*	-0.44***	0.39***	0.56***	-	-0.65***	-0.16	-0.60***	-0.01	-0.34**	-0.14
Glucose	0.02	0.43***	-0.21	0.33**	0.48***	-0.49***	-0.43***	-0.65***	-	0.29*	0.51***	-0.19	0.35**	0.02
Lipase	-0.17	0.19	-0.22	0.37**	0.38**	-0.46***	-0.19	-0.16	0.29*	-	0.39***	-0.35**	0.17	0.03
Energy balance	-0.01	0.25*	-0.23	0.40***	0.30*	-0.31**	-0.56***	-0.60***	0.51***	0.39***	-	-0.16	0.47***	0.12
BMI	0.29*	-0.20	-0.37**	-0.40***	-0.07	-0.03	0.04	-0.01	-0.19	-0.35**	-0.16	-	-0.20	0.47***
BWgain	0.05	0.15	-0.15	0.29*	0.23*	-0.26*	-0.49***	-0.34**	0.35**	0.17	0.47***	-0.20	-	-0.07

*P<0.05
** P<0.01
*** P<0.001

Paper III

Energy status, measured by computer tomography (CT)-scanning, and milk quality of dairy goats fed rations with various energy concentrations

Ingjerd Dønnem, Margrete Eknæs, Åshild T. Randby

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Energy status, measured by computer tomography (CT)-scanning, and milk quality of dairy goats fed rations with various energy concentrations

I. Dønnem, M. Eknæs, Å.T. Randby

Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway.

Corresponding author:

Ingjerd Dønnem

ingjerd.donnem@umb.no

Phone: +47 64966037

Fax: +47 64965101

Abstract

The milk produced by Norwegian goats varies in quality with regard to level of free fatty acids (FFA) and sensory quality. The aim of this study was to relate the milk quality to the changes in the energy status of dairy goats during early and mid lactation when fed rations with various energy concentrations. Twelve goats of the Norwegian Dairy Breed were from lactation week 3 to 18 fed grass silages prepared from the primary growth at a very early or normal stage of maturity (harvesting time (HT) 1 and 3, respectively), supplemented with a low (LC; 0.6 kg daily) or normal (NC; 1.2 kg daily) level of concentrate. Energy status was estimated by changes in body composition measured by computer tomography (CT), calculated energy balance and blood parameters.

Calculated increase in adipose tissue mass of the goats was highest for HT 1 with NC. During the first 18 weeks of lactation only the goats fed the lowest energy diet (HT 3 with LC) mobilized from the adipose tissue (in average 3.26 kg). The other goats deposited body fat throughout this period, and had low (<0.3 mmol/l) blood concentration of non-esterified fatty acids (NEFA). Calculated energy balance was positive throughout the indoor experiment for goats fed HT 1. Both net energy lactation (NEL) intake and milk yield increased with earlier stage of grass maturity and was positively correlated to daily gain in adipose tissue mass. Milk FFA tended to be higher (P = 0.09) for goats offered HT 1 (1.48 mEq/l) than HT 3 (0.99 mEq/l), but was not significantly affected by concentrate level. A high milk FFA concentration was correlated to a high NEL intake. The level of milk FFA increased when the goats were let out on mountain pasture in lactation week 26-30. This study documents that it is possible to avoid fat mobilization in early lactation by feeding the goats high energy diets, based on high quality grass silage. However, a high plane of nutrition during early and mid lactation seemed to increase milk lipolysis. A high-energy diet offered indoors during the 18 first weeks of lactation did neither improve milk quality during the same period nor in the following mountain pasture period.

Keywords: Goat, Energy status, Adipose tissue, Computer tomography, NEFA in blood, Milk quality

Introduction

The quality of the milk produced by Norwegian goats varies, and in parts of the year some of the milk is unsuitable for cheese production. The most prominent problem is a high content of free fatty acids (FFA), which is a measure of lipolysis, i.e. the hydrolysis of fat globule triglycerides into FFA (Chilliard et al., 2003). The total concentration of FFA is found to be correlated to the frequency of off- flavor (Collins et al., 2003). Eknæs et al. (2006) found high FFA concentrations in goats' milk and a specific rancid-tart flavor in mid lactation after a period with substantial energy mobilization. Norwegian goats are kept on natural pasture in 3-4 months during summer, and there is a particular problem with high FFA concentration and poor sensory milk quality during this period (Eknæs and Skeie, 2006).

The energy balance in lactating animals can be estimated by the difference between ingested energy and required energy for body maintenance and for milk secretion. This balance is variable, according to animal milk genetic potential and lactation stage, as well as on composition and energy concentration of the diet (Chilliard et al., 2003). Lactating goats are able to mobilize a considerable amount of adipose tissue in early lactation (Dunshea et al., 1990; Eknæs et al., 2006; Ngwa et al., 2009) to maintain milk production according to their genetic potential. Milk fat synthesis is partly based on mobilized fat and the fatty acid composition of milk in early lactation will therefore differ from that of milk synthesized in later lactation when animals are in positive energy balance (Chilliard et al., 2003).

The energy status of the goats can be estimated by changes in blood constituents (Dunshea et al., 1989), or by directly measuring the body composition. The total body fat of goats is partitioned into different depots, including subcutaneous, intermuscular and visceral fat (Bondi, 1987). As goats deposit most of their body fat as visceral fat (Colomber-Rocher et al., 1992; Marinova et al., 2001) scoring of body condition is more difficult in goats than in sheep. Computer tomography (CT) is found to give reliable information about the body composition of lambs (Jones et al., 2002; Kvame et al., 2004; Kongsro et al., 2008) and pigs (Jopson et al., 1995) both when used on carcasses and on live animals. Eknæs et al. (2006) used CT measurements on goats to predict the changes in body composition throughout a lactation.

It is possible to achieve a high energy content of grass silage by early harvesting time (HT) (Rinne et al., 1999; Randby et al., 2010; Dønnem et al., submitted-b), and concentrate supplementation will increase energy intake. The main objectives of this work was to study the changes in the energy status of dairy goats fed rations with various energy concentrations during early and mid lactation, and relate this to milk quality. Another objective was to study how rations with different energy concentration in early and mid lactation could possibly affect the milk quality in later lactation on mountain pasture.

Materials and methods

Animals and management

The study involved 12 goats of the Norwegian Dairy Breed in 3rd to 7th lactation (mean 4th) which kidded between 10th and 21st of January 2008 (mean 15th). Their average body weight (BW) 2 days after kidding was 60.6 ± 7.6 kg. The goats were housed in individual stalls and were milked twice a day at 06:30 and 16:00 h. Grass silage was given *ad libitum* twice daily at 06:00 and 15:00 h and concentrate was distributed four times per day, at each milking and two hours after each milking. The goats were weighed 2 days after kidding and thereafter every second week at 12.30 h for three consecutive days.

Diets and experimental design

The primary growth of a timothy-dominated sward was harvested at a very early (23- 24 May) and a normal (13 June) stage of maturity in 2007 at Ås, Norway (60°N, 11°E). The silages are referred to as HT 1 and HT 3, respectively. Grass silage harvested in between the experimental silages (4 June), referred to as HT 2, was fed during a pre and post experimental period. Detailed information about the preparation of the silages is given by Dønnem et al. (submitted-b). The experiment started about 3 weeks after mean kidding date, and lasted for 16 weeks. During the pre experimental period between kidding and the start of experiment all goats were fed the same ration, which consisted of HT 2 *ad libitum* plus 0.9 kg concentrate daily (Formel Geit, Felleskjøpet Agri, Norway, with a calculated energy content of 7.8 MJ net energy lactation (NEL)/kg dry matter (DM) and contents (g/100 g) of CP and fat of 16.9 and 8.3, respectively). The goats were allocated to 3 blocks according to their body condition (poor, medium or high) 5

days before the beginning of the experiment. CT scanning with a visual rapid evaluation of the scans was used to assign the goats into the blocks according to body condition. Within each block, goats were randomly assigned to the four diet treatments. The experimental diets were arranged in a 2 x 2 factorial design, consisting of the two silage qualities and either a low (LC; 0.6 kg daily) or normal (NC; 1.2 kg daily) level of concentrate. The ingredient composition of the concentrate is given by Dønnem et al. (submitted-b). The goats were kept on the same diet throughout the 16 weeks. The genetic status of the goats was known, and the distribution of goats with casein α-s1 genotype FF (or "null null") was as following: one goat in each of dietary groups with HT 1 and two goats in each of the dietary groups with HT 3. Goats of FF genotype are more prone to secrete milk with lower fat content and higher levels of lipoprotein lipase activity (LPL) than goats of other casein α-s1 genotype (Chilliard et al., 2003).

After the experimental period the goats were housed indoors for five weeks and group fed HT 2 *ad libitum* plus 0.9 kg concentrate daily (Formel Geit). The goats were let out to mountain pasture the 26th of June. The pasture was located in Folldal (62°N; 10°E), above the treeline, 900- 1000 m.a.s.l. The goats grazed both day and night and were supplemented with 0.5 kg concentrate/goat/day (Formel Favør 30, Felleskjøpet Agri, Norway, with a calculated energy content of 6.6 MJ NEL/kg DM, and contents (g/100 g) of CP and fat of 15.7 and 4.4, respectively).

Computer tomography

The goats were subjected to CT four times during the experiment (31 January, 14 March, 18 April and 19 May) at mean lactation week 2, 8, 13 and 18, respectively.

Prior to scanning the goats were fasted for minimum 16 h and then sedated with an intravenous injection of Xylacin (0.12 mg/kg BW. They were scanned lying on their right lateral in a cradle. A series of cross-sectional images were taken at 40 mm distance between the first cervical vertebra and the hock joint using a Siemens Somatom Emotion CT scanner (Siemens AG, Erlangen, Germany). An average of 30 images was taken per animal. Instrumental settings of the CT were: voltage 130 kV, current 170 mAs, 512 x 512 pixels per image, slice thickness 3 mm, field of view 500 and kernel B50s. The exposure time was 1.0 s per scan. Images were analyzed

using the program 'Autocat' (Jopson et al., 1995), which quantifies the areas of different tissues. This required the images to be rescaled to a 256 grey scale. The level of gray scale values in a CT image is expressed in Hounsfield Units (HU), which range from -1024 (no absorption) to +1023 (complete absorption). Before using the program, all images were dissected manually on screen. The non-fat visceral components (which contain all abdominal and thoracic organs) were removed from all scans, leaving the protein tissue and the adipose tissue (subcutaneous, intermuscular and visceral) of the soft tissues. Tissue area from each scan was numerically integrated to estimate tissue volume for each depot (Gundersen et al., 1988). These were corrected for density to provide estimates of tissue weight based on the relationship between HU and density (Fullerton, 1980). All CT scans included in the analysis (n = 1440 recorded scans) were analyzed by the same operator. The repeated scans within the same animal were used to estimate the changes in body composition of the animals.

Measurements and samplings

Samples of silage were collected during feed out once a week in the experimental period, and stored for -20°C until analysis. Concentrate was sampled once a week in the experimental period and composited to one sample for every fourth week. Individual feed intake was recorded four days per week. Milk yield was measured for three consecutive days every week. For each goat, the samples taken within the 3-day period were mixed prior to chemical analysis. In addition to the weekly milk samples, individual samples for chemically analyzed FFA, sensory analysis and fatty acid composition were collected for one day every fourth week (mean lactation week 6, 10, 14, 18). Milk production and milk FFA concentration were also measured one day before and after the experimental period in mean lactation week 2 and 22, respectively. Additionally, one-day milk production, FFA concentration and sensory quality were measured twice during the mountain grazing, in mean lactation week 26 and 30. Blood samples were collected before morning feeding one week before the start of the experiment and thereafter every fourth week. Table 1 gives an overview of the different times for measurements and samplings.

Chemical and sensory analysis

Chemical analyses of silage and concentrate were done as reported previously by Dønnem et al. (submitted-b). Weekly milk samples from each goat were preserved with 2-bromo-2-

nitropropane-1,3-diol and stored at 4°C before analysis of fat, protein, lactose and urea content with an infrared (IR) milk analyzer (Milkoscan 6000, Foss- Electric, Hillerød, Denmark) 4-7 days after sampling. The one-day milk sample was stored non-preserved at 4°C for 2.5 days before sensory analysis and chemical FFA analysis, and further storage at -20°C for fatty acid composition. Free fatty acids in milk were analyzed by Autoanalyzer 3 (Bran + Luebbe GmbH, Norderstedt, Germany), as described by Bråthen (1984). Analysis of the blood samples and fatty acid composition and sensory analysis of the milk was performed as described by Dønnem et al. (submitted-a). Sensory analysis was performed by a permanent panel of trained goat-milk assessors. In lactation week 26, another trained panel had to step in due to summer vacation.

Calculations of NEL, amino acids absorbed from the small intestine (AAT), protein balance in the rumen (PBV), energy corrected milk (ECM) and energy balance were performed as described by Dønnem et al. (submitted-b).

Statistical analysis

Statistical analysis was performed using the MIXED procedures of SAS (SAS, 2003). Samples were collected several times from each animal during the experimental period, and therefore a repeated measurement procedure was used. The covariance structure of the repeated measurements was chosen by comparing potential structures using Akaike's Information Criterion and Schwarz' Bayesian Criterion and a variance components structure was found to be the best fit for all variables. Statistical analysis of the milk production (included yield and milk IR analysis) and intake data was performed according to the following model:

$Y_{ijklm} = \mu + B_i + L_j + S_k + C_l + G_m(S_k, C_l) + SC_{kl} + LS_{jk} + LC_{jl} + LSC_{jkl} + E_{ijklm}$

where Y_{ijklm} = the dependent variable; μ= the overall mean; B_i= fixed effect of block i, i=1,…3; L_j = fixed effect of lactation week j, j = 1,…16; S_k = fixed effect of silage harvest time k, k = 1, 2; C_l = fixed effect of concentrate level l, l = 1, 2; $G_m(S_k, C_l)$ = random effect of goat m within silage k and concentrate level l; E_{ijklm} = random residual.

Sensory milk quality, FFA chemically and blood data were analyzed according to the same model except that L_j was the fixed effect of lactation week 6, 10, 14, 18. Covariates from the preliminary period were used when found significant. These variables were BW, and milk

concentrations of FFA, fat, protein and milk solids (the sum of fat, protein and lactose). Body composition was analyzed according to the same model where L_j was the fixed effect of lactation week 8, 13, 18, and where lactation week 2 was used as covariate. Results are presented as least square means (LSmeans) and standard error of means. Significance level $P < 0.05$ was used to indicate significance differences. A tendency was stated when $P < 0.1$. Measurements of milk production variables after the 16-weeks experimental period are not included in the statistical analysis nor reported in any tables; they are only presented in Figure 4.

Results

Feed chemical composition and feed values

The silages and the concentrate fed are characterized in Table 2. Delayed harvesting time decreased the concentrations of CP and fat, and increased the concentrations of NDF in the silages. Both digestible organic matter per kg DM (D- value) in silage and the silage energy value (NEL) decreased markedly from HT 1 to HT 3. The value of AAT and PBV decreased with delayed harvest time.

Energy status and blood metabolites

The BW increased for all treatments during the experimental period (Table 3), with the strongest increase for goats fed NC. Also the goats fed the low energy diet, HT 3 with LC, gained BW in total, although they lost 41.7 g BW per day from lactation week 1 to 8. Two weeks after kidding, before the beginning of the experiment, the average adipose tissue mass was 9.69 kg (Figure 1). At lactation week 18 the goats fed HT 1 with LC, HT 1 with NC and HT 3 with NC had increased their adipose tissue mass to 12.9 kg, 16.1 kg and 10.9 kg, respectively, while the goats fed HT 3 with LC had decreased their adipose tissue mass to 7.1 kg. For the goats fed HT 3 with LC the mobilization of body fat was in average 3.26 kg (29.7 g fat per day) which constituted 32 % of their adipose tissue mass, and the majority of it (97 %) was mobilized from lactation week 2 to 8 (Figure 2). An example of CT images for one animal fed HT 1 with NC and one animal fed HT 3 with LC, both in lactation week 2 and 18, is shown in Figure 3. All goats, except those fed HT 3 with LC deposited proteinous tissue from lactation week 2 to 18. The proteinous tissue

of the goats fed HT 3 with LC decreased markedly during the period from lactation week 2 to 8 (31 g per day), while it increased during the rest of the indoor experiment.

Calculated energy balance was positive for goats fed HT 1 throughout the experimental period. The goats offered HT 3 with LC was primarily in negative energy balance, while the goats offered HT 3 with NC reached energy balance at lactation week 8 and maintained mainly in positive energy balance through the remaining experimental period (Figure 1). The goats offered HT 1 with NC were in very high energy balance, but decreased their energy balance after lactation week 10.

Postponing the harvest time decreased serum BHBA and increased serum NEFA (Table 3). The NEFA concentration also tended to increase with LC, and was especially high (0.93 mmol/l) in lactation week 5 for the goats fed HT 3 with LC. It decreased to similar levels as for the goats fed the other rations from week 9 and onwards (Figure 1). Serum glucose, triglyceride and cholesterol concentrations increased for all dietary treatments throughout the experimental period, while serum BHBA had no steady direction (results not shown).

Energy intake

Silage and total DM intakes and NEL intake increased with earlier stage of grass maturity (Table 4). The goats fed HT 3 increased their NEL intake from lactation week 3 to 8-11, where they reached their maximum intake (Figure 1). Goats fed HT 1 slightly decreased their intake after lactation week 10.

Milk production

There was a positive effect of earlier stage of grass maturity on the daily yield of milk and ECM and the milk concentration of protein and lactose (Table 4). Increased concentrate level did not influence milk production significantly. The interaction between harvest time and lactation stage had a clear effect on milk yield, fat and lactose concentration, meaning that the harvest time affected these parameters differently during the first 18 weeks of lactation. Figure 4 shows the development of milk and fat yield and milk quality of goats fed the different diets throughout these weeks. The goats fed HT 1 with LC had highest milk yield in week 3-7 (approximately 4.3

kg) but decreased the yield thereafter, to approximately 3.5 kg. By contrast, the other groups had rather flat yields throughout the experimental period, but with strongly different levels (Figure 4). When all goats were fed the same standard diet from week 19, the yield differences between the previous treatment groups seemed to decrease. The goats fed the highest energy diets during the experimental period decreased their milk yield when let out to mountain pasture. Milk fat secretion remained quite steady for the goats fed HT 1 with NC during the experimental period, while milk fat secretion for the other groups decreased during this period. Both milk fat, protein and lactose concentration decreased throughout the experimental period.

Milk quality

Milk quality, measured by milk FFA concentration and sensory milk quality, was not significantly affected by dietary treatment (Table 4). Milk FFA concentration tended, however, to be higher for goats offered HT 1 than HT 3, with the highest FFA values for goats fed HT 1 with NC. Numerically, these goats also obtained the poorest sensory milk quality. In general, milk FFA concentration increased from week 2 (preliminary period) to week 6, but was thereafter rather stable until the end of the experiment in week 18 (Figure 4). It increased slightly in week 22 and in the mountain pasture period (week 26 and 30). Sensory quality decreased from week 2 to week 6 and continued to decrease slightly until the end of the experiment in week 18. Sensory quality was thereafter rather stable during the mountain pasture period. There was no effect of block (initial body composition) on the milk FFA and sensory quality.

The fatty acid composition in milk (Table 5) was only to a small extent affected by dietary treatments. The proportion of C4, C18:1-t11 and C18:2-c9t11-CLA fatty acid was highest when HT 1 was fed, and the short-chain fatty acids C6- C12 and C18:3-c9c12c15 also tended to be highest for HT 1. The proportion of short and medium chained fatty acids increased throughout the experimental period for the goats fed HT 3 with LC, and mainly decreased for the other dietary treatments. It was mainly opposite regarding the proportion of long chained fatty acid (results not shown).

Discussion

Contrary to most previously reported research the goats in this experiment mainly deposited adipose tissue in early lactation. Only the goats fed the lowest energy diet (HT 3 with LC) mobilized body fat. From lactation week 2 to 8 they mobilized 74 g fat per day, which was comparable to the results found by Dunshea et al. (1989) where primiparous Saanen goats had a fat loss of 64 g per day from lactation week 2 to 5. During lactation week 2 to 11 Eknæs et al. (2006) measured the body fat loss to be 49 g per day in Norwegian dairy goats fed indoors the first month, then grazing cultivated pasture until week 11. Ngwa et al. (2009) estimated a high fat mobilization in the first 2 months of lactation, but a gain of fat tissue in month 3 to 6. The correspondence between these reported results and the fat mobilization by the goats fed HT 3 with LC could be that the goats in all these situations were offered a shortage of energy compared to their demand in that part of lactation. By contrast, a high energy intake in the present experiment, caused by either highly digestible silage or a high level of concentrate, or both, prevented fat mobilization during the 18 first weeks of lactation. Similarly, the gain of proteinous tissue of the goats fed other diets than HT 3 with LC indicated a high energy supply. While the goats fed HT 3 with LC mobilized both body fat and proteinous tissue from lactation week 2 to 8, they also lost BW. Otherwise, goats measured to deposit fat and proteinous tissue also gained BW. Although measures of BW changes in dairy goats not always reflect changes in body energy stores (Dunshea et al., 1990; Eknæs et al., 2006) this study indicates that measurements of BW changes sometimes may be well related to the energy balance of dairy goats.

Ngwa et el. (2009) compared the effect of two dietary forage levels and stage of lactation on body composition of Alpine dairy goats. Body composition was determined by slaughter measures. A low forage (40 %) diet, which had highest energy concentration, resulted in greater body fat mass than a high forage (60 %) diet. This is in line with the present experiment, where higher energy intake increased the body fat mass. In the study by Ngwa et al. (2009) the daily change in body fat mass was not significantly affected by the dietary level, in contrast to the present study.

Blood concentrations of NEFA are closely linked to the goats' energy balance. If goats are in negative energy balance there is a high rate of lipolysis in adipose tissue which elevates the concentration of blood NEFA (Dunshea et al., 1989). The NEFA concentration at zero energy balance, excluding the preliminary period, was calculated to be 0.249 mmol/l ($y = -0.0278x + 0.249$, $P < 0.001$, $R^2 = 0.20$, where y= NEFA mmol/l, x= calculated energy balance in MJ NEL, n=48). This corresponds well with the results of Dunshea et al. (1989), where NEFA concentration was 0.217 mmol/l at zero energy balance. The goats fed rations with HT 3 with LC had an elevated concentration of NEFA at lactation week 5, which agrees well with their mobilization of adipose tissue and calculated energy balance at this time. The NEFA concentration decreased to the same level as the goats fed HT 1 at lactation week 9 and onwards, and reflected the increasing energy balance of the goats fed HT 3 (Figure 1). Goat adipose tissues are rich in C18:1-c9, C16:0 and C18:0 (Banskalieva et al., 2000), which are incorporated into milk fat during fat mobilization (Palmquist et al., 1993). Chilliard et al. (2003) reported that 59% of the variability of milk C18:0 + C18:1 fatty acids was linked to changes in energy balance of the goats. Both Dønnem et al. (submitted-a) and Eknæs et al. (2006) found a strong positive correlation between both C18:0 and C18:1-c9 in milk and plasma NEFA concentration. In the present study such correlations were not detected, however, for the goats fed HT 3 with LC, C18:0 and C18:1-c9 in milk was highest at lactation week 6 (as the serum NEFA concentration) and then decreased throughout the experimental period.

The high energy intake of the goats fed the highest energy rations provided a high supply of substrates from ruminal digestion which supported both high milk yield, milk fat secretion and deposition of adipose tissue. Goats have their maximum DMI from 6 to 10 weeks after parturition (Sauvant et al., 1991). This is in accordance to the present experiment, where the goats reached their peak energy intake at lactation week 8 to 10 (Figure 1). After reaching its maximum the intake decreased, with about 0.16 MJ NEL per week as an average of all dietary treatments. Simultaneously with the decrease in energy intake, the goats fed HT 1 with NC decreased their energy balance after lactation week 10 because milk yield was maintained (Figure 4).

In line with Dønnem et al. (submitted-a) both the earliest harvested silage (HT 1) and the highest concentrate level (NC) seemed to cause higher milk FFA concentrations than normal harvested silage and LC, but in the present experiment with continuous design and few animals, these differences did not reach significance ($P = 0.09$ for silage maturity stage and $P = 0.14$ for concentrate level). During the present conditions it was observed that milk FFA was positively correlated to NEL intake ($n = 12$, $r = 0.60$, $P = 0.04$) and tended to be positively correlated to adipose tissue mass ($n = 12$, $r = 0.55$, $P = 0.06$) and milk yield ($n = 12$, $r = 0.56$, $P = 0.06$). This suggests that milk lipolysis in goat during early and mid lactation occurs more likely in high yielding animals in a high plane of nutrition than in low yielding animals on poorer nutrition.

The level of milk FFA was clearly lowest in the pre experimental period at lactation week 2. It increased until week 6 when peak lactation was obtained, and remained thereafter at a quite steady level the first 18 weeks of lactation. When the concentration of milk FFA exceeds 2.0 mmol/l, goat milk is considered as unsatisfactory for cheese production (Skeie, personal communication). None of the dietary treatments resulted in concentrations above this limit during the indoor experimental period. The level of FFA increased when the goats were let out to mountain pasture. Eknæs et al. (2006) reported that goats started to produce milk of inferior quality (FFA > 2.0 mmol/l) when they were let out to mountain pasture, around 11 weeks after kidding. At this point approximately 40 % of their prepartum adipose tissue mass had been mobilized. In the present experiment the goats who deposited adipose tissue the first 18 weeks of lactation also increased their milk FFA concentration on mountain pasture. Goat milk lipolysis and LPL activity are at their highest level after the lactation peak, and are, according to Chilliard et al. (2003), low in very early and in late lactation. The stage of lactation was confounded with the mountain pasture period both in the study of Eknæs et al. (2006) and in the present study. However, while the goats started to produce milk of inferior quality already in week 11 on mountain pasture in the first study, the milk quality remained quite stable and good until week 22 in the present study, at least for the poorest fed animals. When let out on mountain pasture, the milk quality decreased also in the present study, although this was as late as in lactation week 26 and 30. At that point the goats in the first study were back home on cultivated pasture, and produced milk of high quality. Together, these two studies suggest that the mountain pasture period is a great challenge as regards goat milk quality. Whether the reason for it is related to

energy reserves, energy intake, energy balance, high physical activity, special mountain pasture plants or brushes or other factors remains unknown.

Milk lipolysis and milk LPL activity are highly correlated in goat milk, because the milk LPL are bound to the cream (Chilliard et al., 1984). Milk LPL originates from either adipose tissue LPL or mammary LPL which have leaked into the milk (Chilliard et al., 1979). Chilliard et al. (1979) found that when fasting goats for 2 days milk LPL activity decreased sharply, together with adipose tissue LPL activity. This lowered the milk lipolysis. Similarly, Eknæs and Skeie (2006) observed that goats fed restricted amounts of roughage in a 2 day period had lower milk FFA and higher sensory milk quality than goats with free access to pasture. The restricted fed goats were in clearly lower energy balance than those with free access to pasture. These short time fasting experiments had similar outcome as 16 weeks of low energy feeding (HT 3 with LC) in the present experiment. The activity of LPL in adipose tissue is stimulated by insulin and supplies adipose tissue with fatty acids from circulating triglycerides (Borensztajn et al., 1972). The LPL activity is found to be low in early lactation, when the anabolic activity of adipose tissue usually is low, and to increase when animals return to a positive energy balance (Chilliard et al., 1977). A high lipogenesis in adipose tissue is assumed to limit the flow of fat to the mammary gland (Griinari et al., 1997). Adipose tissue LPL activity is found to be positively correlated ($r = +0.61$) to milk LPL activity (Chilliard, 1985), and Chilliard et al. (2003) hypothesized that a low flow of blood fat reduces the need of LPL at the basal membrane and increases the availability of LPL for secretion into milk. Consequently, as the adipose tissue mass deposition and calculated energy balance were highest for the goats fed HT 1 in the present experiment, the tendency of highest milk FFA when feeding HT 1 could partly be due to a high adipose tissue LPL activity at high energy supply and positive energy balance.

Besides the milk LPL activity, the stability of the milk fat globule membrane (MFGM) would influence the susceptibility of milk to lipolysis (Deeth, 2006). Eknæs (2009) hypothesized that goat milk with high concentrations of FFA has lower concentrations of MFGM components than normal goat milk. One of the important precursors in the synthesis and stability of MFGM is cholesterol, which is partly supplied by the blood plasma, and partly synthesized in the mammary gland (Nielsen and Jakobsen, 1994). No differences in blood serum concentration of

cholesterol were detected in the present study (Table 3). When mammary *de novo* synthesis of fatty acids is high, cholesterol synthesis is found to be low (Smith et al., 1986). The milk fat of the goats fed HT 1 in the present experiment tended to have highest proportion of short and medium chain fatty acids, which suggests that those goats synthesized less cholesterol for the maintenance of MFGM stability and may therefore have produced milk with higher FFA concentration.

Conclusion

Feeding lactating goats a diet with a high energy concentration obtained by early harvest of grass silage and increased concentrate level, increased energy intake and milk yield. The mass of adipose tissue of the goats increased with increasing energy content of the diet. The goats fed the lowest energy diet (HT 3 with LC) mobilized from the adipose tissue until lactation week 8 only, after which they obtained zero energy balance. The rest of the goats deposited body fat throughout the 16 experimental weeks. It was clearly possible to feed goats in early lactation with sufficient energy to give a high milk yield and at the same time avoid fat mobilization. During the present conditions, a high plane of nutrition during early and mid lactation increased lipolysis in milk, maybe caused by increased milk LPL activity and reduced stability of the MFGM. A high energy ration indoors during the first 18 weeks of lactation did not improve milk quality neither simultaneously nor later on mountain pasture.

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Table 1. Time (lactation week) for measurements and sampling of the goats

	Period			
	Pre experimental	Experimental	Post experimental	Mountain pasture
Feed intake	2	3-18		
Milk yield and composition (IR)	2	3-18	22	26, 30
Milk FFA[1]	2	6, 10, 14, 18	22	26, 30
Sensory milk quality	2	6, 10, 14, 18		26, 30
Milk fatty acid composition		6, 10, 14, 18		
Blood samples	2	5, 9, 13, 17		
CT-scanning	2	8, 13, 18		
Weighing of the goats	1	Every second week from 3-18	22	

[1]Chemically determined free fatty acid concentration

Table 2. Chemical composition and feeding value of silage and concentrate

	Harvesting time		
	1	3	Concentrate
Date of harvest in 2007	22-24 May	13 June	
Dry matter, g/kg	236	238	881
Chemical composition (g/kg DM)			
Crude protein	156	105	177
Fat	34.3	28.7	43.5
Starch			427
Neutral detergent fibre	433	584	181
Water soluble carbohydrates	36.6	18.5	53.6
NH_3-N g/kg of Total N	64.3	73.4	
pH	4.19	4.26	
Feeding values			
D-value[1]	771	619	763
NEL[2] (MJ/kg DM)	7.18	5.26	7.10
AAT[3] (g/kg DM)	78.0	69.5	109
PBV[4] (g/kg DM)	46.8	-20.4	2.59

[1] Digestible organic matter, g per kg DM

[2] Net energy lactation.

[3] Amino acids absorbed in the small intestine.

[4] Protein balance in the rumen

Table 3. Effect of harvesting time and concentrate level on body weight, body composition, energy balance and blood metabolites as means over the 16 weeks experimental period.

Harvesting time	HT 1		HT 3			P-value[2]			
Concentrate level[1]	LC	NC	LC	NC	SEM	HT	C	HT*C	L
Body weight gain, g/day	41^{ab}	89^{a}	32^{b}	66^{ab}	12.34	NS	0.01	NS	
Adipose tissue g/day	24.7^{b}	65.3^{a}	-29.7^{c}	15.0^{b}	10.52	0.003	0.007	NS	
Proteinous tissue g/day	13.0^{b}	25.3^{a}	-0.3^{c}	17.3^{ab}	3.439	0.02	0.005	NS	
Energy balance, MJ NEL	1.31^{ab}	2.29^{a}	-1.16^{c}	-0.27^{bc}	0.526	0.003	NS	NS	<0.001
Blood metabolites									
Glucose mmol/l	3.31	3.39	3.08	3.18	0.123	NS	NS	NS	0.007
NEFA[3] mmol/l	0.17^{c}	0.12^{c}	0.40^{ab}	0.26^{bc}	0.015	0.006	0.08	NS	<0.001
BHBA[4] mmol/l	0.78^{a}	0.68^{a}	0.38^{b}	0.39^{b}	0.081	0.006	NS	NS	0.002
Cholesterol mmol/l	2.66	2.60	2.48	2.41	0.507	NS	NS	NS	<0.001
Triglyceride mmol/l	0.18	0.18	0.22	0.16	0.042	NS	NS	NS	<0.001
Lipase units/l	53.3^{ab}	49.8^{b}	89.3^{a}	38.1^{b}	11.41	NS	0.05	0.08	NS

[a.b.c] Means without a common letter within the same row differ (P<0.05)

[1]LC= low concentrate level (0.6 kg/day), NC= normal concentrate level (1.2 kg/day)

[2]Effect of harvesting time (HT), concentrate level (C), interaction (HT*C), and lactation week (L).

Table 4. Effect of harvesting time and concentrate level on daily feed intake, milk yield and milk composition, measured each week

Harvesting time	HT 1		HT 3			P-value[2]			
Concentrate level[1]	LC	NC	LC	NC	SEM	HT	C	HT*C	L
Intake									
Silage, kg DM	1.97[a]	1.76[a]	1.46[b]	1.31[b]	0.090	0.002	NS	NS	
Concentrate, kg DM	0.53	0.96	0.53	1.05	0.027				
Total, kg DM	2.49[ab]	2.72[a]	1.98[c]	2.36[b]	0.091	0.003	0.02	NS	
NEL, MJ	17.9[a]	19.5[a]	11.4[c]	14.3[b]	0.579	<0.001	0.008	NS	
Milk production									
Milk, kg	3.89[ab]	3.93[ab]	2.59[c]	3.36[bc]	0.238	0.008	NS	NS	<0.001
ECM[3], kg	3.58[ab]	3.69[a]	2.33[b]	2.88[b]	0.234	0.005	NS	NS	<0.001
Fat, g/kg	40.2	32.8	37.0	36.2	2.171	NS	NS	NS	<0.001
Protein, g/kg	28.8	28.9	26.8	27.9	0.562	0.06	NS	NS	<0.001
Lactose, g/kg	44.5[ab]	45.4[a]	44.2[ab]	43.3[b]	0.512	0.05	NS	NS	<0.001
Milk solids, g/kg[4]	114	107	108	107	2.100	NS	NS	NS	<0.001
Urea, mmol/l	7.74	8.60	8.88	9.33	0.590	NS	NS	NS	<0.001
FFA, mEq/l	1.22	1.73	0.86	1.12	0.222	0.09	NS	NS	NS
Milk taste[5]	3.82	3.00	3.83	3.88	0.450	NS	NS	NS	0.04

[a.b.c] Means without a common letter within the same row differ ($P<0.05$)

[1, 2] See Table 3

[3]Energy- corrected milk

[4]Calculated by adding up fat, protein and lactose concentration

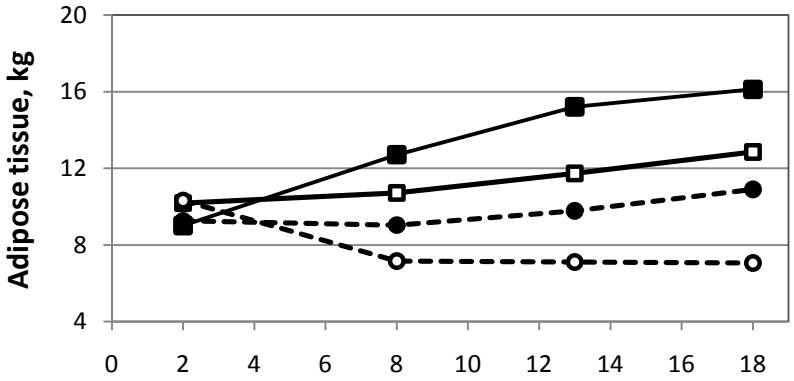
[5]Five point scale where 1= poor quality milk to 5= high quality milk with no deviation from normal aroma or taste

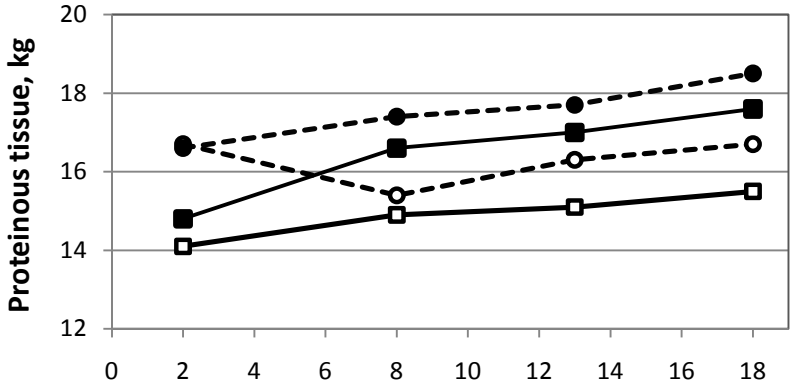
Table 5 . Effect of harvesting time and concentrate level on fatty acid composition (g/ 100 g of total fatty acids) in milk

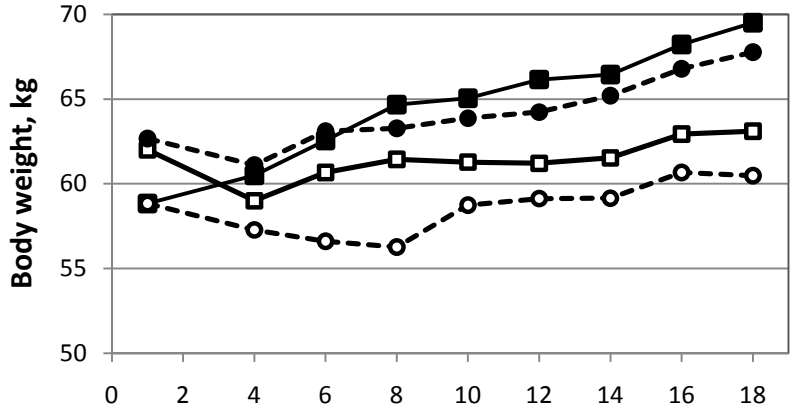
Harvesting time	HT 1		HT 3			P-value[2]			
Concentrate level[1]	LC	NC	LC	NC	SEM	HT	C	HT*C	L
C4:0	3.06[a]	3.10[a]	2.79[b]	2.94[ab]	0.058	0.009	NS	NS	<0.001
C6:0	2.63	2.58	2.32	2.48	0.086	0.06	NS	NS	0.03
C8:0	2.76	2.64	2.27	2.49	0.159	0.09	NS	NS	<0.001
C10:0	9.44	9.02	7.70	8.30	0.616	0.09	NS	NS	<0.001
C12:0	3.88	3.68	3.04	3.43	0.270	0.09	NS	NS	<0.001
C14:0	10.9	9.9	10.2	10.6	0.290	NS	NS	NS	<0.001
C14:1-c9	0.15	0.16	0.16	0.16	0.008	NS	NS	NS	<0.001
C16:0	28.2	29.5	31.1	30.8	1.241	NS	NS	NS	<0.001
C16:1-c9	0.50	0.57	0.65	0.59	0.076	NS	NS	NS	0.03
C18:0	9.50	7.92	8.30	8.32	0.576	NS	NS	NS	0.002
C18:1-c9	18.6	19.2	21.0	20.1	0.836	NS	NS	NS	0.003
C18:1-t11	0.94[a]	1.01[a]	0.73[ab]	0.60[b]	0.087	0.02	NS	NS	NS
C18:2-c9c12	1.19	1.55	1.48	1.67	0.139	NS	0.09	NS	<0.001
C18:3-c9c12c15	0.59	0.56	0.53	0.40	0.047	0.06	NS	NS	<0.001
C18:2-c9t11 (CLA)	0.60[ab]	0.75[a]	0.52[b]	0.46[b]	0.045	0.01	NS	0,06	<0.001
C20:0	0.18	0.18	0.22	0.22	0.017	0.06	NS	NS	<0.001

[a.b.c] Means without a common letter within the same row differ ($P<0.05$)

[1, 2] See Table 3


Adipose tissue, kg
20
16
12
8
4
0
2
4
6
8
10
12
14
16
18


Proteinous tissue, kg
20
18
16
14
12
0
2
4
6
8
10
12
14
16
18


Body weight, kg
70
65
60
55
50
0
2
4
6
8
10
12
14
16
18

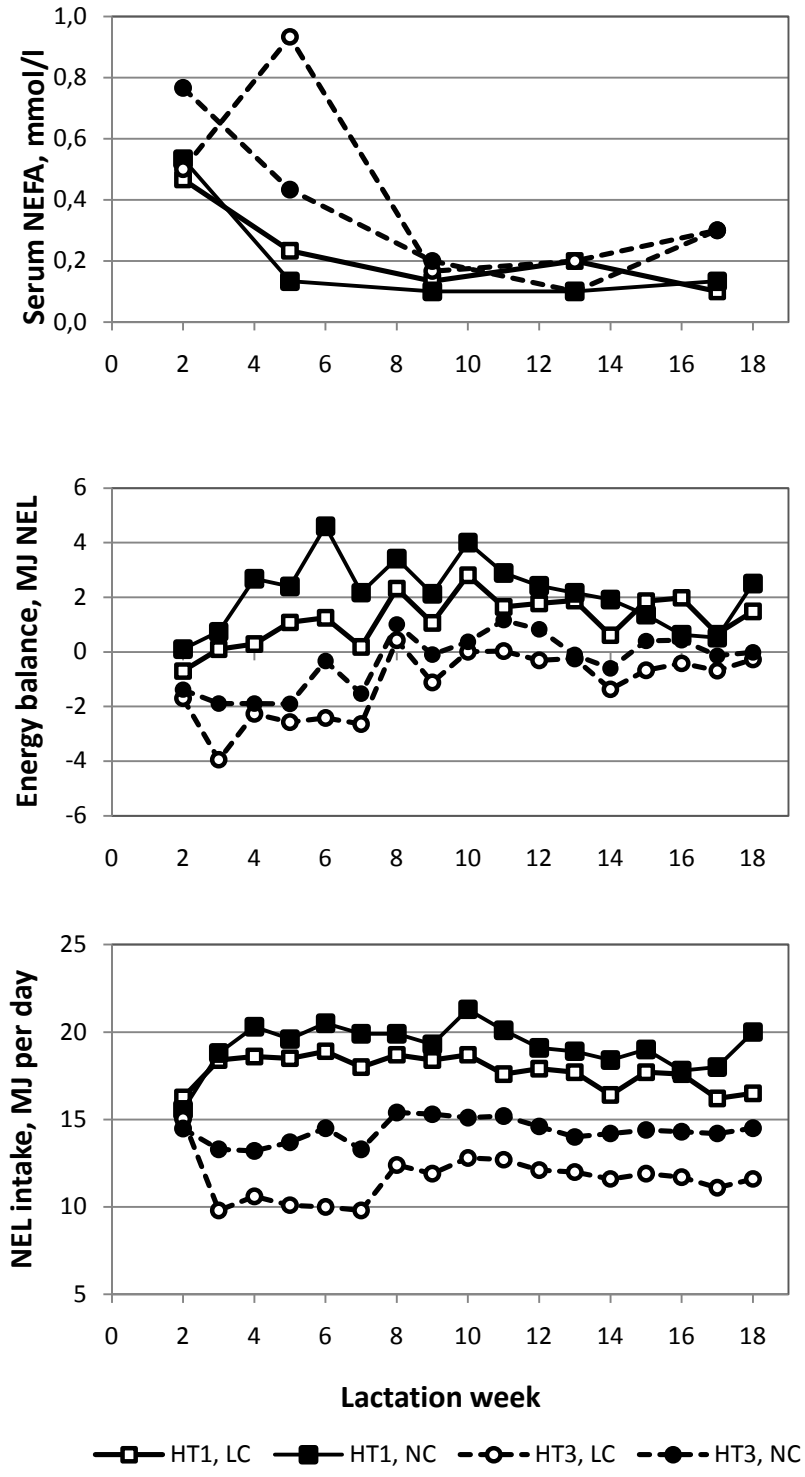


Figure 1. Various measures related to the energy status of the goats during the pre experimental period (week 2) and indoor experimental period (week 3-18) .

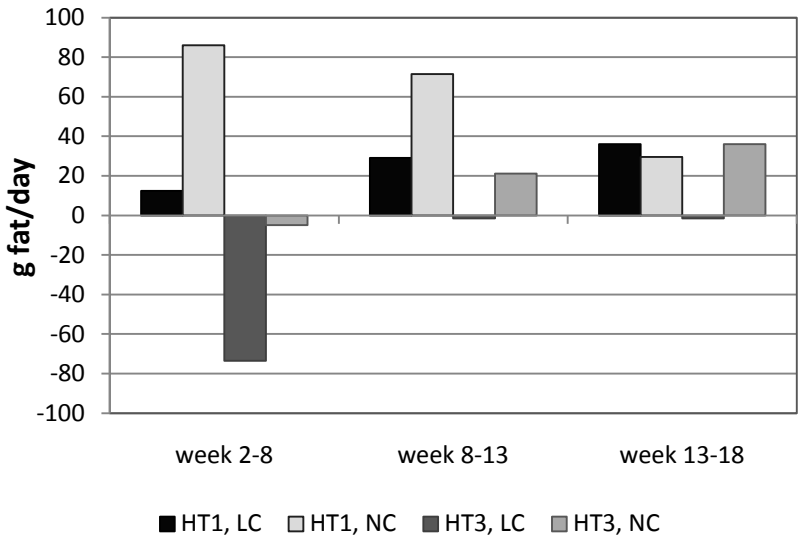


Figure 2. Mobilization and deposition of adipose tissue from the pre experimental period (week 2), and throughout the experimental period.

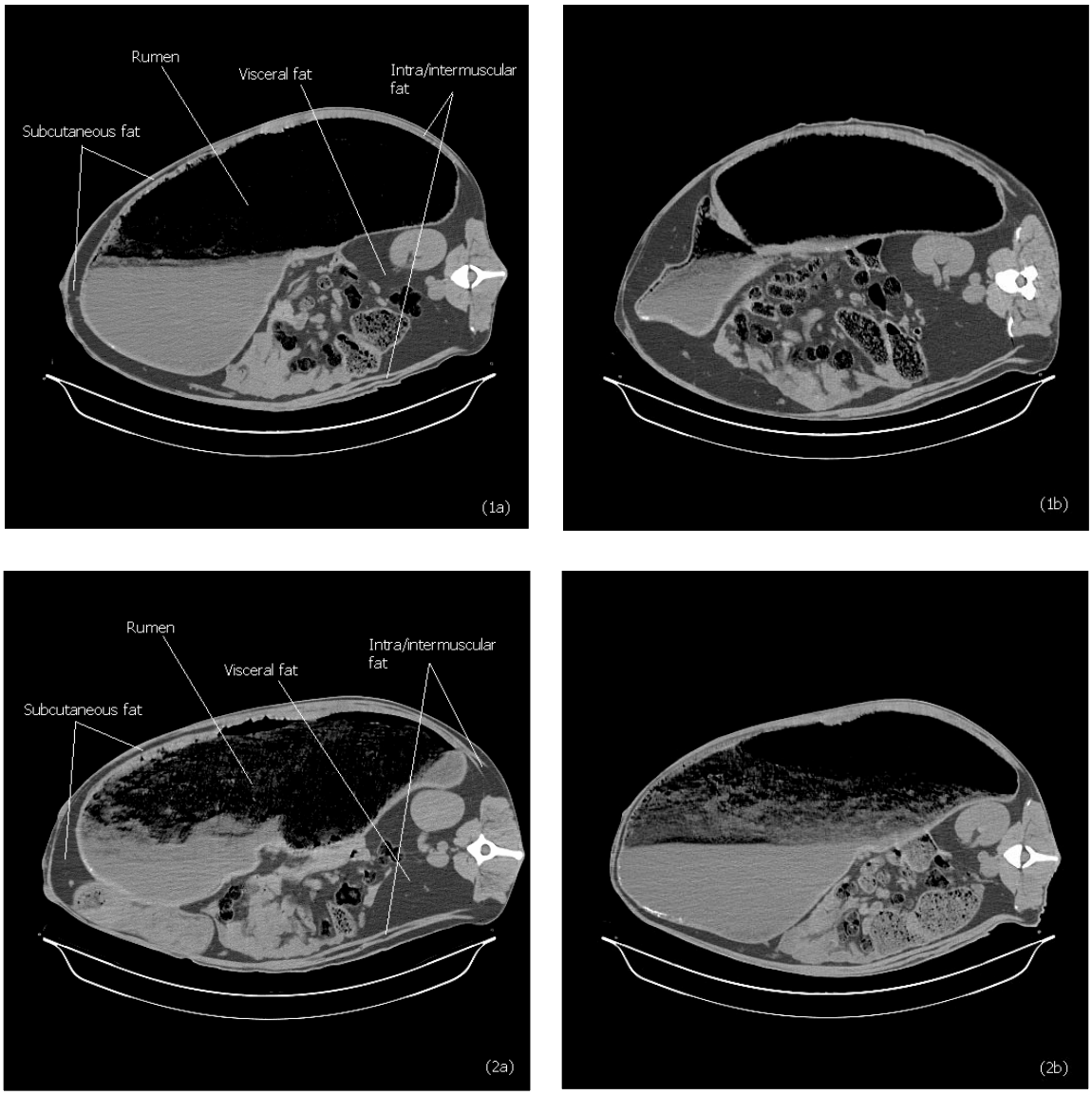
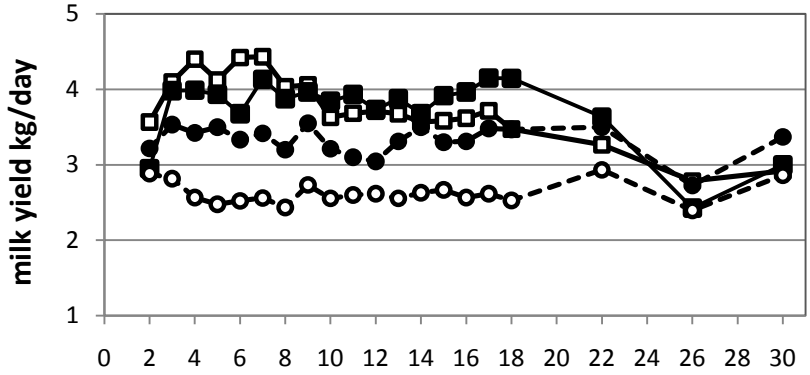
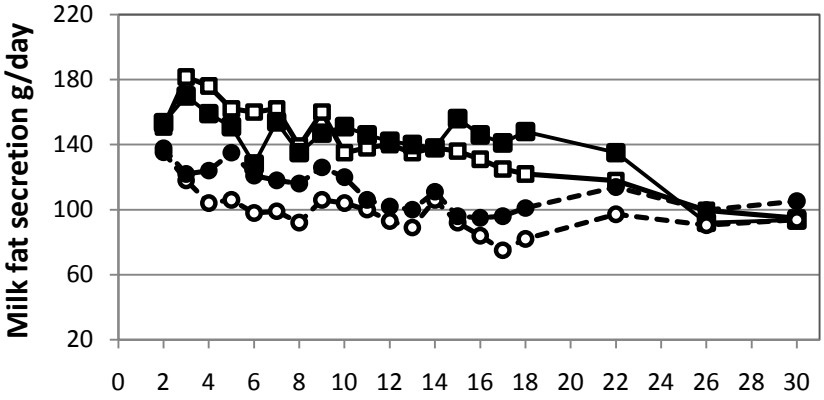
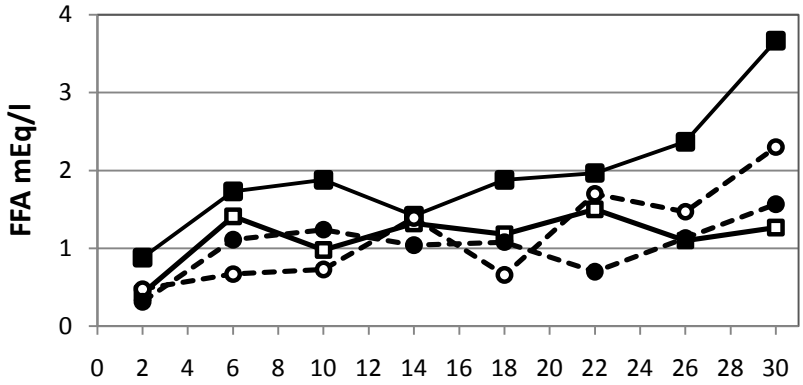


Figure 3. Fat depots in CT images of one goat fed HT 1 with NC at lactation week 2 (1a) and 18 (1b) and one goat fed HT 3 with LC at lactation week 2 (2a) and 18 (2b)


milk yield kg/day
5
4
3
2
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30


Milk fat secretion g/day
220
180
140
100
60
20
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30


FFA mEq/l
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

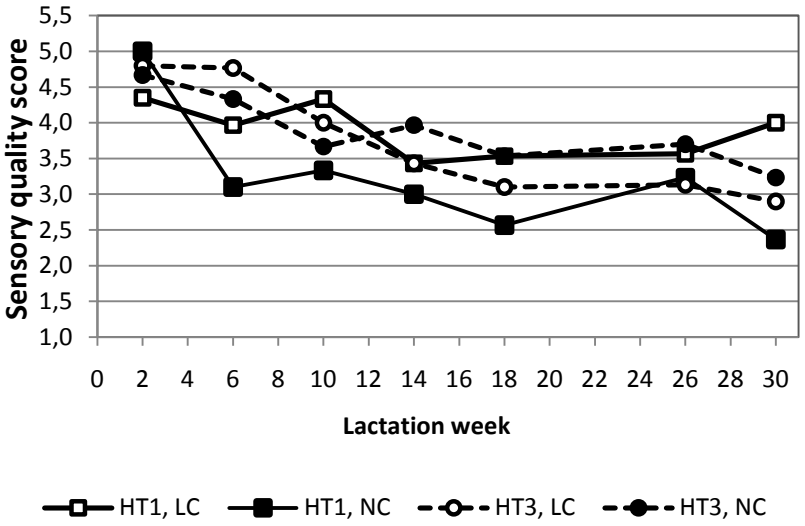


Figure 4. Development of milk and fat yield and milk quality of the goats during the pre experimental periode (week 2), indoor experimental period (week 3-18), post experimental period (week 22) and on mountain pasture (week 26 and 30).