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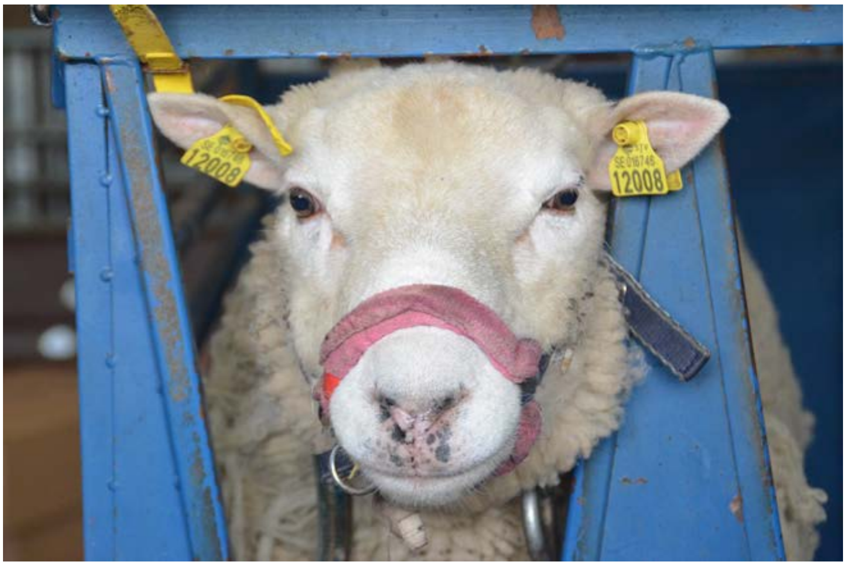
Agricultural Science Programme – Animal Science



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Förord

Detta examensarbete ingår i agronomprogrammet med inriktning husdjur och omfattar 30 hp. Arbetet ingår i ett större projekt som pågick 2012-2014 och finansierades av VL-Stiftelsen, Agroväst Nöt- och lammköttsprogram, Fåreafgiftsfonden, Dansk Fåreavl och Addcon Europe GmbH. Syftet med projektet var att undersöka majs-, gräs- och klöver/gräsensilage samt deras påverkan på konsumtion, ät- och idisslingsbeteende, smältbarhet, partikelstorleksfördelning i träck och proteinutnyttjande hos får för att förbättra foderrådgivningen till idisslare och för att kunna göra mer balanserade foderstatsberäkningar.

Min del av projektet var att undersöka majsensilage med olika mognadsstadier vid skörd, rödklöver med eller utan tillsatsmedel av bakteriepreparat samt gräsensilage och deras påverkan på konsumtion, smältbarhet och proteinutnyttjande hos får. I arbetet ingick att samla in foder-, träck- och urinprover samt att bearbeta data från analyser av proven.

Jag vill tacka handledarna Carl Helander och Elisabet Nadeau för deras tålamod och engagemang att svara på mina frågor och att ge vägledning i arbetet. Jag vill också tacka min examinator Birgitta Johansson för kommentarer på arbetet samt Jonas Dahl och Karin Wallin för hjälp med provtagningar från djur och foder. Även Annika Arnesson ska ha ett tack för hjälp med analyser av prover och sammanställning av data samt Jan-Eric Englund, SLU Alnarp, får ett tack för hjälp med den statistiska modellen. Jag vill även tacka Wolfram Richardt och Stefanie Muche, LKS mbH, Lichtenwalde, för analyser av näringsinnehåll i foder, träck och urin, Kirsten Weiss, Humboldt University, för analyser av ensilagens fermentationsegenskaper samt Börje Ericson, HUV, SLU Uppsala för analys av VOS i foder. Slutligen vill jag tacka familj och vänner för deras tålamod och att de peppat mig till att bli färdig med arbetet.

Summary

Sheep in Sweden are usually fed grass/clover forage supplemented with concentrate during the winter season. Red clover forage fed to ruminants has shown to increase dry matter (DM) intake and give a lower *in vivo* DM digestibility compared to grass forage. Whole-crop maize silage is increasing in use in Sweden and can complement grass silage and grass/red clover silage or replace grain concentrate due to high starch content. Maize has shown to give slightly higher live weight and carcass gain in ruminants when fed separately compared to a mix with grass/red clover silage. The intake and digestibility of silage in sheep is mostly affected by the fibre digestibility. Also, time of harvest affects intake and digestibility of the silages as advancing maturity increases the proportion of less digestible fibre where neutral detergent fibre (NDF) affects the intake negatively. In addition, well-preserved silage affects the intake and digestibility positively. To combine different feedstuffs into a balanced diet, the digestibility, nutrient content and utilization ability by the sheep of each individual feedstuff must be known. Then different forages can be combined and complemented with the right amount of concentrates to increase the production in growth or milk per feed unit, which gives decreased emissions of nitrogen (N).

The aim of this study was to investigate feed intake, *in vivo* digestibility and protein utilization of silages fed to rams with or without protein supplementation. The relative differences between the forages are applicable to other ruminants and contribute to improved feed formulations. The silages used were whole-crop maize harvested at the dough (28 % DM) and the dent (38 % DM) stage of maturity, grass (31 % DM) and red clover/grass (32 % DM) ensiled with or without microbial inoculant. Ten rams were divided into duplicated 5 x 5 Latin squares with five rams (one treatment to each ram) and five periods in each square. One of the groups was supplemented with rapeseed meal. Each period was four weeks long and divided into sub periods with *ad libitum* and 80 % of *ad libitum* intake of silages. When the five periods were completed, all rams had been fed the five different silages. The live weight (LW), body condition score and feed intake were continuously registered during the experiment. Samples of feed, refusals, urine and faeces were collected during the four last days in each period and sent to analysis of fermentation quality and nutrient content in silage and nutrient contents in faeces and nitrogen compounds in urine.

Supplementation of rapeseed meal increased silage intake of all nutrients, crude protein (CP) digestibility, urea and total N in urine. However, N in faeces in % of N intake was lower when supplementation was used. Grass and red clover silages gave generally higher intakes of DM and NDF than maize silage. Grass silage generally gave higher digestibility of DM, organic matter (OM), NDF and acid detergent fibre (ADF) than the red clover and maize silages. Furthermore, grass silage had the highest excretions of allantoin, purine derivatives (PD) and hippuric acid in urine. Red clover silage gave the highest intakes of ADF and CP, similar CP digestibility to grass silage, the highest N and urea excretion but the lowest hippuric acid excretion in urine. Early harvested maize silage had lower DM intake than the grass and red clover silages and both maize silages had the lowest intakes of CP, NDF and ADF. Late harvested maize silage had lower digestibility of DM, OM, CP, NDF and ADF than grass and red clover silages. The excretion of urea was lowest for the maize silages. There was no effect of inoculant addition to red clover silages and no effect of time of harvest of maize silage on feed intake, *in vivo* digestibility or protein utilization by the rams. The LW in rams were not affected by silage diet, but by supplementation of

rapeseed meal, giving higher LW loss at 80 % of *ad libitum* feed intake compared to the un-supplemented group.

In conclusion, grass silage is suitable forage for optimizing diets to ruminants as it had the highest nutrient digestibility and microbial protein efficiency. Red clover silage is suitable when balancing the protein concentration while maize silage can give a higher energy concentration in the diet for growth and production. Red clover and maize silage can preferably be combined in a diet because of the high protein content in red clover silage and the high energy content in maize silage.

Sammanfattning

Under vintersäsongen i Sverige utfodras ofta får med grovfoder i form av gräs/klöver-blandningar som kompletteras med kraftfoder. Rödklöver utfodrat som grovfoder till idisslare har ökat intaget av torrsubstans (ts) medan *in vivo* ts smältbarhet har visat sig vara lägre än för gräs. Helsädesensilage av majs ökar i användning i Sverige och kan komplettera gräs- och gräs/klöver-ensilage eller ersätta kraftfoder av spannmål pga. högt stärkelseinnehåll. Majs har gett något högre ökning av levande- och slaktkroppsvikt hos idisslare när det utfodras separat jämfört med en blandning av gräs/rödklöver-ensilage. Intaget och smältbarheten av ensilage hos får påverkas till stor del av fibersmältbarheten. Även tidpunkten för skörden påverkar intaget och smältbarheten av ensilage eftersom mognaden av växten ökar proportionen av mindre smältbara fibrer, varav neutral detergent fibre (NDF) påverkar intaget mest. Dessutom påverkar väl ensilerat ensilage intaget och smältbarheten positivt. För att kunna kombinera olika fodermedel till en foderstat i balans måste smältbarhet, näringsinnehåll och fårets förmåga att kunna utnyttja varje fodermedel vara känt. Då kan olika grovfoder kombineras och kompletteras med rätt mängd kraftfoder för att öka produktionen i tillväxt eller mjölk per foderenhet, vilket minskar utsläppen av kväve (N).

Syftet med studien var att undersöka foderintag, *in vivo* smältbarhet och proteinanvändning av ensilage hos baggar med eller utan tillskott av protein. De relativa skillnaderna mellan grovfodren kan användas till andra idisslare och bidra till förbättrade foderstatsberäkningar. Ensilagen som användes var helsädesensilage av majs skördat vid deg- (28 % ts) och mjölmognad (38 % ts), gräs (31 % ts) och rödklöver/gräs (32 % ts) ensilerat med eller utan mjölksyrabakterier som tillsatsmedel. Tio baggar delades in i ett duplicerat 5 x 5 romersk kvadrat med fem baggar (en behandling per bagge) och fem perioder i varje kvadrat. Ena gruppen fick tillskott av rapsfrömjöl. Varje period var fyra veckor lång och indelad i delperioder med fri tillgång och 80 % fri tillgång av ensilagen. Efter de fem perioderna var avslutade hade alla baggar utfodrats med de fem olika ensilagen. Levande vikt, hull och foderintag registrerades kontinuerligt under experimentet samt prover samlades in under de fyra sista dagarna i varje period från foder, rester, urin och träck. Proverna analyserades för ensilagens hygien och näringsinnehåll, träckens näringsinnehåll och urinens innehåll av kväveföreningar.

Tillskott av rapsfrömjöl ökade foderintaget av alla näringsämnen, råproteinets smältbarhet samt utsöndringen av urea och totalkväve hos baggarna. Däremot var mängden N i träck i % av N-intag lägre vid tillskott. Gräs och rödklöverensilage gav generellt högre intag av ts och NDF än majsensilage. Gräsensilage gav generellt högre smältbarhet av ts, organisk substans, NDF och acid detergent fibre (ADF) än rödklöver och majsensilage. Dessutom hade gräsensilage högst utsöndring av allantoin, purinderivat och hippursyra i urinen. Rödklöverensilage gav högst intag av ADF och råprotein, liknande smältbarhet av råprotein som gräsensilage, den högsta N- och ureautsöndringen men den lägsta hippursyrautsöndringen. Tidigt skördat majsensilage hade lägre ts-intag än gräs- och rödklöverensilage och båda majsensilagen hade lägsta intagen av råprotein, NDF och ADF. Sent skördat majsensilage hade lägre smältbarhet av ts, organisk substans, råprotein, NDF och ADF än gräs och rödklöverensilage. Utsöndring av urea var lägst för majsensilagen. Tillsats av bakteriepreparat till rödklöverensilage och olika skördetidpunkter av majsensilage hade inga effekter på foderintag, *in vivo* smältbarhet eller proteinutnyttjande hos baggarna. Olika foderstater med ensilage påverkade inte levandevikten hos baggarna, men tillskott av rapsfrömjöl gav ökad viktminskning vid 80 % fri tillgång till ensilage jämfört med gruppen utan tillskott.

Sammanfattningsvis, gräsensilage är lämpligt att använda för att balansera foderstater till idisslare eftersom det hade den högsta smältbarheten av näringsämnen och effektivaste mikrobproteinsyntesen. Rödklöverensilage är lämpligt att använda för att få upp proteinhalten i foderstaten medan majsensilage kan öka energikoncentrationen i foderstaten för ökad tillväxt och mjölkavkastning. Rödklöver och majsensilage kan med fördel kombineras för att få en lämplig balans mellan protein och energi i foderstaten.

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Introduction

Sheep in Sweden are usually fed grass/clover forage supplemented with concentrate during the winter season (Eggertsen & Arnesson, 2007; Eggertsen, 2008). Red clover (*Trifolium pratense* L.) has been shown to increase dry matter (DM) intake, compared with perennial ryegrass (*Lolium perenne* L.) and hybrid ryegrass (*Lolium hybridicum*), when fed as the sole forage to mature rams and young lambs (Paul *et al*., 2001; Marley *et al*., 2007). Also, red clover increased intake in sheep when fed mixed with grass silage, with or without concentrate, compared to grass and red clover fed separately (Niderkorn *et al*., 2012). In addition, red clover potentially can contain more crude protein (CP) and minerals than grass and can thereby, theoretically, replace some of the concentrate (McDonald *et al*., 2002). However, the *in vivo* DM digestibility of red clover is generally lower than in grass, mainly due to the lower extent of neutral detergent fibre (NDF) degradation in rumen (Paul *et al*., 2001).

Whole-crop silage from maize (*Zea mays* L.) is an alternative feed that is increasing in use in Sweden. In 2013, 15 890 ha were used to crop maize, mostly as whole-crop silage (SJV, 2014). Maize has a high–yielding potential and is only harvested once per year whereas grass leys give several smaller harvests. Whole-crop maize silage (WCMS) can complement grass silage (Juniper *et al*., 2005; Keady *et al*., 2007) and grass/clover silage when fed to growing ruminants and works also as a replacement for grain concentrate due to the high starch content (Johansson, 2010). Maize has shown to give higher live weight (LW) gain and carcass weight gain in growing cattle when fed separately compared to when fed in a mix with grass silage (Nadeau *et al*., 2013; Zaralis *et al*., 2014). The stage of maturity can also matter where early harvested WCMS can tend to give higher LW gain compared to late harvested WCMS (Zaralis *et al*., 2014).

The digestibility of the silage is mostly affected by the fibre digestibility, and silage made from grass, clover or maize has higher fibre concentration and more variation in the fibre digestibility than commercial feedstuffs or cereals (McDonald *et al*., 2002). The differences in fibre digestibility depend on the chemical structure (i.e. the proportions of hemicellulose, cellulose and lignin) and quantity of fibre. The time of harvest affects digestibility in maize, grass and clover. At advancing maturity the proportion of less digestible fibre increases in the stem of the plants. The stems consist of more fibre compared to the leaves, especially of lignin, which makes the forage less digestible (Wilman *et al*., 1977; Nordkvist, 1987; McDonald *et al*., 2002).

Ewes can consume at maximum 1.9 % of their LW in NDF from forage during lactation when supplemented with concentrate (Helander *et al*., 2014). Therefore, it is important to choose the right harvesting time and plant species in the ley to ensure a low-to-medium NDF concentration and, thereby, promoting a high feed intake. An increased feed intake increases the nutrient intake, which is especially important during growth in lambs and lactation in ewes to keep normal body condition (Eggertsen & Arnesson, 2007; Nadeau & Arnesson, 2008).

The protein content in the diet can affect feed intake; especially feed intake of high fibre content is favoured by supplementation of protein (Galyean & Goetsch, 1993; Matejovsky & Sanson, 1995). The availability of nitrogen (N) for sheep is a balance between the intake of available amounts of carbohydrates and protein for the rumen microbes to successfully convert the feed into energy without too much N lost in urine (Hvelplund *et al*., 1987; Pettersson, 2006). The ensiling process is one important factor for the protein utilization in the animal. At wilting and ensiling, true protein is broken down to non-protein nitrogen (NPN), which can contribute to 50-60 % of the CP concentration in silage. The highest CP concentration is achieved by a quick pre-drying of early harvested forage and by preserving correctly (Pettersson, 2006; Nadeau *et al*., 2012), preferably with additives to avoid secondary fermentation, especially in red clover silage (Pahlow *et al*., 2001; Nadeau & Auerbach, 2013). A limited amount of extra protein feed is needed when the silage is well preserved, which also increases palatability of the silage. An early harvest gives silage with high energy and low fibre concentration as well, which is positive for the microbial degradation of the feed in rumen (Pettersson, 2006). By using additives, such as formic acid, lactic acid bacteria and molasses, the DM losses decrease during fermentation and storage of the silage (Hetta *et al*., 2003; Jatkauskas & Vrotniakiene, 2005).

By knowing the diet's digestibility, a balanced diet can be set up (Särkijärvi *et al*., 2012) and increase the production in growth or milk per feed unit, which gives decreased emissions of N and methane (Nadeau *et al*., 2007; Kumm, 2011). Kumm (2011) has found that leys for pasture and mowing gives a better storage of carbon in the soil and less emissions of carbon dioxide equivalents than grain cultivation. This means that a diet with a high ration of forage gives less total emission of carbon dioxide equivalents than a diet with a high ration of concentrate (Kumm, 2011), even if the emission of methane by eructation decrease with a diet rich in concentrate (McDonald *et al*., 2002).

There are some studies done on comparing maize silage digestibility with grass and red clover silage digestibilities in ruminants and how they can be combined to make a fulfilled diet with only a small proportion of concentrates (e g. Margan *et al*., 1994; Vranić *et al*., 2008; Zaralis *et al*., 2014). There also are studies done on comparing red clover with grass (e g. Laforest *et al*., 1986; Paul *et al*., 2001; Dewhurst *et al*., 2003; Speijers *et al*., 2005; Marley *et al*., 2007; Niderkorn *et al*., 2012). To combine different feedstuffs into a balanced diet, the digestibility and nutrient content of each individual feedstuff must be known. Then different forages can be combined in a correct way and be supplemented with the right amount of concentrates (Eggertsen, 2008).

This study of feed intake, *in vivo* digestibility and protein utilization is part of a larger project also investigating how the *in vivo* digestibility can be related to the faecal particle size and how the chewing activity is affected by the forage digestibility. Furthermore, in the larger project, the *in vivo* protein digestibility values are related to the *in situ* protein digestibility according to NorFor, which is compared with the Cornell Net Carbohydrate and Protein System for wet chemistry protein fractionations.

Objectives

The aim of this study was to investigate feed intake, *in vivo* digestibility and protein utilization of different types of silages fed to rams with or without protein supplementation. Also, effects of type of silage and protein supplementation on live-weight changes of the rams were studied. The silages used were whole-crop maize harvested at the dough and dent stage of maturity, grass and red clover-grass ensiled with or without microbial inoculant. The relative differences between the treatments are applicable to other small and large ruminants as well and contribute to future feed counselling and calculation modules for diets. By knowing the forage *in vivo* digestibility and protein utilization advisors and farmers can combine different forages and supplement the diet with concentrate to get an optimal feed conversion with healthier and more productive animals, which is environmentally friendly and economically sustainable for the farmer.

Hypotheses

- Feed intake will differ between the forages, with red clover giving higher intake compared to the other silages.

- Rams fed protein supplementation will have a higher silage intake and live weight gain than rams fed without protein supplementation.

- The digestibility of organic matter, fibre and protein will be different between forages and be affected by protein supplementation. The fibre digestibility will be higher for grass, the organic matter digestibility will be higher for maize and the protein digestibility will be higher for red clover compared to the other silages.

- Protein utilization will be different between the forages regardless of supplementation of protein. Red clover will have a lower protein utilization compared to the other silages.

- Forage type and protein supplementation will affect live-weight changes of the rams.

Literature review

Cultivation traits, nutrient values and ensiling ability of the silages

Grass

The most common forage grasses in Sweden are timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and perennial ryegrass (Eggertsen, 2008; Halling, 2008). Timothy is winter hardy and gives a high DM yield in first harvest. If harvested early, the sugar content and palatability will be high, but because of a fast maturity development, the energy concentration can decrease rapidly. Meadow fescue has a better regrowth than timothy. Perennial ryegrass is competitive to other plants with high DM yields. The regrowth is fast and several harvests can be taken each year, but the winter hardiness may be poor (Eggertsen, 2008; Halling, 2008).

When comparing grass with maize and red clover, grass is richer in sugar and NDF, but contains no starch, whereas red clover has a small amount and maize has a larger amount of starch at late maturity stages (Table 1; Spörndly, 2003). The NDF has a higher digestibility in grass at early harvest than in maize (Browne *et al*., 2005; Vranić *et al*., 2008). Generally, grass also has higher protein concentration than maize (Juniper *et al*., 2005; Keady *et al*., 2007; Vranić *et al*., 2008).

Grasses have high sugar concentrations compared to legumes, which contributes to the higher ensilability of grasses than of legumes. The relatively high sugar concentration gives enough nutrients for the lactic acid bacteria to ferment sugars to lactic acid, causing a decrease in the pH to a level close to 4.0, where unwanted microorganisms cannot grow. The DM concentration of the forage is also important, for example a short wilting time to a low DM concentration (<280 g/kg) increases the risks of losing DM during the ensiling by seepage and low fermentation quality, while a higher DM concentration gives almost no losses of DM (McDonald *et al*., 2002).

Red clover

Red clover is the most common forage legume in Sweden (Taylor & Smith, 1995; Halling, 2008). The varieties of red clover can be divided into early, middle late and late depending on their rate of development. In the south of Sweden early types fit best because of their early flowering and good regrowth ability when taking several harvests, but they are not persistent enough to survive in stricter climates in the north of Sweden. In the north, the late types are more suitable because they give one large first harvest and are more persistent to the colder climate (Taylor & Smith, 1995; Halling, 2008).

When comparing red clover with maize and grass, red clover has higher concentrations of protein and ash, while the sugar concentration is lower. The NDF concentration in red clover is lower than in grass and similar or lower to the NDF concentration in maize depending on maturity stage (Table 1; Spörndly, 2003). Lignin and mineral concentrations are higher in red clover than in grass, especially of the minerals Ca, P, Mg, Cu and Co (Table 1; Van Soest, 1994; Spörndly, 2003). The lignin is concentrated to the vascular bundles of the legume while the grass has a larger distribution of lignin in the stem (McDonald *et al*., 2002). In the cell walls of legumes, there are higher concentrations of pectin compared to grasses (Van Soest, 1994). The pectin is a heteroglycan with gel forming qualities, which is easy to degrade in the rumen and not included in the NDF as it is solubilized during the NDF analysis (Van Soest, 1994; McDonald *et al*., 2002). Although the lignin and pectin concentrations are higher in legumes, the legumes have less cell wall contents than grasses during their whole growth (Van Soest, 1994; McDonald *et al*., 2002) and change less in nutrient value during maturity compared to grasses (McDonald *et al*., 2002). The low cell wall content and more concentrated distribution of lignin into the vascular bundles give legumes a faster rate of digestion of NDF in the rumen and, thereby, a higher intake in ruminants, than grasses even if grasses are more digestible (Wilson & Hatfield, 1997).

The low sugar concentrations and high protein concentrations make red clover difficult to ensile (Pahlow *et al*., 2001). The lactic acid bacteria get fewer nutrients in the form of sugars to produce lactic acid to decrease the pH while the protein gives a buffering effect on the pH. The protein in red clover also contributes to the formation of butyric acid and ammonia during deamination of amino acids. To avoid low fermentation quality and to increase the number of lactic acid bacteria in the forage, silage additives and a fast wilting is required (Pahlow *et al*., 2001; Nadeau & Auerbach; 2013).

Maize

Maize needs a growing season of five-six months (Carr & Hough, 1978) and the harvest occurs in September/October or when the first frost occurs (Bunting, 1978a; Carr & Hough, 1978). Recommended maturity stage at harvest is the dent stage. The plant is sensitive to cold temperatures and is, therefore, difficult to cultivate in Sweden north of Stockholm (Weidow, 1998). With plant breeding, early maturing varieties have been developed that can stand our cold climate in northern Europe (Bunting, 1978b; Eriksson, 1999). The plant is also sensitive to weeds; therefore weed control must be performed with herbicides or mechanically, especially during the first period of cultivation (Bunting, 1978a; Weidow, 1998). The cob proportion and nutrient value is affected by the harvesting time. At the dent stage of maturity (late maturity) there is higher starch content than at the dough stage (early maturity) because of a higher proportion and a later maturity of cobs and is, therefore, a better choice to harvest when taking into account the nutrient value and digestibility (Joanning *et al*., 1981; Masoero *et al*., 2006). The most common DM content at harvest is 30-32 % (between dough and dent stage) in Sweden (Nadeau *et al*., 2010).

Maize differs mainly from grasses and red clover with its high concentration of starch and low concentration of CP, ash and minerals. As maize has a low protein concentration, it must be supplemented with protein when fed in large amounts to ruminants (Kilkenny, 1978). The NDF in maize is less digestible than in other forages, but the total DM digestibility is higher because of its content of digestible starch (Browne *et al*., 2005; Moorby *et al*., 2008). With increasing maturity, the water-soluble carbohydrates (WSC) in the stem and leaves are transformed into starch in the cob and the DM concentration increases (Wilkinson, 1978; Svensson, 2010). The stem and leaf proportion decreases while the cob proportion increases, but the lignification of the cell walls increase in the stem with increasing maturity, which results in more indigestible NDF of the whole plant at delayed harvest (Wilkinson, 1978).

Whole-crop maize is easy to ensile compared to grasses and red clover because of a low buffering capacity (Wilkinson, 1978), high DM concentration (McDonald *et al*., 2002) and relatively high sugar concentration (Weidow, 1998). Thereby, whole-crop maize requires less lactic acid to decrease the pH and restrict the activity of undesirable bacteria (Wilkinson, 1978). Maize silage is susceptible to heating after opening of the silo. To prevent this, the use of an additive containing active substances against yeast and mould, such as heterofermentative lactic acid bacteria, sodium benzoate, potassium sorbate and propionic acid, will improve aerobic stability of the silage and thereby heating after opening (Svensson, 2010; Nadeau *et al.*, 2011; Auerbach & Nadeau, 2013).

Table 1. Nutrient composition per kg DM in red clover, whole-crop maize and a mixture of grass species (after Spörndly, 2003).

	Red clover, 2nd harvest, middle early harvest	Maize, whole-crop silage >25 % DM	Timothy, meadow fescue, perennial ryegrass, cocksfoot, 2nd harvest, early harvest
Metabolizable energy, MJ	9.9	11.0	11.0
Digestible energy, MJ	12.4	13.0	13.2
Digestible crude protein, g	160	54	130
Crude protein, g	203	91	171.5
Ash, g	90	44	80
Sugar, g	65	75	110
Starch, g	25	223	0
Neutral detergent fibre, g	430	496	550
Lignin, g	70	–	30
Calcium, g	15.0	2.4	6.0
Phosphorous, g	3.0	2.3	3.5
Magnesium, g	2.8	1.2	1.4
Potassium, g	25.0	11.1	27.0
Sodium, g	0.4	0.4	1.5

Feed intake

The DM intake is affected by the concentrations of acid detergent fibre (ADF) and NDF in the forage, especially of the NDF (cell wall; Van Soest, 1965; Reid *et al.*, 1988) that is known to limit voluntary intake of DM and energy (Waldo, 1986; Jung & Allen, 1995). With advancing maturity of the forage, the NDF concentration increases and affects the feed intake negatively (Mertens, 1994; Särkijärvi *et al.*, 2012). One exception is WCMS, which gets a decreasing total amount of NDF and ADF with maturity because of the increasing cob proportion in the plant (Aufrère *et al.*, 1992).

The filling of the rumen is affected by which group of grass the forage belongs to. The groups are divided into C_3 grasses, C_3 legumes and C_4 grasses (e g. maize; Reid *et al.*, 1988). The differences in filling of the rumen depends on the chemical conformation of the fibre, which affects the animal's ability to chew the feed and on how long time the feed takes up space in the rumen before it is passed on to the intestines (Allen, 1996; Mertens, 1997). If the particle size of the feed is reduced fast by chewing, and thereby sent out of the rumen faster, the intake increases (Ulyatt, 1983). Other factors can affect the intake in addition to fibre, such as the amount of the organic acids lactate, acetate and butyrate, which affect the acidity of the silage. Nevertheless, the most important factors are the amount of digestible organic matter (OM) and ammonia N (McDonald *et al.*, 2002). By using additives, such as formic acid, lactic acid bacteria or cellulase, the feed intake can be promoted (Hetta *et al.*, 2003; Vrotniakiene & Jatkauskas, 2004) because the pH and amount of acetate, butyrate, NDF and ammonia N decrease, while the amount of lactate increase (Hetta *et al.*, 2003). However, ethanol can also increase during fermentation and during exposure to air of silages high in sugar. Ethanol is produced by yeast during aerobic conditions or by heterofermentative lactic acid bacteria (McDonald *et al.*, 2002) when carbohydrates are broken down from feed (Hetta *et al*., 2003) and is correlated with decreased feed intake and increased DM loss from the silage when produced in excessive amounts (Krizsan & Randby, 2007).

When supplementing feeds high in fibre, with N, the feed intake increases as the number of microbes increase and, thus, more fibres are digested (Galyean & Goetsch, 1993). Also, the treatment of feed is important for the digestibility and how the rumen will process the feed. Feedstuffs processed to small particles in the form of pellet or crushed are, for example, not digested sufficiently, as they pass through rumen too quickly, leaving space for more feedstuff and are degraded to a large part in the intestines (Fahey & Merchen, 1987; McDonald *et al*., 2002). In addition, the rumen microbes need some time to adapt to new feed, depending on the chemical conformation and type of microbes that need to increase in the rumen to digest the feed (McDonald *et al*., 2002).

As mentioned earlier, legumes have lower proportions of cell wall content than grass (Van Soest, 1994) when compared at the same digestibility and give thereby a higher feed intake in ruminants (Van Soest, 1994; Wilson & Hatfield, 1997; Paul *et al.*, 2001), but at the same time the legumes have a higher content of lignin in the cell wall than grass, which affects the digestibility negatively (Van Soest, 1994). Therefore there are other constituents that affect the DM intake of legumes positively, such as the lower NDF concentration and the higher rate of digestion of the potentially digestible NDF (Reid *et al*., 1988; Van Soest, 1994; Allen, 1996) and the concentrated distribution of lignin that make the digestible content more available for microbes, thereby speeding up the digestibility rate in the rumen (Nadeau *et al*., 1996; Wilson & Hatfield, 1997). The stem of the maize plant has both a thick cell wall and a high content of lignin, which affect the intake negatively, but the total amount of cell wall, when taking the cob into account, is less than by other mature grasses and makes it, thereby, more digestible (Wilkinson, 1978). The starch in the cob is the main reason for the higher total digestibility (Wilkinson, 1978; Svensson, 2010), which leads to a higher intake (McDonald *et al*., 2002).

Red clover vs. maize

Red clover hay and WCMS fed to rams at *ad libitum* have shown to give significantly lower DM intake for WCMS supplemented with or without urea (1.1, 0.9 kg/day) than for red clover hay (1.8 kg/day; Margan *et al*., 1994). Combinations of the forages (2:1, 1:2) gave a DM intake between the intakes of the forages fed separately at *ad libitum* (WCMS/red clover: 1.4, 1.6 kg/day; Margan *et al*., 1994). In a study by Moorby et al. (2008), combinations of WCMS and red clover silage, supplemented with concentrate, were fed to Holstein-Friesian dairy cows. There were three diets fed *ad libitum* in mixtures of 90/10, 50/50 and 10/90 of WCMS and red clover. The mix of 50/50 gave the highest DM intake with a 1.0 kg higher DM intake per day, but there were no significant differences between the diets (Moorby *et al*., 2008).

Grass vs. maize

Aston and Tayler (1980) studied the intake of WCMS and grass silage in bulls and found that WCMS gave a higher DM intake than grass when fed separately at *ad libitum* (17.7 and 13.0 g DM/kg LW respectively). Even supplementation of concentrate promotes the forage intake, which Browne et al. (2005) and Aston and Tayler (1980) showed, when comparing WCMS and early harvested grass silage, both supplemented with concentrate. Vranić et al. (2008) studied WCMS fed in combination with grass silage (mixes of 33/67, 67/33) of different maturity to Charollais rams. The DM intake increased

(33 % WCMS/67 % early grass, 67 % WCMS/33 % early grass: 66.7, 84.3 g/kg $LW^{0.75}$; 33 % WCMS/67 % late grass, 67 % WCMS/33 % late grass: 80.7, 79.2 g/kg $LW^{0.75}$) compared to when the silages were fed separately (early, late grass: 72.5, 59.0 g/kg $LW^{0.75}$; WCMS: 49.6 g/kg $LW^{0.75}$), regardless of early or late (early flowering stage) harvest for grass silage. Also, intake of NDF was higher when WCMS was fed in combination with late harvested grass silage (33 % WCMS/67 % late grass: 54.5 g NDF/kg $LW^{0.75}$; 67 % WCMS/33 % late grass: 50.3 g NDF/kg $LW^{0.75}$) compared to silages fed separate (grass: 42.2 g NDF/kg $LW^{0.75}$; WCMS: 31.3 g NDF/kg $LW^{0.75}$; Vranić *et al*., 2008).

In other studies of WCMS and grass silage mixtures, fed to steers, the opposite has been shown (Browne *et al*., 2005; Juniper *et al*., 2005). When WCMS was fed separately it gave a higher DM intake (9.5 kg/day; Browne *et al*., 2005; 7.8 kg DM/day; Juniper *et al*., 2005) than mixes of WCMS and grass silage (33/67, 67/33) supplemented with concentrate (33 % WCMS/67 % grass: 8.3 kg/day; 67 % WCMS/33 % grass: 9.1 kg/day; Browne *et al*., 2005; 67 % WCMS/33 % grass: 7.4 kg DM/day; Juniper *et al*., 2005). In the study by Juniper et al. (2005), the concentrate contents were compensated on the basis of the silage CP content to give equal amounts of CP in each diet (Juniper *et al*., 2005). Grass silage fed separately gave the lowest DM intake of all the diets (7.7 kg/day; Browne *et al*., 2005; 6.3 kg DM/day; Juniper *et al*., 2005). When looking at the NDF intake, there were no significant differences between diets (Browne *et al*., 2005).

In another study (Matejovsky & Sanson, 1995), protein supplementation (soybean meal and corn gluten meal) and corn grains (maize) were fed with three qualities of grass hay, harvested at different dates, to rams of Rambouillet breed. When supplementing the late harvested grass hay with protein, the DM intake of forage increased (2.36 % of LW) compared to the hay without the protein supplementation (1.95 % of LW), while middle harvested and early harvested grass hay did not give any difference in forage DM intake with or without the protein supplementation (Matejovsky & Sanson, 1995).

Red clover vs. grass

When Marley et al. (2007) compared the intake of silages of red clover and grass by eight-month-old Suffolk-cross lambs; red clover (1.0 kg DM/day) gave a higher feed intake than grass (0.8 kg DM/day; Marley *et al*., 2007). In another study (Laforest *et al*., 1986), timothy and red clover, complemented with mineral blocks, were compared in rams of Dorset, Leicester and Suffolk crosses (21.3-35.3 kg LW). Red clover gave a higher *ad libitum* feed intake (mean 90.9 g DM/$kg^{0.75}$) than the timothy (69.7 g DM/$kg^{0.75}$). The NDF intake was higher for the timothy (mean 44.0 g NDF/$kg^{0.75}$) than red clover (34.7 g NDF/$kg^{0.75}$; Laforest *et al*., 1986). Paul *et al.* (2001) showed in a study where rams of Leine Valley breed (80-100 kg LW) were studied, that red clover gave a mean feed intake of 2.2 kg DM/day, whereas grass gave a mean feed intake of 1.7 kg DM/day (Paul *et al*., 2001).

By supplementing the silages of grass and red clover with concentrate, red clover still gives a higher forage intake than grass in ruminants (Dewhurst *et al*., 2003; Speijers *et al*., 2005). This was shown by Speijers et al. (2005), which studied ewes' intake of grass (perennial ryegrass) and red clover silage, when bearing twins. Ewes of Mule breed (70.3 kg LW) were fed *ad libitum* of the silages separately during the six last weeks of pregnancy and supplemented with molassed sugar-beet shreds (0.25-1.12 kg the closer to lambing) and 35 g of minerals and vitamins. During the whole experimental period of the study, red clover silage (1.3 kg DM/day) gave a higher silage intake than grass silage (0.8 kg DM/day; Speijers *et al*., 2005).

When combining the two silages, the feed intake is further increased (Dewhurst *et al*., 2003). Dewhurst et al. (2003) studied cows, fed red clover or grass silage separately and in a 50/50 mix supplemented with 8 kg of standard dairy concentrate per day. The mix gave the highest silage intake (13.9 kg DM/day), thereafter red clover (12.1 kg DM/day) gave a higher silage intake than grass (10.2 kg DM/day), which had the highest rumen fill (Dewhurst *et al*., 2003). In another experiment, Niderkorn et al. (2012) studied separate and combined feeding of red clover and orchard grass (*Dactylis glomerata* L.) silages without concentrate supplementation to one-year-old Texel rams. Red clover fed separately and mixed with grass (50/50 and 75/25) showed no significant differences in DM intake (1.6 kg DM/day). However, they gave a significantly higher DM intake than grass silage fed separately (1.3 kg DM/day) and a mixture of 25/75 of red clover and grass (1.5 kg DM/day). For the NDF intake, there were only significant differences between the mix of 50/50 and the silages fed separately, where the mix gave the highest intake (680 g/day) followed by red clover (630 g/day) and grass silage (590 g/day; Niderkorn *et al*., 2012).

Nadeau et al. (2007) studied the *ad libitum* intake of grass/red clover silage using different dietary CP concentrations to primiparous and multiparous Swedish red dairy cows. Three diets were fed during four years: normal concentration (168 g CP/kg DM), low concentration (160 g CP/kg DM; fed during two years) and normal concentration of dietary CP (170 g CP/kg DM) with 75 % grass/red clover silage and 25 % whole crop barley silage of forage DM intake. The cows were supplemented with hay, a grain mix, protein concentrate and dried sugar beet pulp depending on their milk yield. The DM intake of silage for multiparous cows were highest for the diets with low concentration of CP (20.7 kg DM/day) and normal concentration of CP supplemented with whole crop barley silage (21.1 kg DM/day) compared to the diet with only grass/red clover silage at normal concentration of CP (20.1 kg DM/day). The primiparous cows showed no significant difference in DM intake. The NDF intake of multiparous cows was highest for the diet supplemented with barley silage (8.4 kg NDF/day) and for primiparous cows when fed only grass/red clover silage at normal amount of CP (7.3 kg NDF/day). The lowest NDF intake was found for the diet with low amount of CP for both multiparous (7.4 kg NDF/day) and primiparous cows (7.0 kg NDF/day; Nadeau *et al*., 2007).

Effect of maturity stage at harvest of maize

When comparing WCMS at the milk stage and at the soft dent stage (early and late maturity) supplemented with 10 % protein and minerals (90 % soybean meal, 8 % ground limestone, 2 % trace mineralized salt) fed *ad libitum* to six Hereford and Hereford-Angus crossbred steers (250 kg mean LW), soft dent stage gave a higher silage DM intake (88.8 g DM/kg$^{0.75}$) than the WCMS at milk stage (83.9 g DM/kg$^{0.75}$), which may depend on the large difference in starch content (Joanning *et al*., 1981). In another study with finishing bulls, WCMS at the dough and dent stage were fed at *ad libitum* as total mixed rations with 40 % concentrate (rolled barley, dried distillers grain, and cold-pressed rapeseed cake) and balanced for protein, energy and NDF between diets. There was no significant difference between the diets in DM and NDF intake (Zaralis *et al*., 2014).

Effect of additives

Additives of homofermentative and heterofermentative lactobacilli (*Lactobacillus plantarum, Lactobacillus brevis, Pediococcus acidilactici, Streptococcus cremoris, Streptococcus diacetylactis*) in silages of 50 % legumes (red clover, lucerne or Landino clover; *Trifolium repens* L.) and 50 % grass silage (timothy) affected the feed intake negatively in Holstein cows, according to Stokes (1992), although the fermentation process was satisfying. The reason could be a bad aerobic stability. Instead, untreated silage (20.9 kg DM/day) and a mix of enzymes (cellulase, xylanase, cellobiase, glucose oxidase) and lactobacilli (20.5 kg DM/day) gave a higher feed intake than the lactobacilli additive (18.8 kg DM/day). The cows were supplemented with concentrate (55.5 % shelled corn, 35.2 % ground oats, 7.0 % soybean meal, 1.0 % minerals + vitamins, 0.3 % vitamins) and fed *ad libitum* of the silages (Stokes, 1992).

In another study by Vrotniakiene and Jatkauskas (2004) grass/legume silage (72 % red clover, 20 % timothy, 8 % other plants), untreated or with additives containing lactic acid bacteria (*Pediococcus acidilactici*, *Lactobacillus plantarum* and cellulase), were fed *ad libitum* to Lithuanian Black and White fattening bulls and supplemented with concentrate of 1.86 kg DM/day. The silages showed no significant difference in intake (Vrotniakiene & Jatkauskas, 2004). Jatkauskas and Vrotniakiene (2005) did another study with ten Lithuanian Black and White dairy cows fed *ad libitum* of a legume/grass silage (64 % red clover, 12 % timothy, 16 % meadow fescue, 8 % other plants) supplemented with a concentrate of 75 % barley, 10 % wheat, 15 % soybean meal and a vitamin/mineral concentrate. The silage was untreated or treated with *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* ssp. *Shermanii*. There was no significant difference between untreated and treated silages (Jatkauskas & Vrotniakiene, 2005).

In vivo digestibility

Organic matter digestibility

The OM in the plants consists of virtually all nutrients found in DM except for some minerals in ash (McDonald *et al*., 2002). The OM digestibility determined *in vitro* is used to calculate the metabolizable energy in the feed in the Nordic Feed Evaluation System NorFor (Åkerlind *et al.*, 2011) and is also called VOS (rumen soluble OM) for forage silage (Eriksson, 2007; Åkerlind *et al*., 2011). A higher OM digestibility is the same as a higher metabolizable energy content. If the ash content increases, the available energy content decreases (Åkerlind, 2011). When analysing whole crop silages for OM digestibility the IVOS (*in vitro* rumen soluble OM) or the VOS method can be used (Åkerlind *et al*., 2011; Åkerlind, 2012). The IVOS method is modified from the Tilley and Terry (1963) method. The digestibility of OM is correlated with the fibre content in the cell walls, where a higher NDF content have shown to give a lower OM digestibility (Aufrère *et al*., 1992). Thus, the OM digestibility decreases with maturity (Särkijärvi *et al*., 2012). The OM digestibility in WCMS is about 71.5 % in normal grown maize, but can vary depending on the cultivar and environmental factors (Andrieu, 1976). Since the ash content is lower in maize than in grass, the OM digestibility can differ between the forages while the DM digestibility still can be the same (Aston & Tayler, 1980; Browne *et al*., 2005). As mentioned before, when comparing grass and legumes in OM digestibility, the legumes have a higher rate of digestibility in the rumen than grass because of a lower content of digestible NDF, but not a higher total OM digestibility as the concentration of indigestible NDF is larger in legumes than in grasses (Beever *et al*., 1986).

Red clover vs. maize

When Margan et al. (1994) compared red clover hay and WCMS, WCMS had lower digestibility (510 and 560 g/kg OM) of the cell wall OM than red clover (640 and 660 g/kg OM) when fed at *ad libitum* and at maintenance to rams. In the study, it was also shown that the OM digestibility of feed was lower at *ad libitum* than at maintenance when forages were fed separately. Still, the highest cell wall OM digestibility was for the mix with a majority of red clover and a smaller part of WCMS (2:1; 690 g/kg OM; Margan *et al*., 1994). Moorby et al. (2008) showed the opposite results. A higher intake of WCMS gave a higher apparent OM digestibility (90/10: 690 g/kg OM) than a majority of red clover silage (10/90: 660 g/kg OM) in the diet for dairy cows supplemented with concentrate. A 50/50 mix of WCMS and red clover gave a digestibility between the other two diets (50/50: 670 g/kg OM; Moorby *et al*., 2008).

Grass vs. maize

In the study by Aston and Tayler (1980), the WCMS had a greater OM digestibility (646 g/kg OM) than grass (594 g/kg OM) in bulls. When feeding supplementation of barley at 2.4 and 5.0 kg DM, the OM digestibility did increase for both silages, still with the highest OM digestibility for WCMS (WCMS: 671 and 703 g/kg OM; grass: 653 and 696 g/kg OM; Aston & Tayler, 1980). If grass is harvested early WCMS supplemented with concentrate still has a higher OM digestibility than grass silage supplemented with concentrate when fed to steers (726 and 704 g/kg OM respectively; Browne *et al*., 2005). In the Keady et al. (2007) study, the OM digestibilities of grass silage and WCMS in sheep were not significantly different (662 and 675 g/kg OM respectively). As well, the beef diets of grass and grass/WCMS mix supplemented with concentrate showed no significant difference (689 and 684 g/kg OM respectively). However, there was a significant difference when feeding different levels of concentrate (3 and 5 kg/day), where a higher intake of concentrate gave a higher OM digestibility (3 kg: 668 g/kg OM; 5 kg: 692 g/kg OM; Keady *et al*., 2007).

Red clover vs. grass

In the study by Paul et al. (2001), grass and red clover had an OM digestibility of 717 g/kg OM and 707 g/kg OM, respectively. Laforest et al. (1986) found that the apparent OM digestibility for rams was higher for red clover (692 g/kg OM) compared to timothy (mean 667 g/kg OM). Speijers et al. (2005) investigated the *in vitro* OM digestibility of red clover and grass silage fed to pregnant ewes and found that red clover gave the highest digestible OM in DM (722 g/kg DM vs. 673 g/kg DM).

Effect of additives

Hetta et al. (2003) studied the difference in *in vitro* degradability of OM in timothy and a mix of timothy and red clover silages when treated or untreated with lactobacilli and molasses. The degradability was significantly higher for the treated silages (910 g/kg OM) than the untreated silages (872 g/kg OM; Hetta *et al*., 2003). Jatkauskas and Vrotniakiene (2005) found no significant differences in *in vitro* OM digestibility of red clover-grass (64/36 mix) silage treated with or without lactic acid bacteria.

Fibre digestibility

The digestibility of cell walls (fibre) is depending on how the constituents are bound to each other (Wilkinson, 1978; Jung & Allen, 1995) and how much lignin there is that inhibits the enzymatic hydrolysis and microbial fermentation of carbohydrates (Wilkinson, 1978; Jung & Deetz, 1993). This is, as mentioned earlier, affected by the plant maturity stage at harvest (Wilman *et al*., 1977; McDonald *et al*., 2002), but also by the amount of indigestible fractions and by how the rate of digestion and passage out of rumen competes with each other (Tamminga, 1993). The cell wall is divided into two parts (Wilson, 1993) where the outer cell wall is prolonged with pectin, xylan (base of hemicellulose) and cellulose among others (Chesson *et al*., 1985; Åman, 1993) while the internal cell wall is thickened with more cellulose, xylan and lignin, but no pectin (Nordkvist, 1987). The amounts of nutrients are different between different plants where, for example, legumes have more pectin and cellulose than grasses in the outer wall (Åman, 1993). In both legumes and grasses increase the contents of NDF, ADF, lignin and indigestible NDF (iNDF) with maturity (Jalali *et al*., 2008; Jalali *et al*., 2012; Särkijärvi *et al*., 2012). Legumes are easy to digest because their outer cell walls are not lignified during advanced maturity. Only the internal cell wall (xylem) is lignified and thereby not digestible. In grass, the internal cell wall and the middle of the outer cell wall is thickened with lignin (Wilson & Hatfield, 1997). This makes it difficult for the microbes to get in to the inner parts of the stem for digestion if there are no broken ends of the stem from chewing or chopping (Wilson & Hatfield, 1997).

The amount of cell wall affects the digestibility of the plant and the passage rate out of rumen. The thicker cell wall of grasses makes the digestion slower in rumen because of a more extensive lignification even if the total lignin content is lower than in legumes (Van Soest, 1994). Legumes give thereby a faster digestion than grass even if grasses have a higher amount of digestible fibre than legumes (Smith *et al*., 1972). Also, maize has a thick cell wall in the stem, but also a high content of lignin, which makes it less digestible. On the other hand the leaves and cob area do contribute with a large part of the DM and have a high digestibility of starch and WSC, which compensate for the less digestible stem (Wilkinson, 1978). The rate of digestion of feed is dependent on a couple of factors, such as how much indigestible fibre (iNDF) there is, how fast the digestible particles are digested, how compact the particles are, the particle size and how long time the particles retain in the rumen (Uden & Van Soest, 1982; Allen, 1996). Mature grass has shown to have smaller particles after chewing than younger grass, apparently because they are weaker when having more lignin (Ulyatt, 1983) or are being more chewed (Jung & Allen, 1995).

Red clover vs. maize

Moorby et al. (2008) compared three diets with mixes of WCMS and red clover silage supplemented with concentrate fed to dairy cows and found that a mix of 10/90 of WCMS and red clover gave the highest apparent NDF digestibility of 610 g/kg NDF compared to 560 g/kg NDF and 470 g/kg NDF for mixes of 50/50 and 90/10 of WCMS and red clover.

Grass vs. maize

When comparing different silages, as mentioned earlier, the time of harvest is important to consider. For example when Vranić et al. (2008) compared the fibre digestibility of grass silage in Charollais rams at late harvest (early flowering stage) and WCMS, the digestibility of ADF was higher in the WCMS (562 g/kg ADF) than in the grass silage

(454 g/kg ADF), while the digestibility of NDF showed no significant difference with a mean of 555 g/kg NDF. The authors related the results to the lower NDF content (582 g/kg DM) of WCMS than the late harvested grass silage (705 g/kg DM). If using the early harvested grass silage (697 g/kg NDF) instead, in comparison, the grass silage had a higher digestibility of fibres (754 g/kg NDF; 698 g/kg ADF) than the WCMS (595 g/kg NDF, 562 g/kg ADF; Vranić *et al*., 2008). Browne et al. (2005) also showed that early harvested grass silage had a higher fibre digestibility (641 g/kg NDF, 596 g/kg ADF) than the WCMS (529 g/kg NDF, 481 g/kg ADF) when fed separately with concentrate to steers (Browne *et al*., 2005). When Vranić et al. (2008) studied the late harvested grass silage combined with WCMS the ADF digestibility increased instead to a higher value (617 g/kg ADF) than when the silages were fed separately, while there was no significant difference between NDF digestibilities. This was the opposite to when the WCMS was fed in combination with early harvested grass silage. In that case the fibre digestibility decreased (NDF: 699 g/kg NDF, ADF: 666 g/kg ADF) compared to separate feeding, apparently because of the lower digestibility in the WCMS than in the early harvested grass silage (Vranić *et al*., 2008).

In another study (Keady *et al*., 2007), continental-cross beef steers were fed grass silage separately and in combination with WCMS in a 40/60 ratio, both diets supplemented with 3 or 5 kg concentrate (barley, maize meal, sugar-beet pulp, soya bean, and molasses). A study was also done in sheep to get the digestibility for the separate forages. The grass gave higher digestibilities for NDF and ADF (717 g/kg NDF, 762 g/kg ADF) than WCMS did (569 g/kg NDF, 582 g/kg ADF) in the sheep. When looking at the digestibilities in beef, grass still gave higher digestibilities (728 g/kg NDF, 747 g/kg ADF) than the mix of 40/60 (684 g/kg NDF, 709 g/kg ADF). When comparing the groups fed 3 and 5 kg concentrate, no significant difference was found (Keady *et al*., 2007).

Red clover vs. grass

When comparing red clover and grass in NDF (cell walls) and ADF apparent digestibility in rams, Laforest et al. (1986) found that the timothy gave a higher fibre digestibility (563 g/kg NDF and 519 g/kg ADF) than the red clover (492 g/kg NDF and 469 g/kg ADF).

Effect of maturity stage at harvest of maize

When WCMS was harvested at the milk stage and at the soft dent stage (early and late maturity stage) the NDF and ADF digestibilities were higher in the early stage (635 g/kg NDF, 591 g/kg ADF than in the late stage of maturity (489 g/kg NDF, 408 g/kg ADF) when fed to crossbred steers supplemented with 10 % of a mix of protein and minerals (Joanning *et al*., 1981).

Effect of additives

The use of additives to the silage can also affect the digestibility of fibres. In a study by Hetta et al. (2003) timothy and mixtures of timothy and red clover (70.3 and 92.0 % of red clover) were compared between untreated and treated silages with additives of lactobacilli and molasses. The *in vitro* degradability of NDF was significantly lower in the untreated mixtures than in the treated mixtures (621 g/kg NDF vs. 687 g/kg NDF). The degradability of NDF in timothy was not affected by the inoculant plus molasses treatment (Hetta *et al*., 2003).

Protein digestibility

When talking about CP in the feed it does not show how the animal can utilize the feed, only how much N it contains (McDonald *et al*., 2002). To define the digestibility of protein, the N intake and output in faeces must be compared and that is most correctly done when feed output in the terminal ileum is examined. In the calculations, also endogenous protein must be considered. The endogenous N is dependent on how much DM that passes through the system and which quality and quantity the protein in feed have (McDonald *et al*., 2002). For example, if the ruminant animal eats large amounts of easily digested feed, the passage rate out of rumen increases and the digestion in the rumen is reduced. Instead, protein degradation increases in the intestine and the amount of amino acids absorbed in the intestine (AAT) increases (Pettersson, 2006). The extent of protein digestibility depends on whether the microbes can come close to the feed and start to break it down or if other component structures are defending the surface. It also can depend on how the physical and chemical structure of the protein looks (McDonald *et al*., 2002).

Red clover vs. maize

When comparing red clover hay and WCMS supplemented with urea, the N digestibility was higher in red clover hay than in WCMS when fed *ad libitum* (780 g/kg N vs. 660 g/kg N) and at maintenance (810 g/kg N vs. 660 g/kg N) to rams (Margan *et al*., 1994).

Grass vs. maize

In a study with Charollais rams, the WCMS was fed as the sole forage and in combinations with grass silage, consisting mainly of orchard grass (Vranić *et al*., 2008). The grass silages were harvested at early and late (early flowering stage) maturity. When WCMS was fed in combination with the grass silages (mixes of 33/67, 67/33), the CP digestibility increased (WCMS/early grass mixes: 668 g/kg CP; WCMS/late grass mixes: 569 g/kg CP) compared to when the silages were fed separately (early grass: 596 g/kg CP; late grass: 489 g/kg CP; WCMS: 469 g/kg CP; Vranić *et al*., 2008). This can depend on a higher energy intake, which gives more available energy for the microbes to digest protein (Cottrill *et al*., 1982). However, in the study by Browne et al. (2005), WCMS supplemented with concentrate did give the highest apparent N digestibility (667 g/kg CP) when fed as sole forage compared to when early grass silage, supplemented with concentrate, was fed as sole forage (641 g/kg CP) or in combination with WCMS to beef steers (653 g/kg CP). In the study by Keady et al. (2007), the N digestibility was compared between grass silage and a mix of grass/WCMS fed to beef steers with no significant effect of mixing. Also, there was no effect of concentrate level on the protein digestibility (Keady *et al*., 2007).

Red clover vs. grass

Dewhurst et al. (2003) studied the apparent digestibility in the rumen and the total degradability of N in cows fed grass and red clover silage separately and in a mix of 50/50 supplemented with concentrate, but found no significant differences between the diets. Laforest et al. (1986) found that red clover gave the highest apparent digestibility of protein (750 g/kg CP) compared to timothy (639 g/kg CP) in rams.

Effect of maturity stage at harvest of maize

When the N digestibility in beef steers was compared between milk stage and soft dent stage (early and late maturity) of WCMS, the silage harvested at the milk stage gave the highest N digestibility (634 g/kg N vs. 596 g/kg N; Joanning *et al*., 1981).

Protein utilization

The microbes play an important role in the contribution of useful protein to sheep. The microbes' main task is to convert carbohydrates into volatile fatty acids that give energy to the sheep. To handle this, they need energy from carbohydrates and N to grow and multiply by creating microbial protein (Hvelplund *et al*., 1987; Pettersson, 2006). The N comes from amino acids, peptides and ammonia from amino acids in the feed (McDonald *et al*., 2002; Sjaastad *et al*., 2003). Excess of ammonia in the rumen, due to excess of protein or deficit of carbohydrates, enables the ammonia to be absorbed by the rumen wall and transported on to the bloodstream. The ammonia is transported in the blood to the liver and transformed into urea, a NPN compound, which can be transported back to the rumen directly via the rumen wall or via the salivary glands in the mouth. However, most of the urea is transported to the kidneys, excreted and lost in urine (McDonald *et al*., 2002; Sjaastad *et al*., 2003). Therefore, a balanced diet, where the microbes can utilize the feed efficiently with a minimum of lost N is desirable (McDonald *et al*., 2002). Other NPN, such as the uric acid and allantoin in urine show how efficient the synthesis of microbial protein is in the rumen and are end products from the degradation of the microbes' nucleic acids in the intestines (Chen *et al*., 1990). Hippuric acid is another N compound in urine that is associated with the amount of phenyl propionic acid or phenolic acids in feed like cinnamic acid, quinnic acid and hydrocinnamic acid (precursors of lignin) that are digested by the rumen bacteria into benzoic acid, which is, by the conjugation with glycine, converted into hippuric acid in the kidneys (Martin, 1982). Grasses have a higher content of phenolic acids compared to legumes (Jung & Deetz, 1993) and give a decreasing content of lignin precursors that are excreted in urine with maturity (Martin, 1970). Also, hippuric acid is associated with degradation of protein by the rumen microbes and with a decrease in the urine with lower protein intake from feed. However, the proportion of hippuric acid in urine has shown to increase at a diet with low protein content (Szanyiová *et al*., 1995). The content of hippuric acid in urine is used to determine the amount of emissions of N_2O, which is a highly potent greenhouse gas, equivalent to 310 CO_2 (Cederberg, 2002). The emissions of N_2O decrease with a higher content of hippuric acid in urine (Groenigen *et al*., 2006).

The production of microbial protein gets higher when carbohydrates, such as starch and sugar are fed (Hvelplund *et al*., 1987). The WCMS has a high starch content and works as a supplementation of energy when fed together with other forages and gives a better utilization and good availability of N for the rumen microbes and by that less N losses in urine. This is especially useful when fed with red clover or grass of high protein contents (Hvelplund *et al*., 1987; Margan *et al*., 1994; Vranić *et al*., 2008). However, when WCMS is fed separately, the N balance (amount of N retained in the body when taking the difference between N intake and N outtake from the body; McDonald *et al*., 2002) is negative because of the low N content in WCMS, which results in lost N in urine because the N utilization is ineffective by the microbes in the rumen. This indicates that N supplementation as concentrate or silage with high protein content must be used when feeding WCMS to ruminants (Vranić *et al*., 2008). When the microbes pass from the rumen into the small intestine, sheep can use their microbial protein as a N source for growing and storage in the body. The undegraded proteins that are digested in the intestines can also be used as a resource of N (McDonald *et al*., 2002). A negative N balance can give a higher amount of creatinine in urine, because the body muscles are degraded when no N is added by feed intake (McDonald *et al*., 2002).

Red clover vs. maize

In the study by Margan et al. (1994), the N intake by sheep was higher for red clover than for WCMS supplemented with urea when fed at *ad libitum* (61.8 g/day vs. 16.8 g/day) and at maintenance (28.3 g/day vs. 16.8 g/day). Combinations of WCMS and red clover (2:1, 1:2) gave N intakes between the separately fed silages, with highest intake for a majority of red clover fed at *ad libitum* and maintenance (1:2: 44.5, 23.0 g/day vs. 2:1: 27.9, 15.3 g/day). The urinary losses of N were on average 37.5 and 20.1 g/day for *ad libitum* and maintenance fed diets, respectively, of red clover hay. For WCMS the mean N losses for *ad libitum* and maintenance fed diets were 7.7 and 6.0 g/day. The N balance was higher for the red clover diets than for the WCMS diets at *ad libitum* and maintenance (10.3, 2.8 g/day vs. 1.1, 0.01 g/day). Urinary N losses for the combinations of WCMS and red clover (2:1, 1:2) were 12.9 and 25.8 g/day at *ad libitum* and 10.0 and 16.5 g/day at maintenance. The N balance was highest for a majority of red clover in the diet, both at *ad libitum* and maintenance (5.8, 1.3 g/day vs. 6.8, 1.7 g/day; Margan *et al*., 1994).

When Moorby et al. (2008) studied the feed intake of combined WCMS and red clover silage (90/10, 50/50, 10/90) in dairy cows, the same was shown for the N intake. The N intake was lowest for a majority of WCMS (90/10: 366 g N/day) and highest for a majority of red clover in the diet (10/90: 528 g N/day). The urinary losses of N were higher with a majority of red clover in the diet compared to a majority of WCMS (90/10: 74 g N/day; 50/50: 120 g N/day; 10/90: 173 g N/day). There were no significant differences between the diets in the total N balance (Moorby *et al*., 2008).

In a study by Auldist et al. (1999), Friesian cows were fed silages of white clover and a mix of 70 % white clover and 30 % WCMS. The diet with only white clover fed gave the highest mean N intake (582 g/day vs.450 g/day), N in urine (205 g/day vs. 150 g/day) and urea N in urine (140 g/day vs. 80 g/day) compared to the mix (Auldist *et al*., 1999).

Grass vs. maize

Browne et al. (2005) studied the N intake by steers and found that there were no significant differences between silage intakes of WCMS and grass (silage total N concentration was 15.4 and 18.8 g/kg DM respectively) when fed separately and in combinations. However, when compared for the total N intake of silage and concentrate, the N intake increased with more WCMS in the diet (grass: 171.2 g/day; 33/67: 184.3 g/day; 67/33: 196.1 g/day; WCMS: 205.8 g/day). The WCMS gave a N loss in urine of 86.3 g/day whereas grass gave a N loss of 77.5 g/day with no significant difference. WCMS gave the highest N balance compared to grass silage (247 g N/kg N intake vs. 188 g N/kg N intake; Browne *et al*., 2005).

The intake of N has also been improved in rams when WCMS was fed in combination with early and late harvested grass silage compared to silages fed as sole forage (early grass: 24.6 g N/day; early grass/WCMS mixes: 23.8 g N/day; late grass: 16.2 g N/day; late grass/WCMS mixes: 19.2 g N/day; WCMS: 9.46 g N/day). There were no significant differences in urine losses when comparing the early and late harvested grass silage diets separately. However, there were significantly higher N losses in early harvested grass silage, fed as sole forage and in combinations with WCMS, than for late harvested grass silage (early grass, 33/67, 67/33: 7.3, 8.0, 4.2 g N/day; late grass, 33/67, 67/33: 4.9, 4.0, 5.8 g N/day; WCMS: 3.9 g N/day; Vranić *et al*., 2008).

In a study by Burke et al. (2007), Holstein Friesian cows were fed grass silage and a mix of WCMS and grass silage (67/33) at *ad libitum,* supplemented with 8 kg concentrate, adjusted in CP content to give each diet a content of 160-170 g CP/kg DM. The N intake was highest for the mix with 456 g/day while grass gave an intake of 415 g/day. When comparing the allantoin content in urine, there was no significant difference between the diets (Burke *et al*., 2007). In another study by Bristow et al. (1992), Friesian dairy cows and British Saanen dairy goats were fed grass or WCMS with concentrate as supplementation. The N in urine had for the cows fed grass silage a range of 6.8-9.6 g N/L and for the cows fed WCMS a range of 9.1-12.2 g N/L. The goats were only fed grass silage and had a range of 12.0-16.9 g N/L urine. Urea in urine for cows fed grass were 59.3-71.5 % of total N and 60.6-66.8 % of total N when fed WCMS. For goats, the content of urea was 44.9-77.8 % of total N. When looking at the contents of allantoin and uric acid in cows fed grass silage the contents were 4.5-10.9 and 0.62-1.88 % of total N respectively. Feeding WCMS gave ranges of allantoin and uric acid between 2.2-11.8 and 1.0-1.7 % of total N respectively. In goats, the contents were 3.3-4.0 and 0.2-0.6 % of total N respectively (Bristow *et al*., 1992).

Red clover vs. grass

In a study by Marley et al. (2007), Suffolk crossed lambs had a higher N intake when fed red clover compared to perennial ryegrass silage (30.3 g/day vs. 11.9 g/day and 2.1 g/kg $LW^{0.75}$ vs. 0.9 g/kg $LW^{0.75}$). In a study by Dewhurst et al. (2003) similar results were shown where dairy cows had a higher N intake when fed red clover (632 g/day) compared to grass silage (455 g/day) at *ad libitum*. When fed in a 50/50 mix, the N intake was higher than for grass silage (571 g/day; Dewhurst *et al*., 2003). Also, pregnant ewes fed red clover and grass silage, supplemented with molassed sugar-beet shreds, had a total CP intake during the six last weeks in pregnancy that was higher when fed red clover than when fed grass (344 g/day vs. 191 g/day; Speijers *et al*., 2005). When comparing red clover and timothy in CP intake of rams, red clover gave a higher CP intake than the timothy (20.7 g CP/$kg^{0.75}$ vs. 9.9 g CP/$kg^{0.75}$; Laforest *et al*., 1986).

In a study by Nadeau et al. (2007), Swedish red dairy cows fed diets of grass/red clover silage, with different CP concentrations, showed no significant differences in N efficiency between the diets. However, the N intake was significantly different for the primiparous cows with the highest intake for the diet with low amount of CP and lowest for the diet with inclusion of barley silage (primiparous cows normal CP, low CP, normal CP + barley silage: 488, 506, 453 g/day). This was caused by a higher intake of grains and dried sugar-beet pulp giving a lower NDF and CP intake and a higher starch intake, which is positive for the microbes' utilization of the forage. Despite a low CP content in the diet, the protein degraded and undegraded in the rumen was enough to give the most efficient utilization by the microbes (Nadeau *et al*., 2007). Beever et al. (1986) studied the N intake and N losses in urine when Friesian steer calves were fed perennial ryegrass or white clover harvested at three maturity stages in diets of 18 or 24 g silage DM/kg LW. The N intake for the grass had a range between 0.34-0.69 g/kg LW, while the white clover gave a range between 0.77-1.08 g/kg LW. The average N losses in urine were 0.46 and 0.20 g/kg LW for white clover and grass respectively, which gave a mean N retention that was higher for white clover than for grass (0.28 vs. 0.14 g N/kg LW; Beever *et al*., 1986).

Santoso et al. (2003) studied the feed intake of orchard grass and lucerne silage supplemented with β 1-4 galactooligosaccharides at 2 % of DM in Holstein cows. Orchard grass was also fed without supplementation. The N intake and N output in urine were not significantly different between the diets. When looking at the contents of allantoin in urine the grass with supplementation gave a higher excretion than the grass alone and supplemented alfalfa silages (grass: 69.7 mmol/day; grass/supplement: 92.0 mmol/day; lucerne/supplement: 60.0 mmol/day). There were no significant differences between the diets in uric acid content in urine (Santoso *et al*., 2003). In another study by Carro et al. (2012), Merino ewes and Granadina goats were fed lucerne or perennial rye grass/clover hay combined with concentrate, in mixes of 70/30, and vitamin/mineral blocks. The diets were fed with 0.56 g DM/kg $LW^{0.75}$. The N intakes and output in urine were highest for the lucerne hay mix in the ewes and goats (N intakes: 26.8 and 27.8 g/day respectively; vs. 18.6 and 16.1 g/day respectively; output 10.4 and 16.1 g/day respectively, vs. 6.6 and 8.7 g/day, respectively). The allantoin content in urine was higher for the lucerne hay mix than for the grass hay mix in both ewes and goats (416 and 636 μmol/kg $LW^{0.75}$ respectively vs. 379 and 440 μmol/ kg $LW^{0.75}$ respectively), while there were no significant differences between diets in uric acid contents in urine (Carro *et al*., 2012).

Effect of maturity stage at harvest of maize

Joanning et al. (1981) studied the N retention in steers fed WCMS harvested at the milk stage and at the soft dent stage of maturity (early and late maturity) supplemented with concentrate, but found no significant difference. Johnson et al. (1998) studied mixes of 13 % lucerne and 37 % WCMS, at blackline stage (late maturity) or one-half milkline stage (early maturity), supplemented with 50 % concentrate fed *ad libitum* with 10 % refusals to Holstein cows. The N intake and allantoin content in urine did not differ between the diets. However, the diet with WCMS at the one-half milkline stage gave a higher uric acid content in urine compared to the WCMS of blackline stage (69 mmol/day vs. 58 mmol/day; Johnson *et al*., 1998).

Materials and methods

Animals and housing

Ten 9-month old rams of meat breed crosses (maternal line: Swedish Finewool/Dorset, paternal line: Texel) were used in this experiment at Götala Beef and Lamb Research Centre, SLU Skara Sweden during January-June 2013. The average LW and body condition score (BCS) were 63 ± 2.65 kg and 2.85 ± 0.17 respectively at the start of the experiment. The unsupplemented group had a mean LW of 61.9 ± 2.33 kg and the supplemented group a mean LW of 64.1 ± 2.70 kg at the start of the experiment. The rams were housed in an uninsulated barn in ten separate pens during the whole experimental period except during the last seven days in each period when they were in ten separate metabolic cages. The pens had an area of 6 m^2 with straw bedding and the metabolic cages had a dimension of 1,5 x 0,8 m with meshed floors, rubber mat in the front and separate collection of urine and faeces.

Experimental design

The experiment was designed as a duplicated 5 x 5 Latin square with five rams (one treatment to each ram) and five periods in each square (see Tables 2 and 3). Each period was four weeks long. In square 1, which is shown in Table 2 no rapeseed meal was supplemented to the rams, while in square 2, which is shown in Table 3 the rams were fed untreated rapeseed meal supplemented in equal amounts to each of the five rams. The supplementation of rapeseed meal was used to evaluate how the protein level affected the feed digestibility and protein utilization. Thus, two rams were fed the same silage treatment in each period with the difference that one of them was not supplemented with rapeseed meal whereas the other one was. At the start of each period the silages were changed according to the Latin squares in Tables 1 and 2. When the five periods were completed, all the rams had been fed with the five different silages. The rams were randomly assigned to the treatments and the sequence of the treatments differed between the two squares.

Table 2. A 5 x 5 Latin square with five treatments, without addition of rapeseed meal distributed to five rams in five periods. T= treatment

	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5
Period 1	T 1	T 3	T 2	T 4	T 5
Period 2	T 2	T 1	T 5	T 3	T 4
Period 3	T 3	T 5	T 4	T 1	T 2
Period 4	T 4	T 2	T 3	T 5	T 1
Period 5	T 5	T 4	T 1	T 2	T 3

Table 3. A 5 x 5 Latin square with five treatments, with addition of rapeseed meal distributed to five rams in five periods. T= Treatment

	Ram 6	Ram 7	Ram 8	Ram 9	Ram 10
Period 1	T 1	T 2	T 3	T 4	T 5
Period 2	T 3	T 1	T 5	T 2	T 4
Period 3	T 2	T 5	T 4	T 3	T 1
Period 4	T 4	T 3	T 1	T 5	T 2
Period 5	T 5	T 4	T 2	T 1	T 3

Each period was four weeks long (29 days) and divided into sub periods. During the first two weeks the animals were adapted to the silages and fed individually in the pens at *ad libitum* with at least 10 % refusals per day. The daily *ad libitum* feed intake, allowing 10-15 % refusals per day, was registered for each ram during the third week. After three weeks the rams were moved to the metabolic cages and fed 80 % of *ad libitum* DM intake to avoid refusals. During the first three days in the fourth week the rams were adapted to the restricted allowance and during the last four days the feed intake was registered and the urine and faeces were collected. Refusals were weighed and sampled when they occasionally occurred.

Silages and diets

The experimental treatments used in the experiment were:
1. Grass silage (G)
2. Red clover/grass silage, without inoculant (RC)
3. Red clover/grass silage, with an inoculant as an additive (RCI)
4. Whole crop maize silage, dough (early) maturity (EM)
5. Whole crop maize silage, dent (late) maturity (LM)

The grass (G) in treatment 1 was harvested as a first harvest on 4 June 2012 at 31 % DM and treated with the bacterial inoculant Kofasil Duo containing the homofermentative lactic acid bacteria (LAB) *Lactobacillus plantarum* DSM 3676, 3677 and the heterofermentative LAB *Lactobacillus buchneri* DSM 13573) at 2 x 10^5 cfu/g herbage at ensiling. The red clover/grass forage consisted of 75 % red clover and 25 % grass and was harvested as a second harvest on 4 September 2011 at 32 % DM. The red clover/grass forage in treatment 2 was ensiled without an additive (RC) while treatment 3 was ensiled with the additive Kofasil Duo (RCI; *Lactobacillus plantarum* DSM 3676, 3677 and *Lactobacillus buchneri* DSM 13573) at 2 x 10^5 cfu/g herbage at ensiling (Addcon Europe GmbH). The maize at the dough stage of maturity (EM), when the maize kernel is doughy at finger top pressure, was harvested as whole crop on 14 September 2010 at 28 % DM. The maize at the dent stage of maturity (LM), when the maize kernel is harder and its content is mealy, was harvested as whole crop on 12 October 2010 at 38 % DM. Both maize silages were ensiled with the additive Kofasil Stabil at 2 litres per tonne herbage (potassium sorbate 150 g/L and sodium benzoate 250 g/L; Addcon Europe GmbH). The maize was direct cut whereas the grass and the red clover/grass swards were wilted by wide spreading in the field to a DM content of ca 30 %. All forages were precision chopped with a Jaguar (Nya Fagerås Lantbruk, Åsarp) and ensiled in hard-pressed round bales with a stationary baler (Orkel; AHA Lantbrukstjänst HB, Ålstorp, Laholm). See Tables 4 and 5 for chemical composition of the silages during days 15-21 and days 25-28. The fermentation characteristics of the silages are shown in Table 6. To half of the rams untreated, hexane extracted with low fat content (45 g/kg DM according to Lantmännen table value), rapeseed meal from Lantmännen was fed at 150 g when the silage was fed at *ad libitum* and at 120 g when the silage was fed at 80 % of *ad libitum* intake. See Table 7 for chemical composition of the rapeseed meal. All of the rams were fed 20 g of minerals and had free access to salt block and water. In addition, the four rams that were fed WCMS were given 10 g of limestone daily.

Table 4. Chemical composition (g/kg DM unless stated otherwise) of grass silage (G), red clover/grass silage without inoculant (RC) or with an inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) during *ad libitum* period (days15-21). Mean and standard deviation (SD) within parenthesis (n=5).

	G		RC		RCI		EM		LM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM[1] (%)	33.6	(0.5)	28.9	(0.8)	33.3	(2.1)	29.6	(0.9)	36.4	(1.3)
Ash	75	(2.1)	104	(2.5)	101	(2.8)	39	(3.0)	41	(2.2)
OM[2]	925	(2.1)	896	(2.5)	899	(2.8)	961	(3.0)	959	(2.2)
Crude protein	118	(5.0)	176	(6.9)	168	(6.4)	87	(4.1)	89	(3.9)
Starch	-		-		-		290	(31.8)	324	(38.7)
NDF[3]	500	(8.4)	479	(10.8)	458	(12.7)	395	(29.2)	394	(24.1)
ADF[4]	288	(5.9)	350	(10.7)	341	(6.8)	203	(14.8)	196	(24.8)

[1]DM = dry matter determined after 20 h in 60°C
[2]OM = organic matter
[3]NDF = neutral detergent fibre
[4]ADF = acid detergent fibre

Table 5. Chemical composition (g/kg DM unless stated otherwise) of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) during the restricted feed intake period (days 25-28). Mean and standard deviation (SD) within parenthesis (n=5).

	G		RC		RCI		EM		LM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM[1] (%)	33.7	(0.8)	28.6	(1.0)	32.2	(1.4)	29.8	(1.7)	37.1	(1.5)
Ash	73	(2.2)	103	(3.7)	97	(3.1)	44	(3.6)	49	(1.2)
OM[2]	927	(2.2)	897	(3.7)	903	(3.1)	956	(3.6)	951	(1.2)
VOS[3] (%)	88.4	(1.1)	73.9	(3.1)	77.6	(0.8)	85.3	(1.3)	83.9	(1.4)
ME[4] (MJ/kg DM)	11.3	(0.2)	9.6	(0.3)	10.1	(0.1)	11.2	(0.2)	11.0	(0.2)
Crude protein	117	(0.8)	170	(4.7)	165	(5.2)	85	(3.6)	88	(3.0)
Starch	-		-		-		299	(61.2)	312	(57.8)
NDF[5]	507	(16.7)	483	(9.4)	465	(12.1)	407	(19.4)	413	(23.9)
ADF[6]	286	(5.2)	360	(4.3)	344	(4.5)	221	(21.9)	216	(16.2)
ADL[7]	25	(2.7)	53	(2.5)	50	(1.9)	20	(2.3)	20	(2.5)

[1]DM = corrected dry matter for volatile organic compounds (Weissbach & Strubelt, 2008)
[2]OM = organic matter
[3]VOS = rumen soluble organic matter
[4]ME = metabolizable energy
[5]NDF = neutral detergent fibre
[6]ADF = acid detergent fibre
[7]ADL = acid detergent lignin

Table 6. Fermentation characteristics (g/kg DM unless stated otherwise) of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) during days 15-21. Mean and standard deviation (SD) within parenthesis (n=5).

	G		RC		RCI		EM		LM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	4.1	(0.01)	4.6	(0.1)	4.3	(0.01)	3.8	(0.03)	3.9	(0.03)
WSC[1]	28	(3.6)	2.6	(0.4)	6.2	(0.9)	43	(8.6)	28	(2.6)
Lactic acid	69	(6.8)	64	(14.2)	91	(11.2)	53	(2.7)	55	(4.7)
Acetic acid	17	(3.0)	26	(5.3)	21	(2.9)	17	(4.2)	14	(0.8)
Propionic acid	0.5	(0.0)	2.7	(0.9)	0.7	(0.1)	0.4	(0.1)	0.3	(0.0)
Butyric acid	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.1	(0.3)	0.0	(0.0)
Ethanol	8.7	(2.1)	4.1	(1.0)	2.5	(0.3)	1.7	(0.4)	1.4	(0.4)
NH_3-N (% tot N)[2]	11.2	(1.3)	11.7	(0.4)	9.0	(1.8)	9.5	(0.5)	11.0	(0.9)

[1]WSC = water soluble carbohydrates
[2]Ammonia-nitrogen, % of total nitrogen

Table 7. Chemical composition (g/kg DM unless stated otherwise) of rapeseed meal. Mean and standard deviation (SD) within parenthesis (n=2).

	Rapeseed meal	
	Mean	SD
DM[1] (%)	88.5	(0.8)
Ash	72	(0.7)
OM[2]	929	(0.7)
Crude protein	347	(0.3)
Starch	60	-
NDF[3]	307	(4.1)
ADF[4]	240	(9.4)
ADL[5]	109	(2.1)

[1]DM = dry matter
[2]OM = organic matter
[3]NDF = neutral detergent fibre
[4]ADF = acid detergent fibre
[5]ADL = acid detergent lignin

Registrations and sample collection

Registrations of live weight and body condition score and feed sample collection

Registrations were done on the LW of the rams at trial start and before and after the fourth week in each period. The mean LW for *ad libitum* period was calculated as the mean of the LW at start and at the end of the three-week *ad libitum* period. The mean LW during the restricted period was calculated as the mean of the LW before and after the fourth week. For the *ad libitum* period the mean LW gain was calculated as the difference between the LW at the end and the start of *ad libitum* period and the mean LW loss during restricted period was calculated as the difference between the LW at the end and the start of the restricted period. The BCS was estimated at the start and at the end of the trial. During week three and four in each period, the feed and refusals were weighed every day for each ram and sampled for chemical analyses. One feed sample of 600 g was taken from each treatment and one refusal sample was taken from each ram. In week 3 the feed samples were taken during days 15-21 and the refusals were sampled during days 16-22. During week 4 the feed samples were taken during days 25-28 and the refusals were sampled during days 26-29 if there were any. The rapeseed meal was sampled once each period. All the feed and refusal samples were frozen immediately.

Collection of urine and faeces

Urine and faeces were collected individually during week 4 (days 26-29). The metabolic cages separated the urine and faeces, which were collected in metallic and plastic containers, respectively, on the floor. The containers were replaced every morning at feeding. A 10 % sulphuric acid solution was added to the urine containers every day to decrease the pH and thereby avoiding the N to evaporate from the urine. The rams fed red clover/grass silage got 300-400 ml 10 % sulphuric acid and the rams fed the other silages got 200 ml sulphuric acid in the metallic containers right after feeding in the morning. The rams fed red clover/grass silage got a higher volume of the 10 % sulphuric acid because of the higher N content in the urine, which gives a higher buffering capacity of the urine.

The urine weights and volumes were measured before 190-200 ml of urine was poured into a sample jar and frozen for further analysis. The weight was measured with the container weight on a scale. The urine was then mixed while stirring with a spoon and cleared from wool and feed through a sieve. The volume was measured with plastic measuring cylinders and a decanter of 2000 ml, 1000 ml and 500 ml. The measuring cylinders and decanters were rinsed with water between each urine collection.

The faeces were brushed from the cages down to the containers of faeces each morning before changing of containers. Then the faeces were sorted from wool and feed and collected in plastic bags, which were sealed and put into other plastic bags with a label in each. Two cages were leaking urine into the containers of faeces, why a metallic net was placed into these rams' containers to separate the urine from the faeces. The urine in the faeces container was only measured for the volume and then discarded. The faeces were weighed on a scale (AND HP-12K Max 12 kg Precision Industrial Balance) and then frozen for further analysis.

Analysis of dry matter and chemical composition in feed, refusals and faeces

After thawing, the DM of the daily feed samples and the daily refusals from weeks 3 and 4 were determined by weighing out 150 g or less of each sample in aluminium trays. The samples were first dried in 60 °C for 20 h and taken out to be weighed before they were dried again in 105 °C for 3 h and thereafter weighed again. The daily DM determinations after drying in 60 °C for 20 h of feed and refusals, were used for calculation of DM intake. The rest of the feed were merged into one sample for each silage, week and period and the refusal samples were merged into one sample for each ram, week and period. From the merged samples 200 g were taken from the feed and refusal samples for each week from each period and sent to LKS mbH, Lichtenwalde, Germany and Kungsängen SLU Uppsala, Sweden for nutrient analyses. Only refusal samples from week 3 were analysed for nutrient content, since there were not enough refusals during week 4 to analyse. Two samples of 50-51 g from each merged feed sample were sent to the Humboldt University, Berlin, Germany for fermentation characteristics and WSC analyses.

Before the faeces were merged into one sample for each ram and period, the DM was determined by weighing out 150 g in aluminium trays from each ram and day and drying the samples in 60 °C for 48 h before weighing again. Thereafter 200 g of the merged faeces samples were sent to LKS mbH, Lichtenwalde, Germany for analysis of chemical composition.

The feed, refusals and faeces samples were analysed for CP, NDF, ADF, acid detergent lignin (ADL; feed only), ashes and starch (maize silages and rapeseed meal only). These analyses were performed at LKS mbH, Lichtenwalde, Germany. The CP was determined by using the Kjeldahl nitrogen determination procedure and by calculating the CP content by total N x 6.25. The NDF, ADF and ADL were determined by the Fibre Technology method, excluding sodium sulphite, after Van Soest *et al.* (1991). The ash was determined by drying the samples at 525 °C for 16 h. Starch was analysed enzymatically according to the Boehringer & Mannheim test. The silage intake of starch was not statistically analysed, but the average and standard deviation for intakes of different silages were calculated for starch from *ad libitum* intake.

The OM content in silages was calculated from the ash contents. The VOS (*in vitro* OM digestibility) for silages were analysed at Kungsängen Research laboratory, SLU in Uppsala, Sweden by incubation at 38 °C for 96 h of 0.5 g dried sample in 49 ml buffer and 1 ml rumen fluid (Lindgren, 1979; Lindgren, 1983). The fermentation characteristics (pH, ammonia-N, organic acids and ethanol) and WSC were analysed at Humboldt University in Berlin, Germany. Ammonia concentration was determined colorimetrically based on the Berthelot reaction by use of a continuous flow analyser (SKALAR analytical B.V., Breda, Netherlands) and determination of pH was determined potentiometrically using a calibrated pH electrode. The WSC concentration was determined by the anthrone method according to Lengerken and Zimmermann (1991).

Volatile fatty acids and ethanol were ascertained by gas chromatography according to Weiss (2001). Lactic acid was analysed by HPLC according to Weiss & Kaiser (1995). A corrected DM for volatile losses when drying was used, as determined by the procedure of Weissbach and Strubelt (2008).

The *in vivo* digestibilities of DM, OM, CP, NDF and ADF were calculated by taking the difference between the intake and the faeces amount of DM or nutrients divided by the intake of DM or the respective nutrient. The *in vivo* digestibility was apparent because the endogenous nitrogen in faeces was not considered in the calculations.

Analysis of chemical composition in urine

The daily urine samples were merged to one sample per ram and period before analysis of total nitrogen, urea, purine derivatives (PD; allantoin and uric acid) and creatinine at LKS mbH, Lichtenwalde, Germany. The total nitrogen was analysed with the Kjeldahl procedure. Urea was analysed according to LKS (2006) while allantoin, uric acid and creatinine were analysed with HPLC (LKS, 2013). The analyses and the urine volume (adjusted for addition of sulphuric acid) were used to evaluate the total excretion of nitrogen compounds, which implied how much the losses of nitrogen were from the silages each day when fed. Also, the nitrogen balances from the silages were calculated as the intake of nitrogen minus the nitrogen loss from faeces and urine.

Statistical analysis

Data for feed intake, digestibility, protein utilization and LW were analysed by using the PROC MIXED procedure in SAS (ver. 9.3). The statistical model for the duplicated 5 x 5 Latin Square design was:

$$Y_{ijkl} = \mu + F_i + S_j + (FS)_{ij} + P_k + B_{l(j)} + C_{m(ijkl)} + e_{ijkl}$$

Where $\mathbf{Y_{ijkl}}$ = observed response, $\boldsymbol{\mu}$ = overall mean, $\mathbf{F_i}$ = effect of forage (i = 1 to 5), $\mathbf{S_j}$ = effect of supplementation of protein (l = 1 to 2), $\mathbf{(FS)_{ij}}$ = interaction between forage and supplementation of protein, $\mathbf{P_k}$ = effect of period (k = 1 to 5), $\mathbf{B_{j(l)}}$ = random effect of sheep nested within supplementation of protein (j = 1 to 10),,$\mathbf{C_{m(ijkl)}}$ = effect of carry over between periods for the combination of ijkl (m = 1 to 5) and $\mathbf{e_{ijkl}}$ = residual error.

As no significant carry over effect or interaction between forage and supplementation of protein (except for daily N intake $P < 0.048$) were found ($P > 0.10$), the $C_{m(ijkl)}$ and $(FS)_{ij}$ were excluded from the model. When significant effects were shown at $P \leq 0.05$ in the *F*-test, pairwise comparisons were done between the least square means with Tukey-Kramer adjustment. The pairwise differences were declared significant at $P \leq 0.05$ and stated as a tendency to significant at $0.05 < P \leq 0.10$.

Results

Feed intake

When comparing *ad libitum* silage intake of different silages, G and RCI gave higher DM intakes than the EM and, when expressed in % of LW, the RC also had higher silage DM intake than the EM (Table 8). The NDF intakes in g/day and in % of LW were higher for G, RCI and RC compared to LM and EM. When expressed in % of LW, the NDF intake of G also was higher than the NDF intake of the red clover silages (RC and RCI). The intakes of CP and ADF were highest for the red clover silages and lowest for the maize silages with G in between. There were no significant differences between RCI and RC or between LM and EM in intakes (Table 8).

Table 8. Effects of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) and of late maturity (LM) on silage *ad libitum* intake by rams. Least square means and standard error of the mean (SEM) when averaged over rapeseed supplementation (n=10)

	Experimental diets						
Silage intake of	G	RC	RCI	EM	LM	SEM	*P*-value
DM[1] (kg/day)	1.84[a]	1.73[ab]	1.80[a]	1.59[b]	1.71[ab]	0.056	<0.01
DM (% of LW[2])	2.42[a]	2.28[a]	2.34[a]	2.07[b]	2.24[ab]	0.051	<0.001
OM[3] (kg/day)	1.70	1.55	1.63	1.53	1.64	0.053	0.062
CP[4] (g/day)	219[b]	309[a]	307[a]	138[c]	152[c]	8.3	<0.001
NDF[5] (g/day)	907[a]	818[a]	819[a]	624[b]	663[b]	28.7	<0.001
NDF (% of LW)	1.19[a]	1.08[b]	1.07[b]	0.81[c]	0.86[c]	0.029	<0.001
ADF[6] (g/day)	521[b]	601[a]	611[a]	320[c]	330[c]	19.5	<0.001

[1]DM = dry matter
[2]LW = live weight
[3]OM = organic matter
[4]CP = crude protein
[5]NDF = neutral detergent fibre
[6]ADF = acid detergent fibre
[a,b,c] Least square means in a row with different superscripts differ significantly at $P \leq 0.05$

When the diets were offered at *ad libitum*, the inclusion of rapeseed meal increased silage intake in kg/day of DM, OM, NDF, CP and ADF compared to diets without the protein supplementation. However, silage intakes of DM and NDF in % of LW were not affected by rapeseed supplementation (Table 9).

Table 9. Effect of supplementation of rapeseed meal on silage *ad libitum* intake by rams. Least square means and standard error of the mean (SEM) when averaged over silage types (n=25)

	Rapeseed meal			
Silage intake of	Without	With	SEM	*P*-value
DM[1] (kg/day)	1.63	1.84	0.052	<0.05
DM (% of LW[2])	2.24	2.30	0.038	0.299
OM[3] (kg/day)	1.52	1.71	0.050	<0.05
CP[4] (g/day)	212	238	5.6	<0.05
NDF[5] (g/day)	721	811	27.1	<0.05
NDF (% of LW)	0.99	1.01	0.023	0.477
ADF[6] (g/day)	446	508	18.5	<0.05

[1]DM = dry matter
[2]LW = live weight
[3]OM = organic matter
[4]CP = crude protein
[5]NDF = neutral detergent fibre
[6]ADF = acid detergent fibre

Starch intake from maize silage increased with increased maturity at harvest but the effect of rapeseed supplementation was minor (Tables 10 and 11).

Table 10. Effects of whole crop maize silage of early maturity (EM) or of late maturity (LM) on silage *ad libitum* intake of starch by rams. Mean and standard deviation (SD) in parenthesis when averaged over rapeseed meal supplementation (n=10)

	Experimental diet			
Silage intake of	EM		LM	
Starch (g/day)	473	(113.7)	581	(134.9)

Table 11. Effect of supplementation of rapeseed meal on maize silage *ad libitum* intake of starch by rams. Mean and standard deviation (SD) in parenthesis when averaged over maize silage treatments (n=25)

	Rapeseed meal			
Silage intake of	Without		With	
Starch (g/day)	502	(114.3)	552	(152.0)

***In vivo* digestibility**

When the rams were offered the silage diets at 80 % of *ad libitum*, the digestibility of DM and OM were higher for G than for RC, RCI and LM diets (Table 12). The EM had higher DM digestibility and both EM and LM had higher OM digestibility than the red clover diets. The CP digestibility was higher for G and RC compared to the maize diets and the RCI had a higher CP digestibility than the LM diet. When comparing NDF and ADF digestibilities, G had higher digestibility than the other diets, which did not differ significantly except for RC that had higher NDF and ADF digestibility than the LM diet. There was no effect of the inoculant on the digestibility of the red clover diets and stage of maturity did not affect digestibility of the maize silage diets (Table 12). Inclusion of rapeseed meal in the diets affected only the CP digestibility significantly with a higher digestibility in supplemented diets (Table 13).

Table 12. Effect of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) and of late maturity (LM) on the *in vivo* digestibility by rams fed at 80% of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed supplementation (n=10)

	Experimental diets						
Digestibility of	G	RC	RCI	EM	LM	SEM	*P*-value
DM[1] (%)	73.1[a]	63.4[c]	62.9[c]	69.8[ab]	66.2[bc]	0.97	<0.001
OM[2] (%)	76.0[a]	64.9[c]	65.2[c]	72.6[ab]	69.0[b]	0.94	<0.001
CP[3] (%)	65.7[a]	64.0[a]	61.8[ab]	56.3[bc]	52.4[c]	1.64	<0.001
NDF[4] (%)	73.9[a]	60.2[b]	59.1[bc]	58.9[bc]	53.9[c]	1.34	<0.001
ADF[5] (%)	73.1[a]	59.2[b]	56.4[bc]	57.7[bc]	51.6[c]	1.69	<0.001

[1]DM = dry matter
[2]OM = organic matter
[3]CP = crude protein
[4]NDF = neutral detergent fibre
[5]ADF = acid detergent fibre

Table 13. Effect of supplementation of rapeseed meal on *in vivo* digestibility by rams fed 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over silage types (n=25)

	Rapeseed meal			
Digestibility of	Without	With	SEM	*P*-value
DM[1] (%)	66.4	67.8	0.74	0.205
OM[2] (%)	68.8	70.3	0.65	0.139
CP[3] (%)	57.5	62.6	1.13	<0.05
NDF[4] (%)	60.7	61.7	0.96	0.447
ADF[5] (%)	59.1	60.1	1.29	0.582

[1]DM = dry matter
[2]OM = organic matter
[3]CP = crude protein
[4] NDF = neutral detergent fibre
[5]ADF = acid detergent fibre

Protein utilization

Nitrogen intake, excretion and retention

The red clover diets had the highest N intakes, followed by G and maize silage diets (Table 14). The N yield in faeces (g/day) was higher for the red clover diets compared to the other diets, which did not differ significantly. When comparing N yield in faeces in % of N intake, the maize diets had higher faecal N yield than G and the red clover diets, which did not differ significantly. The N in urine (g/day) was significantly different between diets with the highest content for red clover diets and lowest for maize diets, with the grass diet in between. When comparing N in urine in % of N intake there were only a significant difference between RCI and EM with the highest yield for RCI. The RC gave a significantly higher N retention in g/day compared to the maize diets, while the N retention in % of N intake only gave a significant difference between the G and LM diets with a higher retention for the G diet (Table 14).

Table 14. Effect of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) on the total N intake, excretion of N in faeces and urine and the N retention from feed by rams fed at 80% of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed supplementation (n=10)

	Experimental diets						
	G	RC	RCI	EM	LM	SEM	*P*-value
Total N intake (g/day)	29.4^{b}	42.6^{a}	39.1^{a}	20.1^{c}	20.9^{c}	1.11	<0.001
N in							
faeces (g/day)	9.9^{b}	15.1^{a}	14.8^{a}	8.7^{b}	9.7^{b}	0.56	<0.001
urine (g/day)	12.7^{b}	17.4^{a}	18.9^{a}	7.5^{c}	8.6^{c}	0.68	<0.001
faeces (% of N intake)	34.3^{c}	36.0^{c}	38.2^{bc}	43.8^{ab}	47.6^{a}	1.64	<0.001
urine (% of N intake)	43.3^{ab}	42.2^{ab}	48.9^{a}	38.5^{b}	41.6^{ab}	2.17	<0.01
N retention (g/day)	6.8^{ab}	10.1^{a}	5.4^{ab}	3.9^{b}	2.6^{b}	1.20	<0.01
N retention (% of N intake)	22.4^{a}	21.8^{ab}	12.9^{ab}	17.8^{ab}	10.8^{b}	2.86	<0.05

[a,b,c] Least square means in a row with different superscripts differ significantly at $P \leq 0.05$

When giving supplementation of rapeseed meal to the rams the total N intake and N excretion in faeces and urine increased compared to rams fed silages only (Table 15). However, when comparing the excretion of N in faeces and urine in % of N intake, the supplemented rams got a lower output in faeces compared to rams with no supplementation and N output in the urine showed no significant effect of rapeseed supplementation. The N retention was higher for rams fed rapeseed meal compared to rams fed diets without supplementation. The N retention in % of total N intake only tended to be higher for diets supplemented with rapeseed meal compared to unsupplemented diets (Table 15).

Table 15. Effect of supplementation of rapeseed meal on the total N intake, excretion of N in faeces and urine and the N retention from feed by rams at 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over silage types (n=25)

	Rapeseed meal			
	Without	With	SEM	*P*-value
Total N intake (g/day)	25.6	35.2	0.90	<0.001
N in				
faeces (g/day)	10.4	12.8	0.54	<0.05
urine (g/day)	11.4	14.7	0.64	<0.01
faeces (% of N intake)	42.5	37.4	1.13	<0.05
urine (% of N intake)	43.6	42.3	1.93	0.647
N retention (g/day)	3.82	7.69	0.903	<0.05
N retention (% of N intake)	13.9	20.4	2.05	0.057

Excretion of nitrogen compounds in urine

The excretion of urea in urine was highest for the red clover diets and lowest for the maize diets with the G diet in between (Table 16). The excretion of creatinine in g/day, mmol/day and mg/kg LW and allantoin in % of PD showed no differences between diets. Excretion of allantoin and PD in g/day and mmol/day was higher for the G compared to RC, RCI and EM diets, whereas the LM diet was not different from the other diets. Hippuric acid excretion was highest for G followed by the maize diets and lowest for the red clover diets. The hippuric acid excretion from the rams fed the LM diet was not significantly different from the EM and the red clover diets (Table 16).

Table 16. Effect of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) on the excretion of different N compounds in urine by rams at 80 % of ad libitum DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed supplementation (n=10)

	Experimental diets						
Excretion of	G	RC	RCI	EM	LM	SEM	*P*-value
g/day							
Urea	16.0^{b}	34.3^{a}	33.9^{a}	9.0^{c}	11.0^{c}	0.96	<0.001
Creatinine	2.74	3.16	2.75	2.70	2.71	0.223	0.078
Allantoin	4.19^{a}	3.04^{b}	3.10^{b}	2.94^{b}	3.83^{ab}	0.273	<0.01
PD	4.78^{a}	3.42^{b}	3.45^{b}	3.27^{b}	4.18^{ab}	0.276	<0.001
Hippuric acid	30.0^{a}	7.4^{c}	7.1^{c}	12.4^{b}	11.0^{bc}	1.23	<0.001
mmol/day							
Creatinine	24.2	28.0	24.3	23.8	23.9	1.97	0.078
Allantoin	28.0^{a}	19.2^{b}	19.6^{b}	18.6^{b}	24.2^{ab}	1.69	<0.001
PD	30.1^{a}	21.5^{b}	21.7^{b}	20.6^{b}	26.3^{ab}	1.74	<0.001
Hippuric acid	167.3^{a}	41.1^{c}	39.6^{c}	69.1^{b}	61.6^{bc}	6.85	<0.001
Creatinine (mg/kg LW)	36.4	42.5	37.3	36.5	36.4	3.08	0.086
Allantoin (% of PD)	88.4	88.6	89.5	89.4	91.5	2.04	0.805

[a,b,c] Least square means in a row with different superscripts differ significantly at $P \leq 0.05$
PD =purine derivatives

When comparing the excretion of N compounds in urine from rams fed diets supplemented with rapeseed meal with rams fed unsupplemented diets, there were only significant differences in the excretion of urea. Rapeseed meal increased the excretion of urea compared with a diet of silage only (Table 17).

Table 17. Effect of supplementation of rapeseed meal on the excretion of different N compounds in urine by rams fed at 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over silage types (n=25)

	Rapeseed meal			
Excretion of	Without	With	SEM	*P*-value
g/day				
Urea	18.0	23.7	0.67	<0.001
Creatinine	2.78	2.85	0.268	0.859
Allantoin	3.19	3.65	0.260	0.250
PD	3.52	4.12	0.261	0.141
Hippuric acid	13.0	14.1	1.04	0.483
mmol/day				
Creatinine	24.6	25.2	2.37	0.859
Allantoin	20.2	23.7	1.62	0.166
PD	22.1	25.9	1.65	0.142
Hippuric acid	72.7	78.7	5.79	0.483
Creatinine (mg/kg LW)	39.1	36.5	3.74	0.642
Allantoin (% of PD)	90.2	88.8	1.49	0.529

PD = purine derivatives

Live weight

The mean LW of the rams did not differ between diets when the rams were fed at restricted and *ad libitum* feed intake (Table 18). When feed intake was restricted, the rams lost weight. The LM diet gave a higher LW loss than the G diet. The other diets showed no significant differences in LW losses. During *ad libitum* feeding the rams showed no significant differences in LW gain between diets (Table 18).

Table 18. Effect of grass silage (G), red clover/grass silage without inoculant (RC) or with inoculant (RCI) and whole crop maize silage of early maturity (EM) or of late maturity (LM) on the mean live weight (LW) and LW gain and loss by rams fed at *ad libitum* (*ad lib*) DM intake and at 80 % of *ad libitum* intake (restr intake). Least square means and standard error of the mean (SEM) when averaged over rapeseed supplementation (n=10).

	Experimental diets						
	G	RC	RCI	EM	LM	SEM	*P*-value
Mean LW *ad lib* intake (kg)	75.6	75.4	75.4	75.4	75.7	1.41	0.997
Mean LW restr intake (kg)	75.5	74.4	74.4	74.3	74.5	1.34	0.775
LW gain *ad lib* intake (kg)	1.15	0.70	0.60	0.05	0.60	0.390	0.365
LW loss restr intake (kg)	1.50^{a}	2.65^{ab}	2.55^{ab}	2.40^{ab}	3.00^{b}	0.341	<0.05
LW gain *ad lib* intake (%)	1.55	1.12	0.89	0.25	0.83	0.524	0.480
LW loss restr intake (%)	1.93^{a}	3.53^{ab}	3.31^{ab}	3.21^{ab}	3.91^{b}	0.438	<0.05

^{a,b} Least square means in a row with different superscripts differ significantly at $P \leq 0.05$

The mean LW was higher for rams supplemented with rapeseed meal than for rams not supplemented with rapeseed meal at both restricted and *ad libitum* intakes (Table 19). At restricted feed intake the supplemented rams lost more LW than rams, which did not receive supplementation. At *ad libitum* feed intake protein supplementation did not affect

LW (Table 19). At the end of the trial the mean LW and BCS were 80.6 ± 6.74 kg and 4.5 ± 0.16 respectively.

Table 19. Effect of supplementation of rapeseed meal on the mean live weight (LW) and LW gain or loss by rams at *ad libitum* DM intake and at 80 % of *ad libitum* (restr intake). Least square mean and standard error of the mean (SEM) when averaged over silage types (n=25).

	Rapeseed meal			
	Without	With	SEM	*P*-value
LW *ad libitum* intake (kg)	71.9	79.1	1.73	<0.05
LW restr intake (kg)	71.2	78.0	1.65	<0.05
LW gain *ad libitum* intake (kg)	0.34	0.90	0.290	0.210
LW loss restr intake (kg)	1.90	2.94	0.275	<0.05
LW gain *ad libitum* intake (%)	0.48	1.37	0.384	0.141
LW loss restr intake (%)	2.61	3.75	0.341	<0.05

Discussion

Feed intake

Dry matter intake

The *ad libitum* silage DM intake was lower for EM than for G and RCI in kg/day and also lower than for RC, when comparing DM intakes in % of LW, which is in agreement with others (Aston & Tayler, 1980; Margan *et al*., 1994; Vranić *et al*., 2008). In contrast, Browne et al. (2005) and Juniper et al. (2005) showed that WCMS could give a higher DM intake than grass silage. The potential voluntary forage DM intake in ruminants can be regulated by the amounts of NDF and ADF in the forage, where higher concentrations result in lower intakes (Waldo, 1986; Jung & Allen, 1995; McDonald *et al*., 2002; Särkijärvi *et al*., 2012). However, in this study the results were different from that statement. Instead, the low intake of EM compared to G and RCI could depend on the higher content and more spread distribution of lignin in stem and leaves in EM that makes the feed more difficult to digest by rumen microbes (Wilson & Hatfield, 1997) or by the lower fibre digestibility for EM compared to G, which affects the intake negatively (Allen, 1996). Another reason for the low intake of EM could be the low protein content in proportion to fibre and starch contents and thereby lower intake of protein compared to the other diets. This gives an ineffective N utilization and digestibility of the feed by the microbes, affecting the feed intake negatively (Kilkenny, 1978; Galyean & Goetsch, 1993; Svensson, 2010), but since no interaction was found between silage and protein supplementation, the protein content did not seem to be the only major reason for the lower DM intake for EM. The average intake of starch from maize was highest for LM as expected as the cob proportion, rich in starch, increases with maturity.

The supplementation of rapeseed meal gave a higher silage DM intake compared to rams fed diets without supplementation. This shows that a higher protein intake from the diet affects the silage DM intake positively and depends on more effective N utilization and digestibility by the microbes (Kilkenny, 1978; Galyean & Goetsch, 1993; Svensson, 2010), which can produce more microbial protein and multiply, resulting in higher digestion of fibres and thereby a higher intake (Hvelplund *et al*., 1987; McDonald *et al*., 2002). In addition, other studies have shown that supplementation of protein gives a higher silage intake than diets without supplementation (Aston & Tayler, 1980; Matejovsky & Sanson, 1995). Browne et al. (2005) showed that a diet of WCMS without concentrate supplementation could give a higher intake than diets supplemented with concentrate. The difference between studies could depend on the protein concentration in the diet before supplementation is used. Combinations of WCMS, grass and red clover often affects the intake of different nutrients positively, as concentrates do, because the higher N content in grass and red clover complements the low N and high carbohydrate content in WCMS for the microbial utilization of the forage. In exchange, the WCMS contributes with starch and the grass contributes with high NDF digestibility and sugar to the high N contents in red clover silages for the energy-demanding microbial protein synthesis. (Dewhurst *et al*., 2003; Moorby *et al*., 2008; Vranić *et al*., 2008; Niderkorn *et al*., 2012).

Crude protein intake

In the present study the silage CP intakes were highest for the red clover diets followed by G, and lowest for the maize diets, which is in agreement with other studies where legume silages have given higher N intake compared to grass (Dewhurst *et al*., 2003; Speijers *et al*., 2005; Marley *et al*., 2007) or WCMS (Margan *et al*., 1994; Auldist *et al*., 1999; Moorby *et al*., 2008). However, the N intake from grass and WCMS have been shown to differ between different studies where Vranić et al. (2008) showed the same result as the present study in contrast to Browne et al. (2005), which showed the opposite with higher N intake for WCMS. The reason to differences between studies in nutrient intakes could depend on varying maturity stages of the forage at harvest and thereby different CP concentrations and digestibilities, which affect feed intake (McDonald *et al*., 2002; Särkijärvi *et al*., 2012).

In addition, rapeseed meal as supplement had a positive effect on CP intake in the current study. Other studies have shown the same result when diets were supplemented with concentrate (Santoso *et al*., 2003; Browne *et al*., 2005). This depends, as mentioned before for DM intake, on the more effective utilization and digestibility by the microbes of fibres when more N is added to the diet (Kilkenny, 1978; Galyean & Goetsch, 1993; Svensson, 2010). Another reason could be that rapeseed meal is a small feedstuff that passes through the rumen easy to the intestines without degradation leaving space for more feed intake (Fahey & Merchen, 1987; McDonald *et al*., 2002). However, Nadeau et al. (2007) showed that a diet of low CP concentration can give a higher N intake than a diet with normal CP concentration when the diet is optimally balanced with ruminal degradable and undegradable CP.

Fibre intake

In the current study G and red clover diets gave higher silage NDF intakes in g/day than the maize diets. When looking at the daily silage NDF intake in % of LW, G gave the highest intake of the diets followed by the red clover diets. The maize diets gave the lowest silage intakes of NDF. Silage intake of ADF was highest for the red clover diets, followed by the G and the maize diets. The higher contents of NDF and ADF in G and red clover silages could be limiting feed intake. However, these diets gave the highest intakes in rams. The major reason could be the higher CP content in grass and red clover compared to maize that complements the higher fibre content in grass and red clover for the rumen microbes multiplication, thereby increasing DM intake including the NDF and ADF intakes (Galyean & Goetsch, 1993). The high rate of digestion in rumen of the potentially digestible NDF from red clover could also be a reason for higher intake of fibres, as the fibres passes through the rumen faster, leaving space for more feed intake (Reid *et al*., 1988; Van Soest, 1994; Allen, 1996). In addition, the reason for G giving higher NDF intake than RCI and RC, despite higher NDF content that can limit the feed intake, could be the higher content of lignin in the red clover silages compared to the grass silage, giving higher fibre digestibility and thereby higher intake of grass silage. Lignin is indigestible and makes the NDF less digestible with higher lignin content and gives thereby a lower feed intake (Wilkinson, 1978). The daily intake of NDF in sheep seems to vary significantly between different studies. Grass silage can give a higher NDF intake than WCMS (Vranić *et al*., 2008) and red clover silage (Laforest *et al*., 1986) while WCMS can give a higher NDF intake than grass and red clover silage (Reid *et al*., 1988). A study by Niderkorn et al. (2012) showed that red clover could give a higher NDF intake than grass silage. Differences between studies possibly could depend on differences in maturity stage of the forage at harvest and dietary protein concentrations.

The supplementation of rapeseed meal had a positive effect on the NDF and ADF intakes. This might be related to the higher protein intake from the diet, which make up for the high content of carbohydrates and increases silage DM intake by the rams and microbial activity in the rumen (Kilkenny, 1978; Galyean & Goetsch, 1993; Svensson, 2010). When comparing different amounts of dietary protein concentrations, Nadeau et al. (2007) showed that a normal amount of CP (168 g CP/kg DM) stimulates a higher NDF intake compared to a low amount of CP (160 g CP/kg DM) to dairy cows.

Effects of inoculant in red clover and of different maturity stages of maize silage

There was no effect on inoculant addition to red clover silages on nutrient intakes by rams, which is in agreement with earlier studies (Vrotniakiene & Jatkauskas, 2004; Jatkauskas & Vrotniakiene, 2005). This indicates that the addition of inoculant did not have any major effects on the nutrient intake, as the chemical compositions were similar for the two silages. The fermentation characteristics of the red clover silages were slightly different with higher concentrations of acetic acid, ammonia N and ethanol for RC compared to RCI, which can affect intake negatively (Hetta *et al*., 2003; Vrotniakiene & Jatkauskas, 2004; Krizsan & Randby, 2007), but in the present study this difference did not seem to affect the nutrient intake. In contrast to the current study, a study by Stokes (1992) showed that an inoculant could give a lower DM intake than untreated legume silage.

There was no effect of time of harvest on nutrient intakes from WCMS, probably due to similar chemical compositions. However, in the study by Joanning et al. (1981) the silage with early maturity (milk stage) gave a lower DM intake than the silage with late maturity (soft dent stage), while results by Zaralis et al. (2014), where the same silages as in this study were fed to dairy bulls, agree with results from the current study, showing no significant differences between the maize diets in nutrient intakes. One reason why Joanning et al. (1981) showed lower intake for early maturity compared to late maturity of WCMS could be the larger differences in starch contents compared to the current study and the study by Zaralis et al. (2014).

In vivo digestibility

Dry matter and organic matter digestibility

The *in vivo* DM digestibility was higher for the G and EM diets compared to the RC and RCI diets, likely because of the higher lignin concentration in the red clover silages compared to the other silages. In addition, there was a significant difference between G and LM with higher digestibility for G, probably due to the higher CP intake for G compared to LM that stimulated the digestibility of carbohydrates in the rumen (Kilkenny, 1978; Galyean & Goetsch, 1993; Svensson, 2010). There was no effect of supplementation of rapeseed meal on the *in vivo* DM digestibility, indicating that more protein in the diet did not affect the DM digestibility even though the silage nutrient intake increased with supplementation. This may depend on that the rapeseed meal passes through the rumen without degradation, because of the small particles, leaving space for a higher intake of silage. The silage still had the same digestibility because the extra protein passed through the rumen without helping the microbes to multiply and digest more of the feed (Fahey & Merchen, 1987; McDonald *et al*., 2002). Instead, the protein could to a great extent be absorbed in the small intestine.

The *in vivo* OM digestibility was higher for the G diet compared to the RC, RCI and LM diets. The LM and EM gave higher *in vivo* OM digestibility than RC and RCI, indicating the highest metabolizable energy content in G, LM and EM (Åkerlind, 2011). Likewise, Moorby et al. (2008), found that WCMS gave a higher OM digestibility than red clover while Margan et al. (1994) found the opposite result. Additionally, several other studies showed lower or no significant difference in OM digestibility when comparing grass silage with other forages (Aston & Tayler, 1980; Laforest *et al*., 1986; Browne *et al*., 2005; Speijers *et al*., 2005; Keady *et al*., 2007). In the current study G had both the highest NDF concentration and *in vivo* OM digestibility, but according to Aufrère et al. (1992) a higher NDF concentration gives a lower OM digestibility. One explanation could be a lower ash concentration in G, EM and LM compared to RC and RCI, which increase the *in vivo* OM digestibility (Åkerlind, 2011). Another reason for higher *in vivo* OM digestibility in G compared to the other silages is the higher *in vivo* digestibilities of NDF and ADF in the G diet compared to the other diets. The OM intake did not affect the *in vivo* OM digestibility, as there was no effect of type of forage on OM intake.

There was no effect of supplementation with rapeseed meal on the *in vivo* OM digestibility in rams, indicating, likewise as for DM digestibility, that rapeseed meal passed through the rumen without degradation and thereby did not affect the digestibility by the microbes (Fahey & Merchen, 1987; McDonald *et al*., 2002). However, Aston and Tayler (1980) and Keady et al. (2007) showed that supplementation of concentrate could increase the OM digestibility.

Fibre digestibility

The *in vivo* digestibilities of NDF and ADF were highest for the G diet compared to the other diets. This is in agreement with other studies (Laforest *et al*., 1986; Browne *et al*., 2005; Keady *et al*., 2007; Vranić *et al*., 2008), but vary with the grass maturity at harvest (Vranić *et al*., 2008). When grass was harvested late, and compared with WCMS, in the study by Vranić et al. (2008), the ADF digestibility was low and no significant difference in NDF digestibility was found between the forages. Addition of late harvested (low digestibility) silage can decrease the digestibility in a mix with silage of higher digestibility, and addition of highly digestible silage to a mix based on late harvested silage can improve the total digestibility of the diet (Vranić *et al*., 2008). No studies were found to compare red clover and WCMS in *in vivo* ADF digestibility. In the current study, the RC diet gave a higher *in vivo* fibre (NDF and ADF) digestibility than LM, which is in agreement with Moorby et al. (2008). Maize is known for having thick cell walls and high lignin content in the stem, resulting in lower digestibility of fibres compared to early harvested grass and red clover (Wilkinson, 1978). This was shown in the current study for the LM diet.

Supplementation with rapeseed meal did not alter the *in vivo* NDF and ADF digestibility of the diets. The same was shown by Keady et al. (2007), which fed grass silage and a mix of grass and WCMS (40/60) with various amounts of concentrate (protein levels) to groups of animals for comparison of fibre digestibilities (NDF and ADF) depending on protein level. This indicates, as mentioned before, that the rapeseed meal could be passing through the rumen to the intestines without being degraded by the microbes because of the small particle size, giving capacity to a higher feed intake, but not to a higher fibre digestibility. Instead, the fibres could be passing through the intestines and out from the body with faeces without being degraded (Fahey & Merchen, 1987; McDonald *et al*., 2002).

Protein digestibility

In this study, G and RC gave a higher *in vivo* CP digestibility compared to the maize diets and the RCI gave higher *in vivo* CP digestibility than the LM, but showed no significant difference to the EM diet. The protein digestibility is dependent on the ability of the microbes to approach the feed and break it down (McDonald *et al*., 2002). In agreement with the current study, maize has a thick cell wall and a high lignin content, which prevents the microbes to break it down more than in grass and red clover (Wilkinson, 1978). Margan et al. (1994) and Vranić et al. (2008) also showed that red clover and grass gave a higher N digestibility than WCMS. Other studies have shown no significant differences in N digestibility between red clover and WCMS (Moorby *et al*., 2008) or grass and WCMS (Keady *et al*., 2007). In contrast, Browne et al. (2005) showed that WCMS gave a higher N digestibility than grass. In the current study there was no difference in *in vivo* CP digestibility between G and the red clover diets, which is in agreement with Dewhurst et al. (2003). However, in a study by Laforest et al. (1986) the red clover diet gave a higher N digestibility compared to the grass diet.

When comparing the rams supplemented with rapeseed meal with the rams fed diets without supplementation, there was a positive effect of supplementation on *in vivo* CP digestibility. This could be explained by that the rapeseed meal had small particles, including protein that passed through the rumen faster without being digested or only to a minor part being digested. Instead, the protein was absorbed in the small intestine as AAT, while the other nutrients from rapeseed meal were degraded in the rumen or were passed through the small intestines without being degraded, thereby not showing any difference in digestibility (Pettersson, 2006). In contrast, Keady et al. (2007) found no significant differences in N digestibility when comparing different amounts of concentrate (3 and 5 kg; barley, maize meal, sugar-beet pulp, soya bean, and molasses) in the diet.

Effects of inoculant in red clover and of different maturity stages of maize silage

There was no effect of inoculant addition to red clover silage on nutrient digestibilities, which is in agreement with other studies for *in vitro* OM digestibility of legume silage (Jatkauskas & Vrotniakiene, 2005). Hetta et al. (2003) showed that red clover silage with an additive of lactobacilli and molasses gave higher NDF and OM digestibilities than red clover silage without additive. The major reason for no effect of inoculant addition in the current study could be the similar fermentation characteristics with no clostridia fermentation of the red clover silage without inoculant, giving the same digestibilities. There was no effect of the time of harvest of WCMS on nutrient digestibilities either, depending on the similar chemical compositions. In contrast to this study, Joanning et al. (1981) showed that early maturity (milk stage) WCMS had higher NDF, ADF and N digestibility in steers compared to late maturity (soft dent stage) WCMS.

Protein utilization

N intake, excretion and retention

When comparing the N intake from grass and red clover silage, the current study showed the highest N intake for red clover, in agreement with most of the earlier studies (Laforest *et al*., 1986; Dewhurst *et al*., 2003; Speijers *et al*., 2005; Marley *et al*., 2007). There was an exception for comparison of grass and alfalfa, which gave no significant difference (Santoso *et al*., 2003). In addition, the result for N intake from red clover, grass and WCMS in the present study was in agreement with earlier studies where red clover and grass gave a higher N intake than WCMS (Margan *et al*., 1994; Keady *et al*., 2007;

Moorby *et al*., 2008; Vranić *et al*., 2008). This could be expected because of the higher CP content in grass and red clover compared to maize (Spörndly, 2003; Juniper *et al*., 2005; Keady *et al*., 2007; Vranić *et al*., 2008). Other studies have shown contradictory results where WCMS has resulted in a higher N intake compared to grass when supplemented with protein as concentrate or urea (Browne *et al*., 2005; Burke *et al*., 2007). There has also been shown no difference in N intake between WCMS and grass because of concentrate supplementation (Browne *et al*., 2005). Supplementation of rapeseed meal had a positive effect on the N intake, which was expected because of the higher rationing of CP from supplement (McDonald *et al*., 2002; Sjaastad *et al*., 2003).

The current study showed that RC and RCI gave a higher N loss than G and that LM and EM gave the lowest N losses through faeces and urine. In agreement with the current study, the loss of N from urine has previously been shown to be higher for grass and red clover than for maize (Auldist *et al*., 1999; Moorby *et al*., 2008; Vranić *et al*., 2008). Feeding either legumes or grass silage has not affected the amount of N lost in urine (Santoso *et al*., 2003), but feeding alfalfa could lead to higher urinary N losses (Carro *et al*., 2012). With a higher N intake, the loss of N as urea in the urine increases (McDonald *et al*., 2002; Sjaastad *et al*., 2003) and gives often a higher N retention. Red clover and grass have an excess of protein and gives thereby a higher loss of N in urine compared to maize (Hvelplund *et al*., 1987; Margan *et al*., 1994; Vranić *et al*., 2008). However, too low protein in diets with WCMS could instead increase the loss of N in urine because of ineffective utilization (Vranić *et al*., 2008), which did not seem to be the case in this study as LM and EM gave the lowest N loss. The loss of N in urine and faeces was highest when rapeseed meal was fed, indicating that the N in feed was not fully utilized, but gave an excess of N for the rams even if the N retention also was highest for supplementation of protein. The loss of N increases the N emissions and contributes to the acidification of nature. Also, by overfeeding with losses of N as result give higher feed cost that is not sustainable for the business.

The N retention can be expressed in several ways, such as N balance and N efficiency, as seen earlier in the literature study of this thesis, but hereafter N retention is used. The current study showed a higher N retention for RC compared to LM and EM when expressed in g/day and no difference between the red clover diets compared to the maize diets when expressed in % of N intake. However, RC had almost the double N retention in % of N intake compared to LM. Presumably, this could depend on a large variation in the data as shown by the relatively large SEM because of much variation in the variable studied and a relatively small number of animals used in the study. In agreement with Moorby et al. (2008), red clover and maize silage did not show any significant differences in N retention. This indicates that, even if the N loss in urine was lower for LM and EM, there was a balance of N in the diet giving the same retention of N for the rams to grow and store protein in the muscles when fed maize silages as when fed RC (McDonald *et al*., 2002). However, all the diets still gave an excess of protein for retention in the body of the rams. Comparing white clover with grass gave a higher N retention for white clover (Beever *et al*., 1986). Also, comparison of maize with grass gave a higher N retention for maize compared to grass (Browne *et al*., 2005). In contrast, the current study showed no significant differences between the G diet and the other silages in N retention. However, there was a positive effect of diet on N retention in % of N intake when feeding the G diet compared to the LM diet, indicating a high and easy utilization of the protein for the microbes in rumen and the intestinal absorption in rams in proportion to the protein content in the G diet. As mentioned before, the ability of the microbes to come near the protein is dependent on the chemical structure of the feed components and the protein, showing in this study that the G diet was easy to digest for the microbes (Wilkinson, 1978; Jung & Deetz, 1993; Jung & Allen, 1995; McDonald *et al*., 2002). The current study showed that supplementation of rapeseed meal gave a positive effect on N retention in g/day and there was a tendency to effect of diet on N retention in % of N intake. Different amounts of supplementation of CP seem not to be important for the N retention according to Nadeau et al. (2007). The reason for higher N retention when using protein supplementation was a higher circulation of N in the body from a higher N intake to store in the body (McDonald *et al*., 2002; Sjaastad *et al*., 2003), giving an increasing LW during the whole experiment.

Excretion of N compounds in urine

There was a lack of studies to compare the results of N compounds in urine from the present study with the literature, but several compounds and factors are linked to each other, which made it easier to evaluate the certainty of the results. In this study the RC and RCI diets gave the highest amounts of urea in urine followed by G while urinary urea yield was lowest for LM and EM. This was in agreement with what could be expected according to the protein content and intake of the different silages as the urea content in urine reflects how much N that is in excess in the diet (McDonald *et al*., 2002; Sjaastad *et al*., 2003). As for the current study, Auldist et al. (1999) found that red clover gave more urea in urine than maize. Allantoin in urine was highest when feeding the G diet and lowest when feeding the RC, RCI and EM diets. However, compared with other studies, only Santoso et al. (2003) showed results, which were in agreement with this study, while another study has shown the opposite (Carro *et al*., 2012) or no significant difference between diets for allantoin content in urine (Burke *et al*., 2007). The result for allantoin in the current study indicates a higher microbial protein synthesis in rumen for the G diet compared to the other diets (Chen *et al*., 1990; Margan *et al*., 1994; Vranić *et al*., 2008). Therefore, the proportion of carbohydrates and protein have been most satisfying when feeding G. Looking at PD in urine is the same as putting allantoin and uric acid amounts together and reflects, as just mentioned, the microbe protein synthesis in rumen and the absorption of the microbial protein in intestines. In the current study, PD in urine was highest when feeding G and lowest when feeding RC, RCI and EM, which is in agreement with the N retention of the diets where G gave the highest amount of microbial protein digested in the intestines for the retention to the bodies of the rams. There was no study found looking at PD in urine to compare with.

Hippuric acid is reflecting the protein and lignin intake and decrease with a higher lignin and lower protein intake (Martin, 1970; Szanyiová *et al*., 1995; Groenigen *et al*., 2006). However, red clover seems to be an exception since both the lignin and protein contents are higher, but not the phenolic acid content, compared to grass (Jung & Deetz, 1993), which affect the content of hippuric acid in urine (Szanyiová *et al*., 1995). Therefore, hippuric acid content in urine was lower for RC and RCI compared to grass even if the CP and lignin intakes were higher for the red clover diets compared to G. The CP and lignin content was high and low, respectively, in G because of an early harvest, giving a higher hippuric acid content in urine compared to the other diets. The EM and LM had low CP and lignin contents, giving low CP and lignin intakes, but probably a higher content of lignin precursors compared to the red clover diets, and thereby a lower hippuric acid content in urine compared to the RC and RCI diets, following mostly the CP intake (Martin, 1970). In summary, the G diet should have been the most environmentally friendly diet and the RC, RCI and LM diets the least environmentally friendly diet when estimating the N_2O emissions (Groenigen *et al*., 2006). Hippuric acid was not found in any other studies. Creatinine indicates the size of the body muscle mass. A high content of creatinine in urine could indicate a deficiency of protein from feed (McDonald *et al.*, 2002). In the current study there was no effect of diet on the creatinine content in urine, which could depend on a protein supply in balance and a long adaptation period to new diets before sampling of urine.

In this study rapeseed meal only had effects on urea and total N in g/day with higher amounts in urine. The main reason for this result could be that only urea is affected significantly by the amount of N in the diet. The other components in urine are more affected by the amounts of carbohydrates in the diet, which was not changed to the same extent as the protein content.

Effects of inoculant in red clover and of different maturity stages of maize silage

The addition of inoculant to red clover silage showed no effect on N intake, N excretion and its compounds in urine and faeces and N retention, indicating that addition of inoculant to red clover silage did not improve N utilization of the silage in the sheep. As the inoculant did not alter CP intake or CP content of the two red clover silages, the results could be expected.

Furthermore, in the present study, there was no effect of the time of harvest of WCMS on the N intake, N excretion or N retention. Other studies showed the same pattern, showing no significant differences between early and late maturity (milk stage and soft dent stage) of WCMS in N retention (Joanning *et al.*, 1981), N intake and allantoin excretion (Johnson *et al.*, 1998). However, Johnson et al. (1998) found that uric acid in urine was higher for WCMS with early maturity (one-half milkline stage) compared to late maturity (blackline stage). As the fermentation quality and CP content were similar between silages in the present study and no effect on the CP intake and digestibility depending on harvest date was found, the results could be expected. If the maturity stages in the present study had differed to a larger extent, as in previous studies (e.g. Johnson *et al.*, 1998), the protein utilization of the rams might have been affected.

Live weight and body condition score

There was no effect of diet on the LW gain of the rams, but the supplementation of rapeseed meal affected the mean LW. There was no significant difference between supplemented and unsupplemented rams in gain of LW when fed silages at *ad libitum*, but the rams lost more LW when fed silages at 80 % of *ad libitum* compared to the group not supplemented. Late maize gave the highest loss of LW and G the lowest, most likely because of lack of both protein and carbohydrates for the rumen microbes when fed the LM silage (Hvelplund *et al.*, 1987; McDonald *et al.*, 2002; Pettersson, 2006; Vranić *et al.*, 2008). Also, the differences in LW at the end of the experiment may depend on different mean weights at start between the group not supplemented and the group supplemented with protein and between mean weights at start for rams in each treatment. In addition, the weight loss could depend on that the adjustment period for the rumen microbes and for the rest of the body was too short when fewer nutrients were available when decreasing the feed intake (McDonald *et al.*, 2002).

Further research

This study has shown how sheep utilize different silages separately, supplemented with or without protein. As sheep often are used as a model for different ruminants, the results could be applied on other ruminants as well. Further research should be done to investigate how combining the silages with as little concentrate supplement as possible can optimize a balanced diet. As protein supplementation did only affect feed intake and CP digestibility positively, while the N emissions were increased or not affected in this study compared to diets without protein supplementation, different silage combinations are more interesting to investigate to know how ruminant N balance and utilization could be even more optimized. Further, research should be done on how the balanced diets can be practicable on farms in Sweden to get a high production in animals without too high costs because of a more diverse cultivation.

Conclusions

- The WCMS gave lowest intakes of DM, NDF, ADF and CP. Grass gave the highest intakes of DM and NDF, whereas red clover gave the highest intakes of ADF and CP. Supplementation of rapeseed meal had a positive effect on intakes of DM, NDF, ADF, and CP. There was no effect of bacterial inoculant on nutrient intakes of red clover silage and there was no effect of time of harvest of WCMS on nutrient intakes.

- The grass silage had the highest *in vivo* digestibility of DM, OM, CP, NDF and ADF. The lowest nutrient digestibilities were found for WCMS except for OM and DM digestibility where red clover gave the lowest digestibilities. Rapeseed meal supplementation had no effect on nutrient digestibility except for CP digestibility where the supplemented group had a higher CP digestibility compared to the rams fed unsupplemented diets. Inoculant addition to red clover silages and different time of harvest of WCMS did not alter the *in vivo* digestibility by rams.

- There were large variations in the N utilisation values resulting in numerical but nearly no significant differences between the forages. There was no effect of diet on excretion of creatinine. Supplementation of rapeseed meal gave a decreased N in faeces in % of N intake and increased urea and total N in urine. Rapeseed meal had no effect on the N in urine, creatinine, allantoin, PD and hippuric acid. However, there was a tendency to significantly higher N retention in % of N intake when feeding rapeseed meal.

- No effect was found for inoculant addition to red clover silages and time of harvest of WCMS on protein utilization by rams.

- Grass was the best silage according to intake, digestibility and protein utilization, while maize silages gave a less effective and balanced N utilization compared to the other silages. However, WCMS had a high OM digestibility giving a beneficial energy supplement to mixed diets and red clover gave the highest N intake and a high N retention, giving a good opportunity for the rams to grow.

- There was no effect of silage diet on the LW, but there was an effect of supplementation with rapeseed meal on mean LW overall periods. The group supplemented with rapeseed meal lost more in LW during 80 % of *ad libitum* feeding compared to the group not supplemented.

Implications

The most suitable forage, according to this study, for formulation of optimal diets to ruminants is grass, as the intake, digestibility and protein utilization was highest when feeding grass silage. Red clover showed to be good to balance the protein concentration while WCMS can give a higher energy concentration in the diet for growth and production. Red clover and WCMS are preferable to combine because of the high protein content in red clover and high energy content in WCMS. By analysing the nutrient contents in forage, a balanced diet can be set up by combining the forages and supplementing with concentrate. The supplementation of protein is important to get a higher LW during a shorter period, a higher intake and more easily absorbed protein by the ruminant, but could also give higher losses of N in urine, affecting the environment negatively or has no beneficial effects on growth and production. By feeding the diet with balanced nutrient content, the emissions of N can be decreased and more of the protein can be used by the ruminants for their production potential.

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