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Skara 2017

Animal Science

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Nr. 705

Student report
Swedish University of Agricultural Sciences
Department of Animal Environment and Health

No. 705

ISSN 1652-280X

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Advanced E, 30 ECTS, Animal Science, Degree Project in Animal Sceience EX0566

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Nyckelord: evaluation, forage, nitrogen, wether

Serie: Studentarbete/Sveriges lantbruksuniversitet, Institutionen för husdjurens miljö och hälsa, nr. 705, ISSN 1652-280X

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Förord

Examensarbetet omfattar 30 hp och ingår i agronomprogrammet med inriktning husdjur. Arbetet är gjort som en del i det större projektet ”Helsädesensilage och gräsensilage till får skördade vid olika mognadsstadier – effekt på konsumtion, foderselektion och foderutnyttjande”. Projektet pågick 2013 - 2014 och finansierades av Stiftelsen Svensk Fårforskning, Agroväst Nöt- och lammköttsprogram, och Fåreafgiftsfonden, Dansk Fåravl. Min del i projektet var att undersöka gräsensilage och helsädesensilage av korn skördade vid två olika mognadsstadier med avseende på konsumtion, smältbarhet och proteinutnyttjande hos baggar. Den praktiska delen av arbetet bestod i att utfodra djuren, samla in foder-, urin-, och träckprover och att bearbeta data från analyser av de insamlade proven.

Jag vill rikta mitt tack till handledaren Elisabet Nadeau för engagemang och vägledning i arbetet men framför allt tålamod och stöttning. Tack till Karin Wallin och Jonas Dahl för hjälp med provtagning av foder och baggar. Annika Arnesson som även är biträdande handledare skall ha tack för hjälpen med arbetet med prover och analyser samt sammanställning av data. Slutligen vill jag tacka Susanna Hedlund för gott samarbete med provtagningar och registreringar.

Sammanfattning

I Sverige används gräsensilage och helsädesensilage från bland annat korn som vinterfoder i köttproduktion av både nötkreatur och lamm. Korn kan sås som skyddsgröda när vallar skall anläggas och att skörda kornet som helsädsensilage istället för vid tröskmognad får vallen mer ljus samtidigt som det blir vallskörd redan det första året. Både gräsensilage och helsädesensilage av korn kan skördas vid olika mognadstadier för att möta olika behov hos idisslare. Tidig skörd ger hög smältbarhet medan senare skörd ger högre avkastning av torrsubstans.

Syftet med studien var att ta fram fodervärderingsegenskaper genom att undersöka foderintag, sortering, *in vivo* smältbarhet och proteinutnyttjande av kornhelsädesensilage och gräsensilage skördat vid olika skördetidpunkter och utfodrat med eller utan tillskott av protein till kastrerade baggar. *In vivo* smältbarhetsvärden kan överföras och användas vid foderstatsberäkningar även för andra idisslare. I studien användes två gräsensilage skördade tidigt (31 maj) och sent (17 juni) i första skörd och två ensilage av kornhelsäd skördade vid axgång (30 juni) repsektive mjölkmognad (18 juli). Åtta kastrerade baggar delades in i en duplicerad 4x4 romersk kvadrat med fyra baggar och fyra perioder i varje kvadrat. Rapsmjöl gavs som proteintillskott till den ena gruppen. Efter fyra perioder hade alla baggar utfodrats med alla fyra olika ensilagen. Under försöket registrerades kontinuerligt levande vikt, hull och foderintag. Under de fyra sista dagarna i varje period samlades prover in av foder, foderrester, träck och urin. Ensilagens näringsinnehåll och hygieniska kvalitet, näringsinnehåll i träcken och innehåll av kväveföreningar i urinen analyserades med PROC MIXED of SAS (ver. 9.3).

Konsumtion av torrsubstans (ts) i % av levandevikten var högst för det tidigt skördade kornhelsäden ($P < 0,001$). Det tidigt skördade gräsensilaget hade det högsta intaget av råprotein men det lägsta intaget av neutral detergent fibre (NDF : $P < 0,001$). Det fanns inga skillnader mellan konsumtion av ts mellan det tidiga och det sena gräsensilaget vilket visar att innehållet av NDF i ensilaget inte påverkade konsumtionen av ts hos hamlar med underhållsbehov. Sortering för foderdelar med lägre NDF innehåll förekom för alla foder utom det tidigt skördade gräsensilaget ($P < 0,001$). Foderstaten med det tidigt skördade gräsensilaget hade högst smältbarhet av organisk substans, råprotein och fiber ($P < 0,001$). *In vivo* smältbarhet av organisk substans, råprotein och fiber minskade vid senare mognadsstadier för både helsädesensilage och gräsensilage ($P < 0,001$).

Det högsta råproteinintaget samt den högsta utsöndringen av totalkväve och ureakväve noterades för foderstaten med det tidigt skördade gräsensilaget ($P < 0,001$). Proteinupptaget uttryckt i % av kväveintaget tenderade att bli högre för hamlar som utfodrades med helsädesensilage jämfört med hamlar som utfodrades med det tidigt skörade gräsensilaget men det var ingen skillnad jämfört med det sent skördade gräsensilaget ($P = 0,071$). När rapsmjöl tillsattes foderstaten ökade utsöndringen av kväve i urinen och proteinupptaget uttryckt i % av kväveintaget minskade i genomsnitt över ensilagen ($P < 0,05$).

Foderintag, *in vivo* smältbarhet och proteinutnyttjande påverkas av fysiska och näringsmässiga egenskaper hos både gräsensilage och helsädesensilage skördade vid olika mognadsstadier. Genom att kombinera grovfoder från olika mognadsstadier med rätt nivå av kraftfoder i foderstaten så kan proteinutnyttjandet öka vilket är till fördel för den omgivande miljön.

Summary

In Sweden grass silage and whole-crop silage from barley is used for winter feeding in meat production from cattle and lamb. Barley can be used as nurse crop when establishing leys and harvesting as whole-crop silage instead of harvesting as grain gives sunlight to the undersown ley, which even can be harvested in the establishing year. Both grass silage and whole-crop silage could be harvested at different maturity stages to meet different nutritional demands of the ruminant animals. Early harvest gives high digestibility while harvesting at later stages of maturity give high dry matter (DM) yields.

The aim of this study was to evaluate feed intake, feed sorting, *in vivo* digestibility and utilisation of protein of whole crop barley silage (WCBS) and grass silage (GS) harvested at different maturity stages and fed with or without protein supplementation to wethers. The *in vivo* digestibility values could be transferred and be used for ration calculation for other ruminants. Two GS, harvested early (31st of May) and late (17th of June) in spring growth, and two WCBS, harvested at the heading (30th of June) and at milk stage (18th of July) of maturity, were used in the trial. Eight wethers were divided into a duplicated 4x4 latin square design with four wethers and four periods in each square. Rapeseed meal was fed as a protein supplement in one of the groups. After four periods all wethers had been fed all four silages. Live weight (LW), body condition scores (BCS) and feed intake was registered continuously during the experiment. Samples of feed, refusals, faeces and urine were collected during the last four days in each period. Nutritional and hygienic quality of the silages, chemical composition of faeces and nitrogen compounds in the urine were analysed using PROC MIXED of SAS (ver. 9.3).

Intake of DM in % of LW was highest for the WCBS at heading stage ($P < 0.001$). Early harvested GS had the highest crude protein intake but the lowest intake of neutral detergent fibre ($P < 0.001$). Lack of differences in DM intake between early harvested grass silage (GE) and late harvested grass silage (GL) show that the NDF content of the grass silage did not affect the DM intake of the wethers, which only had maintenance requirements. Sorting for parts with less fibre content appeared for all diets except the early harvested GS diet ($P < 0.001$). The early harvested grass silage diet had the highest digestibility of organic matter, crude protein and fibre ($P < 0.001$). *In vivo* digestibility of organic matter, crude protein and fibre decreased with later maturity stage of whole-crop barley and grass ($P < 0.001$).

The highest CP intake as well as the highest excretion of total-N and urea-N in urine were recorded for the GE diet ($P < 0.001$). Nitrogen retention expressed as % of N intake tended to be higher in wethers fed whole-crop barley silage diets compared to wethers fed the GE diet but did not differ from the GL diet ($P = 0.071$). When rapeseed meal was supplemented to the diets excretion of N increased and retention of N in % of N intake decreased, when averaged over silages ($P < 0.05$).

Feed intake, in vivo digestibility and protein utilistation will be affected of physical and nutritional traits for both GS and WCBS harvested at different stages of maturity. By combining forages from different stages of maturity with correct amounts of concentrate in the ration, the use of N can be more efficient, which is beneficial for the surrounding environment.

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Introduction

In colder climates, such as in Sweden, meat production from both lambs and cattle are dependent on stored forages. Important forages are grass silage from leys and whole crop silages from small grains. When establishing leys, cereals are commonly used as nurse crops and harvesting the nurse crop as whole-crop cereals will give more sunlight to the sward canopy, which enhances its establishment and growth in the establishment year (Rustas, 2009). Both grass silage (GS) and whole-crop cereal silage (WCCS) can be harvested at different stages of maturity, where early harvest is associated with high digestibility and harvesting at later stages of maturity is associated with higher dry matter (DM) yields.

Whole-crop barley silage (WCBS) and GS are important feed stuff for ruminants in Sweden. The efficiency of feed is a combination of protein and energy concentration but it also needs to contain enough amounts of structural fibre to provide a healthy environment for the rumen microbes (Nadeau *et al*., 2016). To calculate feed rations in Sweden today, advisors use the NorFor system. In the NorFor system consideration is taken to total fibre, indigestible fibre, amino acids absorbed in small intestine (AAT) and net energy (NE; Volden, 2011).

Content of nitrogen (N) in the diet and its digestibility are the main factors affecting excretion of N in urine and faeces (Carro *et al*., 2012). Excreted N is a loss that to a great extent could have been used by the animal and minimizing excretion of nutrients is an environmental benefit (Collins *et al*., 2003). In nutrition research, excretion of purine derivatives (PD) in urine can be used as an indicator of microbial protein synthesis in rumen (Chen *et al*., 2004). If N intake and utilisation are controlled, N can be more efficiently used and losses of ammonia from ruminants can decrease (Nadeau *et al.,* 2007).

Wethers can be used as models for measurements of feed digestibility for other ruminants, such as beef and dairy cattle. There are advantages of using small animals, such as sheep, for *in vivo* digestibility trials because they easily can be kept in cages. Using sheep for measurements of *in vivo* feed digestibility values is a well accepted reference method, which is used and has been used for a long time (EAAP, 1969).

Objectives

The aim of this study was to examine feed intake, feed sorting, *in vivo* digestibility and protein utilisation of whole-crop barley silage and grass silage fed to wethers with or without protein supplementation.

Hypotheses

- Feed intake will differ among the forages. Early harvested grass silage and whole crop barley silage harvested at heading stage will give the highest intake.

- *In vivo* digestibility will differ among forages. Grass silage harvested at an early maturity stage early in the season will result in a higher digestibility than forages harvested at later stages of maturity whereas the digestibility of whole crop barley silage will decrease less from heading stage to later maturity stages because of development of a digestible ear.

- Supplementation of protein to the feed will give higher roughage intake and *in vivo* feed digestibility.

- Protein utilisation, regardless of protein supplementation, will differ among forages. Compared to forages harvested at later stages of maturity, early harvested grass and whole crop barley silage harvested at heading will give the highest utilisation of protein.

- Live weight gain of the wethers will change with forage type and protein supplementation.

Literature review

Quality of whole-crop barley and grass harvested at different maturities

Feed value of grass silage is dependent on its nutritive value in combination with how much of the offered silage the animals eat. Digestibility of the silage is what determines intake potential the most (Keady *et al*., 2013).

Whole-crop barley

Whole-crop barley can be harvested at different stages of maturity to meet different production demands by ruminants. Early harvest is associated with high digestibility (Rustas, 2009) while harvesting at later stages of maturity increases dry matter (DM) yield (Nadeau, 2007) and dry matter intake (DMI) (Rustas *et al*., 2010). High digestibility at early harvest depends on high digestibility of neutral detergent fibre (NDF) and high concentration of water soluble carbohydrates (WSC: Rustas *et al*., 2011). Concentration of NDF in cereals increases during stem elongation stage but then it levels off. As the heads fill, NDF concentration decreases as the head:leaf and head:stem ratios increase. With increased maturity, stems and leaves of the forages contain more fibre, which decreases in digestibility with advancing maturity (Khorasani *et al*., 1997). Simultaneously, the NDF content of whole-crop barley decreases while starch content increases as sugar is converted into starch (Nadeau, 2007; Rustas *et al*., 2008). In a study with dairy steers, Rustas *et al.* (2008) used whole-crop barley silage (WCBS) that was harvested at three different stages of maturity; at heading, at milk stage and at dough stage. The NDF content of WCBS decreased by 116 g/kg DM from the heading stage to dough stage of maturity. Content of crude protein (CP) decreased by 14 g/kg DM from the heading stage to the dough stage of maturity (Table 1). The content of starch increased by 137 g/kg DM form heading stage to dough stage of maturity. Intake of DM for the dairy steers increased with increasing stage of maturity and there was a tendency to differences in live weight (LW) gain. The feed conversion ratio (FCR) was 3.7 kg DM /kg LW gain for the silage that was harvested at heading which made it superior to the other two maturity stages (at milk stage FCR was 4.4 and at dough stage 4.3 kg DM/kg LWG: Rustas *et al*., 2008). Wallsten *et al* (2009) also used WCBS harvested at three different stages of maturity; heading, early milk stage and early dough stage. In that study the content of NDF had the lowest level at early milk stage and then increased again to early dough stage (Table 1). The content of CP decreased and concentration of starch, that was only analyzed at early milk and early dough stage, increased (Wallsten *et al*., 2009).

Table 1. Chemical composition of whole-crop barley silage (g/kg DM).

Reference	Harvest date	NDF[1]	Starch	Sugar	Crude protein
Rustas *et al* (2008)					
Heading	June 26	547	40	5	90
milk stage	July 7	508	24	46	82
dough stage	July 21	431	177	10	76
Wallsten *et al* (2009)					
Heading	July 17	477	-	-	126
early milk stage	July 30	396	65	-	110
early dough stage	August 7	430	156	-	96

[1]NDF=neutral detergent fibre

Khorasani *et al* (1997) compared barley, oats, triticale and a barley/triticale mixture as fresh crops at the soft dough stage. In that study concentrations of NDF and acid detergent fibre (ADF) in barley were 499 g/kg DM and 267 g/kg DM, respectively. Concentration of NDF in oat was 547 g/kg DM and concentration of ADF was 341 g/kg DM. The NDF and ADF concentrations for triticale were intermediate (Khorasani *et al*., 1997). Nadeau (2007) compared WCCS from barley, triticale, oats and spring wheat at early milk and early dough stage of maturity. In that study barley had lower NDF concentrations at both maturity stages than spring wheat and oats but higher than triticale. Contents of starch were the same for barley and triticale (38 g/kg DM) at early milk stage whereas oats had higher starch concentration (52 g/kg DM) and spring wheat had lower (27 g/kg DM). When the WCCS were harvested at dough stage, barley had higher starch concentrations (238 g/kg DM) than the other cereals (Nadeau, 2007). When *in vitro* organic matter (OM) digestibility was determined using the enzymatic method, there were no differences between the maturity stages for barley, oats and wheat while triticale had an *in vitro* OM digestibility of 76 % at the early milk stage and 82 % at the early dough stage of maturity. *In vitro* digestibility of OM was highest for barley (59 %) while triticale (58 %) and oats (51 %) had the lowest *in vitro* OM digestibility when the method using rumen fluid was used (VOS; Nadeau, 2007). The almost unchanged OM digestibility at later stages of maturity is associated with higher concentration of starch (with high digestibility) and lower digestibility of NDF (Nadeau, 2007).

Grass

Maturity of the crop at harvest is one of the most important factors that affects nutritive value of grass silage (Bernes *et al*., 2008). As the grass matures the NDF content increases and the CP content decreases. Rinne *et al*. (2002) used four cuttings of grass harvested one week after each other, harvested from the 13th of June to the 4th of July in the first harvest. The contents of NDF and ADF increased while the CP content decreased (Table 2) with later harvest dates (Rinne *et al*., 2002). Särkijärvi *et al* (2012) used a mixed sward of timothy and meadow fescue harvested at three different stages of maturity and harvested with one week in between in the first harvest. Earliest harvest date was the 19th of June and the third and last harvest date was the 3rd of July. In that study the NDF content increased by 52 g/kg DM and content of CP decreased by 39 g/kg DM (Table 2) from the early to the late harvest date (Särkijärvi *et al*., 2012). Nadeau *et al*. (2015) used grass silages harvested at three different maturity stages in the first harvest. At the early harvest, contents of NDF and ADF increased while contents of CP, WSC and metabolisable energy (ME) decreased to the late harvest date (Table 2; Nadeau *et al.,* 2015). Bernes *et al*. (2008) showed the same results when grass was harvested for silage at three different stages of maturity in the first harvest. The NDF content increased while CP, WSC and ME decreased (Table 2) at later stages of maturity (Bernes *et al*., 2008).

Table 2. Chemical composition of grass silage at different harvest dates.

Reference	Harvest date	NDF[1]	ADF[2]	CP[3]	WSC[4]	ME[5]
Rinne *et al* (2002)						
harvest 1	June 13	486	280	172	31	-
harvest 2	June 21	541	298	146	24	-
harvest 3	June 28	641	359	134	20	-
harvest 4	July 4	645	368	113	23	-
Särkijärvi *et al* (2012)						
early harvest	June 19	578	-	155	96	-
medium harvest	June 26	597	-	136	82	-
late harvest	July 3	630	-	110	101	-
Nadeau *et al* (2015)						
early harvest	June 2	472	267	177	202	11.7
medium harvest	June 12	620	357	119	139	10.8
late harvest	June 21	665	383	92	117	9.3
Bernes *et al* (2008)						
early harvest	June 16	470	-	172	38	11.9
medium harvest	June 20	527	-	140	17	11.4
late harvest	June 26	587	-	117	5	10.6

[1]NDF=neutral detergent fibre (g/kg DM)
[2]ADF=acid detergent fibre (g/kg DM)
[3]CP=crude protein (g/kg DM)
[4]WSC=water soluble carbohydrates (g/kg DM)
[5]ME=metabolisable energy (MJ/kg DM)

Feed intake

In trials that intend to measure intake, feed is offered *ad libitum* but the definition of *ad libitum* feeding differs among researchers. It usually ranges between 0 and 30 % of refused feed, the range 5- 15 % of refusals is the most common (Mertens, 1994). If greater refusals are allowed it increases the opportunity for selection and intake can be overestimated (Mertens, 1994). Animal performance is reliant on feed intake but feed intake is also a weak point when feed rations are made because we do not know the feed intake when doing the ration formulations (Huthanen *et al*., 2007). Feed intake depends on many different factors that often are divided in three main areas: animal, feed and management (Mertens, 1994). When rations are made for a specific farm animal where the management factors are rather consistent then properties from the feed is the most important factor affecting feed intake (Huthanen *et al*., 2007). Feed can further be divided into three smaller areas: nutrient availability, physical properties and palatability attributes (Mertens, 1994).

Whole-crop barley silage

When Rustas *et al*. (2010) fed WCBS, harvested at two different stages of maturity (heading and mid-dough stage), to dairy steers, no differences in intakes of DM and NDF were found both expressed as kg/day or in % of LW. There were no differences in intake of OM either (Table 3). Wallsten *et al.* (2009b) fed WCBS harvested at three different stages of maturity (heading, early milk-stage and early dough-stage) to dairy heifers. In that trial intakes of DM, OM and NDF were higher at heading but did not differ between early milk stage and early dough stage (Wallsten *et al*., 2009b).

Rustas *et al.* (2011) fed two different WCBS and one whole-crop wheat silage (WCWS), harvested at three different stages of maturity, to diary heifers. Intake of DM for the WCBS harvested in Skara was higher at dough-stage than at milk-stage, when expressed in % of LW. The WCBS harvested in Uppsala showed no differences in intake of DM at the different

maturity stages. For the WCWS, the DM intake was lower for the silage harvested at heading than at the other maturity stages (Table 3). Intake of NDF for the WCBS harvested in Skara was lower at milk-stage than at heading, both expressed as kg/day and in % of LW. For the WCBS harvested in Uppsala and the WCWS there no differences in NDF intake of between the different maturity stages, both expressed as kg/day or in % of LW (Table 3; Rustas *et al*., 2011).

Table 3. Intake of whole-crop cereal silages harvested at different stages of maturity.

Reference	Harvest date	DM[1]		NDF[2]		OM[3]
		kg/day	% of LW	kg/day	% of LW	
Rustas *et al* (2010) whole-crop barley						
heading	June 27	7.5	-	3.8	1.03	7.1
mid-dough stage	July 18	7.9	-	3.7	1.00	7.4
Rustas *et al* (2011) *barley Skara*						
heading	June 26	7.1^{ab}	2.04^{a}	3.8^{a}	1.10^{a}	-
milk-stage	July 7	6.2^{b}	1.78^{b}	3.4^{b}	0.96^{b}	-
dough-stage	July 21	7.3^{a}	2.09^{a}	3.5^{ab}	1.02^{ab}	-
barley Uppsala						
heading	June 28	6.5	1.91	3.6	1.04	-
milk-stage	July 17	6.6	1.92	3.3	0.97	-
dough-stage	July 24	6.6	1.93	3.5	1.02	-
wheat Uppsala						
heading	June 26	6.1^{b}	1.81^{b}	3.2	0.95	-
milk-stage	July 15	7.2^{a}	2.09^{a}	3.4	1.00	-
dough-stage	July 26	7.7^{a}	2.31^{a}	3.6	1.06	-

$^{a-c}$Means within a feed and column differ significantly ($P < 0.05$).
[1]DM = dry matter
[2]NDF = neutral detergent fibre
[3]OM = organic matter

Grass silage

It has been shown that improved silage digestibility increases silage dry matter intake (SDMI) (Huthanen *et al*., 2002). Increased DM concentration of the forage induces higher intake with a maximum intake at the DM concentration at 500g/kg (Huthanen *et al*., 2007). Rinne *et al*. (2002) were offering grass silage harvested at four different stages of maturity to four ruminally cannulated dairy cows. Intake of DM for the cows decreased with later harvest date, whereas intake of NDF increased with later harvest date (Table 4; Rinne *et al*., 2002).

Jalali *et al*. (2012) fed GS to pregnant ewes when the silages were not supplemented with concentrate. Nadeau *et al*. (2015) used the same ewes and the same silages but that trial was later in pregnancy and in early lactation, when the silages were supplemented with 0.8 kg of concentrate per day. Intake of DM in the trial by Jalali *et al.* (2012) was highest at late heading stage and lowest at early heading, whereas the SDMI later in pregnancy and in lactation was highest for the first harvest date at the leaf stage of maturity (Nadeau *et al.* 2015; Table 4). Jalali *et al*. (2012) reported that intake of NDF in % of LW increased at later stages of maturity. In late pregnancy intake of NDF in % of LW did not differ between maturity stages whereas NDF intake was highest at the early heading stage during lactation (Table 4: Nadeau *et al*., 2015) There is a negative correlation between DMI and NDF concentration of the feed. As NDF concentration increases with later harvest also the

degradability of NDF and OM decline (Bernes *et al*., 2008) When grasses mature the percentage of lignin in the stem increases, which is an important reason to the declining degradability (Südekum *et al*., 1995). Intake of DM is restricted if NDF (fiber) content is high, due to the physical capacities of the ruminants (Mertens, 1987).

Table 4. Intake of grass silage harvested at different stages of maturity.

Reference	Harvest date	DM[1]	NDF[2]		ME[3] (MJ/day)
Rinne *et al* (2002)			kg/day	% of LW	
cows					
harvest 1	June 13	12.5	7.8	-	-
harvest 2	June 21	12.6	8.6	-	-
harvest 3	June 28	11.1	8.9	-	-
harvest 4	July 4	11.1	8.9	-	-
Jalali *et al* (2012)					
pregnant ewes					
leaf stage	June 2	2.4[b]		1.13[c]	28[a]
early heading stage	June 12	2.0[c]		1.27[b]	22[b]
late heading stage	June 21	2.7[a]		1.33[a]	16[c]
Nadeau *et al* (2015)					
ewes in late pregnancy					
leaf stage	June 2	2.5[a]		1.15	38.4[a]
early heading stage	June 12	1.9[b]		1.14	30.2[b]
late heading stage	June 21	1.5[b]		1.11	23.2[c]
ewes in early lactation					
leaf stage	June 2	3.5[a]		1.72[b]	51.2[a]
early heading stage	June 12	2.5[b]		2.02[a]	36.4[b]
late heading stage	June 21	2.2[b]		1.72[b]	30.2[c]

[a-c]Means within a feed and column differ significantly ($P < 0.05$).
[1]DM = dry matter
[2]NDF = neutral detergent fibre
[3]ME = metabolisable energy

In the trial by Rinne *et al*. (2002) when GS, harvested at four stages of maturity, was fed to dairy cows, intake of ADF increased with delayed harvest.

Sorting

When measuring intake, the feed needs to be offered *ad libitum*, which makes it possible for the animals to eat as much as they want to. As animals eat forage selectively measuring intake at *ad libitum* feeding is complicated as increasing availability of feed can increase both intake and digestibility of the feed consumed (Mertens, 1994). At restricted feeding, animals eat all that is offered and digestibility reflects forage composition. When availability increases the animal can select for more desirable parts that often are low in fibre and composition of the eaten forage is not the same as composition of the offered forage (Mertens, 2007).

Bernes *et al*. (2008) showed that growing lambs sorted for the most nutritious parts of GS and against NDF content. In that study, GS from three different harvest dates was offered to growing lambs, and it was shown that the lambs sorted even if the silage was early harvested. These conclusions were based on the fact that refusals from each silage contained higher proportion of NDF and lower proportion of ME and CP than did the fed forage (Bernes *et al*., 2008). Nadeau *et al*. (2015) had similar results as Bernes *et al*. (2008) but in a trial with

pregnant and lactating ewes. In the trial by Nadeau *et al* (2015) grass was harvested for silage at different stages of maturity to get three different feed values (high, medium and low feed value). In late pregnancy, sorting occurred when the ewes were fed the medium feed value silage, pointed out by significantly higher NDF concentrations in the refusals than in the offered silage. There was also a tendency to lower concentrations of CP and ME in the refusals from the medium feed value silage. In lactation, sorting occurred when the ewes were fed low feed value silage, pointed out by significantly lower ME concentrations and a tendency to lower CP concentrations in the refusals than in the offered silage (Nadeau *et al.,* 2015).

Digestibility

When DM digestibility of feed is determined *in vivo,* consumed amounts of DM and DM in faeces are measured and the proportion of DM that has disappeared is calculated. This is called apparent DM digestibility because endogenous faecal losses (EFL) have not been taken into account. To get the true digestibility EFL need to be removed from faecal DM, as faeces contains EFL, true digestibility will be lower than apparent digestibility (Mertens, 2007).
In a trial with wethers offered different hays, Cherney *et al*. (1990) showed that *in vitro* DM digestibility was correlated with *in vivo* DM digestibility. The correlation was stronger when the wethers were fed 1.8 % of their body weight (BW) than when feeding level was at *ad libitum* with up to 10 % refusals. When feeding at 1.8 % of BW the feeding level is close to restricted and the ability for the animals to select for more digestible parts of the feed is decreased (Cherney *et al.*, 1990).

Whole crop barley

Digestibility of WCBS and WCWS was analyzed when used in a trial with dairy heifers. The WCCS were harvested at three different stages of maturity; at heading, at milk stage and at dough stage (Rustas *et al*. 2011). *In vivo* digestibility of DM for WCBS and WCWS was highest at heading and did not differ between milk-stage and dough-stage of maturity of (Table 5). I*n vivo* digestibility of OM was highest at heading for all three silages and there were no differences in *in vivo* OM digestibility between milk-stage and dough stage of maturity (Table 5: Rustas *et al*. 2011). Nadeau (2007) had the same findings when *in vitro* OM digestibility from WCBS, harvested at early milk stage and early dough stage was compared.

The *in vivo* digestibility of NDF was highest at heading for all three silages. In WCWS the *in vivo* digestibility of NDF did not differ from milk stage to dough stage. WCBS harvested in Skara had the lowest *in vivo* NDF digestibility at dough stage and the WCBS harvested in Uppsala had the lowest *in vivo* NDF digestibility at the milk stage of maturity (Table 5). I*n vivo* digestibility of ADF was highest at heading for all three silages. For WCBS harvested in Uppsala *in vivo* digestibility of ADF at milk stage and dough stage did not differ. For the WCBS that was harvested in Skara the *in vivo* digestibility of ADF decreased further from milk stage to dough stage of maturity. *In vivo* digestibility of ADF of the WCWS at milk stage and dough stage of maturity did not differ (Table 5; Rustas *et al*., 2011). One reason for lower digestibility of ADF than digestibility of NDF is that ADF contains higher proportion of lignin than NDF does. The difference between digestibility of ADF and NDF increases with increasing maturity (Wallsten *et al*., 2010).

For WCBS harvested in Uppsala and for the WCWS, the *in vivo* digestibility of CP was highest at heading. For the WCBS harvested in Skara *in vivo* digestibility of CP did not differ between heading and milk stage, at dough stage the digestibility was lower. For WCBS harvested in Uppsala and the WCWS, the *in vivo* digestibility of CP did not differ between milk stage and dough stage (Table 5).

Table 5. Digestibility of whole-crop cereal silages at different stages of maturity (%).

Reference	Harvest date	DM[1]	OM[2]	CP[3]	NDF[4]	ADF[5]
Rustas *et al* (2011)						
barley Skara						
Heading	June 26	67.8[a]	69.7[a]	64.3[a]	68.8[a]	67.7[a]
milk stage	July 7	62.8[b]	65.0[b]	63.2[a]	60.7[b]	59.1[b]
dough stage	July 21	60.7[b]	62.7[b]	57.5[b]	52.9[c]	51.3[c]
barley Uppsala						
Heading	June 28	65.8[a]	68.2[a]	61.9[a]	65.6[a]	64.8[a]
milk stage	July 17	59.4[b]	61.7[b]	56.1[b]	51.5[c]	50.2[b]
dough stage	July 24	58.3[b]	60.4[b]	55.1[b]	55.4[b]	53.1[b]
wheat Uppsala						
Heading	June 26	67.4[a]	70.4[a]	75.5[a]	69.3[a]	66.8[a]
milk stage	July 15	57.8[b]	60.5[b]	63.7[b]	49.1[b]	48.9[b]
dough stage	July 26	60.6[b]	63.3[b]	63.3[b]	49.8[b]	49.0[b]

[a-c]Means within a feed and column differ significantly ($P < 0.05$).
[1]DM = dry matter
[2]OM= organic matter
[3]CP = crude protein
[4]NDF = neutral detergent fibre
[5]ADF = acid detergent fibre

Digestibility of starch was analysed at milk and dough stage of maturity, which for barley was 96.1 % at milk stage and 86.3% at dough stage of maturity. Starch digestibility for wheat silage was 97.7 % at milk stage and 99.1% at dough stage of maturity (Rustas *et al*., 2011).

Grass

In a study with GS harvested at two different stages of maturity *in vivo* digestibility of OM decreased from the earliest harvest date to the last (Kuoppala *et al*., 2009). Bernes *et al*. (2008) used GS from timothy harvested at three different stages of maturity. In that trial *in vitro* digestibility decreased with later harvest dates (Table 6). Kuoppala *et al*., (2009) found that *in vivo* digestibility of NDF was higher at early harvest compared to harvesting at the later stage of maturity. With later harvest dates *in vitro* degradability of both OM and NDF decreased (Table 6; Bernes *et al*., 2008).

Digestibility of protein is affected by how well and fast the microbes can break it down, where physical and chemical structures play a large roll (McDonald *et al*., 2002)
In vivo digestibility of CP decreased with later harvest dates (Table 6; Kuoppala *et al*., 2009). Rinne *et al*. (2002) showed similar results with GS harvested at four different stages of maturity, when digestibility of CP decreased from 74.1 % in the early harvested GS to 66.2 % in the late harvested silage. Digestibility of ADF showed the same pattern as the other components and decreased with harvest at later stages of maturity (Rinne *et al*., 2002).

Table 6. Digestibility of grass silage at different stages of maturity (%).

Reference	Harvest date	OM[1]	CP[2]	NDF[3]
Kuoppala *et al* (2009)				
Early	June 17	75.0[a]	68.1[a]	62.1[a]
Late	June 26	71.9[b]	66.8[b]	58.0[b]
Bernes *et al* (2008)				
Early	June 16	94.6[a]		89.4[a]
Medium	June 20	91.3[b]		85.2[b]
Late	June 26	85.8[c]		77.8[c]

[a-c] = means within a row differ significantly ($P < 0.05$)
[1]OM= organic matter
[2]CP = crude protein
[3]NDF = neutral detergent fibre

Protein

Utilisation

Protein from feed is broken down by proteolytic bacteria in rumen, where it is degraded to peptides or amino acids. Peptides are further degraded to amino acids that can be used directly in microbial synthesis or it is further altered to organic acids or ammonia (Sjaastad *et al.*, 2010). Low levels of protein in the diet will result in slow growth of microorganisms in rumen and, consequently, breakdown of carbohydrates will be weak. An efficient protein utilisation is dependent on sufficient energy supply to the rumen microbes and a synchronization of metabolism of proteins and carbohydrates (McDonald *et al*., 2002). Efficiency of nitrogen (N) utilisation is negatively correlated with concentration of dietary CP (Nadeau *et al*., 2007). When degradation of protein is more rapid than synthesis of microbial protein there will be an excess of ammonia in rumen (McDonald *et al*., 2002). When feeding GS harvested at later stages of maturity excess ammonia in rumen decreases (Rinne *et al*., 1997). Excess ammonia will be transported by the blood to the liver and converted to urea. From the liver some of the urea is recycled and can be used by the microorganisms in rumen but a large part of it will be excreted in the urine (McDonald *et al*., 2002).

In a trial with young dairy cattle, Rinne *et al.* (1997) used four GS from a mixture of timothy and meadow fescue harvested at different stages maturity from prebloom (29th of May) to late bloom stage (25th of June). Intake of N decreased at later stages of maturity but the amount of N that passed by the rumen to the small intestine showed no significant differences among maturity stages even if the trend was a decline with later maturity stages. That trial indicated that when GS is harvested at early maturity stages the use of N in the ruminant is inefficient (Rinne *et al*., 1997).

When Browne *et al*. (2005) fed GS to dairy steers, retention of N was 32.3 g/day or 18.8 % of N intake. Retention of N was higher when maize silage was included in the diet which indicate that use of N is more effective in the presence of easily fermentable carbohydrates (Browne *et al*., 2005). Ahvenjärvi *et al*. (2006) included WCBS to the diet of dairy cows. Four levels of inclusion were used from only GS to 60 % inclusion of WCBS. While WCBS had lower concentration of N in the feed, higher inclusion of WCBS led to decreased levels of N intake (Ahvenjärvi *et al*., 2006). Owens *et al*. (2009) found a higher intake of N when WCWS was fed instead of GS to dairy steers. WCWS had a higher N flow and more efficient microbial N synthesis than the GS (Owens *et al*., 2009). However, Ahvenjärvi *et al*. (2006) reported no increase in microbial N synthesis with increasing levels of WCBS inclusion in the diet.

Proportion of nitrogen in urine compared to protein intake

Contents of N in the feed and N digestibility is what determines excretion of N in faeces and in urine the most (Carro *et al*., 2012). Ahvenjärvi *et al*. (2006) reported decreased levels of ammonia-N in rumen and decreased amounts of N excreted in urine but N excreted in faeces did not differ between treatments, when inclusion of WCBS increased in the GS based diet.

Urea is the dominant form of N content in urine. It has been reported to range from 25 % to 90 % depending on type of diet. Other present N compounds are allantoin, hippuric acid, creatinine and uric acid (Bristow *et al*., 1992).

Excretion of purine derivatives (allantoin and uric acid) in urine

Excretion of purine derivatives (PD) in urine could be used to determine flow of microbial N to duodenum (Carro *et al*., 2012). Purines that originate from the rumen microbes become metabolised and the PD are excreted in the urine (Chen *et al*., 2004). Allantoin and uric acid are the most common PD that are excreted in urine from ruminants (Carro *et al*., 2012) and the only ones excreted by cattle. In sheep, also xanthine and hypoxanthine are excreted in the urine (Chen *et al*., 2004). Xanthine oxidase converts hypoxanthine and xanthine to uric acid in ruminants. Uricase converts uric acid to the end-product allantoin (Chen *et al*. 2004)

In a feeding trial by Carro *et al*. (2012) the amount of PD in urine from sheep was estimated. Of the total PD in urine, allantoin represented between 73.4 and 73.8 %. Hippuric acid is a conjugate compound of the amino acid glycine with benzoic acid. Of total N in urine hippuric acid ranges from 2.6 % to 7.7 % (Bristow *et al*., 1992).

Puchala *et al*. (1992) fed diets differing in protein (0.5 of maintenance, maintenance and 2 times maintenance) and energy (0.5 of maintenance, maintenance and 2 times maintenance) to ewes and found the highest excretion of allantoin and uric acid when the ewes were fed the diet that was twice maintenance for both protein and energy. When high protein and energy was fed the excretion of allantoin was 0.83 g N/day and excretion of uric acid was 0.16 g N/day. When low protein and energy diet was fed the excretion of allantoin was 0.17 g N/day and excretion of uric acid was 0.06 g N/day (Puchala *et al*., 1992).

Creatinine is the cyclic anhydride of creatine, which is a product of metabolism of the amino acids glycine, arginine and methionine. Before excreted in urine creatine is partly converted to creatinine. In general concentrations of creatinine is higher than concentration of creatine in urine (Bristow *et al*., 1992). Increasing levels of protein in the diet increases excretion of creatinine in urine but if intake of energy increases (from maintenance to twice maintenance) creatinine excretion in urine stays rather constant. This shows that increasing energy in the diet do not have any effect on creatinine excretion in the urine. Excreted creatinine in sheep urine is unstable at normal pH (around 8.5) and temperature from 15°C and 39°C. Losses are prevented by lowering pH to less than 6 and storing of the urine samples at temperature at 4°C (Niekerk *et al*., 1963).

Materials and methods

The trial was conducted at the Götala Beef and Lamb Research Centre, SLU Skara, Sweden. Duration of the trial was from 24th of January to the 16th of May 2014. Experimental procedures were approved by the Research Animal Ethics Committee.

Animals and housing

Eight 20-month old cross bred castrated wethers (in the text the term wether will be used) with the sire breed Texel and the maternal line Swedish Finewool/Dorset were used in the trial. The wethers were divided randomly into two groups with four wethers in each group. Average initial live weight was 86.8 kg (SD 7.14) for the first group and 85.6 kg (SD 6.77) for the second group. During the first three weeks (day 1-21) in each period the wethers were housed in single pens of 6 m^2 with deep straw bedding. During the fourth week (day 22-29) the wethers were held in metabolic cages of 1.5 x 0.8 m^2 to enable total collection of faeces and urine (Table 7). The metabolic cages had meshed floors and a rubber mat in the front for better comfort. The wethers were fed the forages and minerals individually once a day in both the single pen and in the metabolic cage. Free access to water and salt block was provided during the trial, both in pens and metabolic cages. All four silage treatments were fed to both groups of wethers.

Table 7. Housing condition, feeding procedure and recording for each period.

Week	Housing	Feeding	Recordings
Week 1	Individual pen	*Ad libitum*	Adaptation to new silage for two weeks
Week 2	Individual pen	*Ad libitum*	
Week 3	Individual pen	*Ad libitum*	Recording of intake
Week 4	Metabolic cages	80 % of *ad libitum*	Adaptation to restricted intake for 3 days followed by collection of faeces and urine during the last four days of the period

Experimental design

The trial was divided into four periods of 29 days each and the wethers switched forages for each period, so at the end of the last period all wethers had been fed all of the forages. Four different forages were used in the trial and the design was a duplicated 4 x 4 Latin square. The forages were two GS and two WCBS (Table 8). In the second group untreated rapeseed meal was added to the diet (Table 9). Thereby, the effect of protein supplementation on silage intake and digestibility and protein utilization by the wethers could be evaluated. Inclusion of rapeseed meal in the diet was constant (150 g during ad libitum feeding and 120 g during restricted feeding in the registration week) and did not differ between forages. Composition of rape seed meal is shown in table 10. This means that at each period two wethers were fed the same forage but one of them was also supplemented with untreated rapeseed meal. During the first two weeks in each period the wethers were adapted to the experimental feed at *ad libitum* feeding. During the third week when feed intake was recorded, they continued to be fed *ad libitum* with ca 15 % refusals. During the fourth week, the collection week, the wethers were fed 80 % of ad libitum. During the first 3 days of the collection week, the wethers were adapted to the restricted intake followed by total collection of faeces and urine during the last four days of the registration week.

Table 8. A 4x4 Latin square with four periods and four treatments without rapeseed meal distributed to four wethers. T= treatment

	Wether 1	Wether 2	Wether 3	Wether 4
Period 1	T 1	T 3	T 4	T 2
Period 2	T 2	T 1	T 3	T 4
Period 3	T 3	T 4	T 2	T 1
Period 4	T 4	T 2	T 1	T 3

Table 9. A 4x4 Latin square with four periods and four treatments with rapeseed meal distributed to four wethers. T= treatment

	Wether 5	Wether 6	Wether 7	Wether 8
Period 1	T 1	T 2	T 3	T 4
Period 2	T 3	T 1	T 4	T 2
Period 3	T 2	T 4	T 1	T 3
Period 4	T 4	T 3	T 2	T 1

Table 10. Chemical composition of rapeseed meal. Mean and standard deviation (SD; n=2).

	Rapeseed meal	
	Mean	SD
DM[1] (%)	88.4	0.14
CP[2] (g/kg DM)	379	14.1
Ash (g/kg DM)	72	0.3
Starch (g/kg DM)	76	6.7
NDF[3] (g/kg DM)	292	7.2
ADF[4] (g/kg DM)	237	7.5

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

Silages and diets

Four silages were used in the trial.

1. Whole-crop barley silage, harvested at heading stage (WCBH)
2. Whole-crop barley silage, harvested at milk stage (WCBM)
3. Grass silage, spring growth, harvested early in season (GE)
4. Grass silage, spring growth, harvested late (GL)

Harvest of forages

GS were harvested at early maturity stage on 31st of May (GE) and at late maturity stage on 17th of June (GL) 2013. Both GS were from spring growth. The WCBS was harvested early on 30th of June at heading stage (WCBH) and at milk stage on the 18th of July (WCBM). Forages were harvested, wilted and ensiled in round bales using salt-based additives; Kofasil LP (sodium nitrite, hexamine, sodium benzoate) for the GS and Kofasil Ultra K (sodium nitrite, hexamine, potassium sorbate, sodium benzoate, sodium propionate, Addcon Europe GmbH) for the WCBS, both at an application rate of 2 l/tonne forage. Silages were stored for at least 4 months before the trial started. All forages were chopped to 40 mm using a mixer wagon and then put in freezer in smaller packages to ensure the quality of the silage at feeding. Silages were thawn thoroughly before feeding.

Registrations and sample collection

Registrations

All wethers were weighed before the trial started, between the different periods and before each time they were moved to the cages. The body condition scores (BCS) were assessed before the trial started and at the end of each period. *Ad libitum* feed intake was registered for 7 days during the third week of each period and *in vivo* digestibility of DM, OM and nutrients and protein utilization were recorded during the fourth week at restricted intake. At restricted intake, chewing and ruminating activity were registered for each wether by a sensor on the nose that were attached to a halter. In addition, particle size distribution in faeces was determined. Results on chewing activity and faecal particle size will be presented in another master thesis (Hedlund, 2017).

Feed sample collection

Feed and refusals were weighed daily for each wether week 3 and 4 in each period. Daily feed samples of 500 g were collected from each treatment in the *ad libitum* week, day 15-21, and during the registration week, day 25-28. In the *ad libitum* week, day 16-22, samples of refusals were taken daily and individually. Refusals from the feed during the collection week were sampled if there were any. Samples from feed and refusals were immediately put in the freezer after sampling.

Collection of urine and faeces

Total collection of faeces and urine was done individually in the metabolic cages day 26-29 in the collection week. Urine was collected in stainless steel bowls that were placed on the floor under the metabolic cages. These bowls were changed daily as the wethers were fed. Every day 300 ml of 10 % sulphuric acid was added to the bowl to lower pH to inhibit microbial activity and to avoid evaporation of N from urine. The urine was stirred and mixed and poured through a strainer to be cleared from feed residue and wool. Volume of the urine was measured using measuring cylinders. Samples of 200 ml urine were taken daily from each wether day 26-29. The samples were poured into sample jars and immediately put in the freezer. Urine from two cages was leaking into the faeces containers. In these faeces

containers a metallic net was placed to separate faeces from urine. Volume from that urine was measured and then it was discarded.

Faeces were collected in plastic containers that were placed on the floor under the metabolic cages. Before changing the containers each morning as the wethers were fed, faeces from cages were brushed down to the container. After the faeces were cleaned from wool and feed it was weighed on a scale. A sample of 1000 g or all faeces if there was not 1000 g was taken from each wether day 26-29. All samples were put into double plastic bags and frozen immediately after sampling.

Analyses

Chemical composition of the silages are shown in tables 11 and 12. Daily feed samples and refusals from *ad libitum* week and collection week were assessed for DM, after thawing. From each sample 150 g were weighed out and put in aluminum trays and dried for 20 h in 60° C. Remaining feed samples were pooled into one sample for each feed, each week in each period. Remains of the refusal samples were pooled into one sample for each wether, each week in each period. From the pooled samples 200 g of feed and refusals were sent to LKS mbH, Lichtenwalde, Germany for nutrient analyses and to the Department of Animal Nutrition and Management, SLU Uppsala, Sweden for analysis of OM digestibility, WSC and fermentation characteristics (acids, alcohols, pH, ammonia-N).

Table 11. Chemical composition of WCBS harvested at heading stage (WCBH) and at milk stage (WCBM) of maturity and GS harvested early (GE) and late (GL). Mean and standard deviation (SD; n=4).

	WCBH		WCBM		GE		GL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM[1] (%)	35.3	0.39	36.7	3.17	27.4	1.05	36.8	2.94
ME[2] (MJ/kg DM)	10.8	0.15	10.5	0.14	10.8	0.18	9.8	0.09
Ash (g/kg DM)	57	0.2	51	2.5	108	10.3	59	1.5
OM[3] (g/kg DM)	943	2.3	949	2.5	892	10.3	941	1.5
VOS[4] (% of OM)	83.7	0.88	81.1	0.85	87.7	0.49	77.3	0.52
CP[5] (g/kg DM)	105	2.4	83	2.1	190	1.8	116	4.1
NDF[6] (g/kg DM)	471	17.2	444	10.6	472	5.1	573	4.1
ADF[7] (g/kg DM)	259	10.7	240	10.1	302	4.8	341	9.1
ADL[8] (g/kg DM)	22	0.9	27	2.1	26	1.8	42	7.2

[1]DM = dry matter determined after 20 h in 60°C
[2]ME = metabolizable energy
[3]OM = organic matter
[4]VOS = rumen soluble organic matter
[5]CP = crude protein
[6]NDF = neutral detergent fibre
[7]ADF = acid detergent fibre
[8]ADL = acid detergent lignin

Table 12. Fermentation characteristics of whole crop barley silage harvested at heading stage (WCBH) and at milk stage (WCBM) of maturity and grass silage harvested early (GE) and late (GL). Mean and standard deviation (SD;n=4).

	WCBH		WCBM		GE		GL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	4.21	0.033	4.23	0.096	4.20	0.005	4.23	0.094
WSC[1] (% of DM)	15.5	1.75	12.6	0.14	0.7	0.55	5.6	0.55
Lactic acid (% of DM)	5.2	0.32	3.9	0.48	8.8	1.21	5.0	0.49
Acetic acid (% of DM)	1.4	0.08	0.6	0.04	2.2	0.36	1.2	0.04
Propionic acid (% of DM)	0.09	0.023	0.04	0.001	0.07	0.005	0.05	0.004
Butyric acid (% of DM)	0.05	0.003	0.04	0.001	0.07	0.005	0.05	0.004
Ethanol (% of DM)	0.17	0.031	0.04	0.144	0.44	0.065	0.36	0.152
NH_3N (% of tot N)[2]	7.0	0.49	5.9	0.61	11.1	1.62	8.3	0.65

[1]WSC = water soluble carbohydrates

[2]Ammonia- nitrogen, % of total nitrogen not corrected for the N content in the silage additive.

Dry matter of the feaces was determined after thawing. Determination was done by weighing out 150 g of faeces in an aluminum tray and dry it for 48 h at 60°C and then weigh it again. DM determination was done for each wether each of the 4 days in the registration period. Faeces samples were pooled for each wether each period and 200 g of the pooled sample were sent to LKS mbH, Lichtenwalde, Germany for analysis of chemical composition.

At LKS mbH, Lichtenwalde, Germany feed, refusals and faeces were analysed for CP, NDF, ADF, acid detergent lignin (ADL) and ashes. Using Kjeldahl nitrogen determination procedure, the CP was determined by calculating total N x 6.25. The Fiber Technology method, according to Van Soest *et al*. (1991) was used to determine NDF, ADF and ADL contents. By drying samples for 16 h at 525°C the ash content was determined. By taking the difference between the intake and the faeces amount of nutrients and DM divided by the intake of the nutrient and DM, the *in vivo* digestibilities of DM, OM, CP, NDF and ADF were calculated. As the endogenous nitrogen in faeces was not considered in the calculations the *in vivo* digestibility was apparent. Sorting was calculated by taking difference between amount of NDF in the feed and the amount of NDF in the refusals.

From the ash content, OM content in the feed was calculated. At the Department of Animal Nutrition and Management, Uppsala, Sweden the VOS (*in vitro* OM digestibility) of silages was analyzed by incubation at 38° C for 96 h of 0.5g dried and ground sample in 49 ml buffer and 1 ml rumen fluid (Lindgren, 1979; Lindgren 1983). The content of metabolizable energy (ME) of the silage was calculated from the VOS value (Lindgren, 1988). Content of WSC, lactic acid, acetic acid, propionic acid, butyric acid and ethanol as well as pH of the silage were anlysed at the Department of Animal Nutrition and Management, Uppsala, Sweden. Content of WSC was assessed by a simplified enzymatic method according to Larsson, K & Bengtsson, S. (1983). Lactic acid, acetic acid, propionic acid, butyric acid and ethanol were assessed with HPLC -applications for agricultural and animal science according to Ericsson & André (2010). The pH was determined in juice squeezed from the silage using a pH-meter Metrohm 654 (Herisau, Schweiz).

At LKS mbH Lichtenwalde, Germany, urine samples pooled per wether and period were analysed for total nitrogen, urea, purine derivatives (allantoin and uric acid) and creatinine. The N concentration of undiluted urine was analyzed with a Kjeldahl procedure. Urine concentrations of creatinine, allantoin and uric acid (samples diluted 1 to 50) were analysed with HPLC as described by Shingfield and Offer (1999), but with the modification of using a second mobile phase containing methanol, acetonitrile and distilled water (45/45/10) and a

Kinetex XB-C18 column (150 x 4.6 mm, 5 µm). Analysis of urea concentration (samples diluted 1 to 50) was made with spectrophotometry according to LKS (2006). Excretion of PD is determined to evaluate flow of microbial N to intestines (Chen and Ørskov, 2003). To evaluate total excretion of nitrogen compounds the urine volume and concentrations of the nitrogen compounds were used. This indicated losses of nitrogen compounds each day from the fed silages. Intake of nitrogen minus the nitrogen loss from urine and faeces were used to calculate nitrogen balances from the feeds.

Statistical analysis

By using the PROC MIXED procedure in SAS (ver. 9.3.) data for feed intake, digestibility, protein utilization and LW were analysed. The statistical model for the duplicated 4 x 4 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 4), $\mathbf{S_j}$ = effect of supplementation of protein (j = 1 to 2), $(FS)_{ij}$ = interaction between forage and supplementation of protein, $\mathbf{P_k}$ = effect of period (k = 1 to 4), $\mathbf{B_{l(j)}}$ = random effect of sheep nested within supplementation of protein (l = 1 to 8), $\mathbf{C_{m(ijkl)}}$ = carry over effect between periods for the combination of ijkl (m = 4) and $\mathbf{e_{ijkl}}$ = residual error.

As no interactions between forage and protein supplementation were found and the carry over effect was not significant, $(FS)_{ij}$ and $C_{m(ijkl)}$ were left out from the model. Pairwise comparisons were done between the least square means with Tukey-Kramer adjustment when significant effects were shown at $P \leq 0.05$ in the *F*-test. At $P \leq 0.05$ the pairwise differences where declared significant and at $0.05 \leq P \leq 0.10$ stated as a tendency to significance.

Results

Feed intake

When wethers were offered the different silages *ad libitum*, the DM intake was higher for WCBH than the GE and GL when expressed in kg/day. When expressed in % of LW, the DM intake was higher for WCBH than for all the other treatments (Table 13). Intakes of OM were higher for the two WCBS than for the GS with the lowest intake for GE. Intake of CP was highest for GE and lowest for WCBM with GL and WCBH being intermediate. Intake of NDF in kg per day was highest for GL and WBCH and lowest for the GE with WCBM being intermediate. When expressed in % of LW the intake of NDF was higher for GL and WCBH than for GE and WCBM, which did not differ. Intakes of ADF and ADL were higher for GL than for the other treatments (Table 13). Sorting of feed is shown by a negative value in the table. Sorting of feed occurred for all feeds except GE (Table 13).

Table 13. Intake and feed sorting by wethers when fed *ad libitum*, affected by silage type. Silages were whole crop barley silage harvested at heading (WCBH) and at milk stage (WCBM) and grass silage harvested early (GE) and late (GL). Least square means and standard error of the mean (SEM) when averaged over rapeseed meal supplementation (n=8).

	Experimental diets					
Silage intake of	WCBH	WCBM	GE	GL	SEM	*P* - value
DM[1] (kg/day)	2.48^{a}	2.25^{ab}	2.01^{b}	2.15^{b}	0.080	< 0.001
DM (% of LW[2])	2.50^{a}	2.25^{b}	2.06^{b}	2.15^{b}	0.059	< 0.001
OM[3] (kg/day)	1.77^{a}	1.75^{a}	1.40^{c}	1.56^{b}	0.056	< 0.001
CP[4] (g/day)	278^{b}	193^{c}	372^{a}	254^{b}	14.6	< 0.001
NDF[5] (kg/day)	1.13^{a}	1.01^{b}	0.91^{c}	1.22^{a}	0.041	< 0.001
NDF (% of LW)	1.15^{a}	1.01^{b}	0.93^{b}	1.21^{a}	0.030	< 0.001
ADF[6](kg/day)	0.49^{b}	0.44^{b}	0.47^{b}	0.57^{a}	0.019	< 0.001
ADL[7] (kg/day)	0.042^{b}	0.048^{b}	0.041^{b}	0.066^{a}	0.0039	< 0.001
Feed sorting (g/kg DM)[8]	-9.74^{b}	-9.68^{b}	17.79^{a}	-11.62^{b}	5.026	< 0.001

$^{a-c}$Least square means within a row differ significantly ($P < 0.05$).
[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
[7]ADL = acid detergent lignin
[8]Calculated as the difference between the NDF content of feed (silage+rapeseed meal) and the NDF content of the refusals. A negative value shows sorting.

There were no significant differences in intake as an effect of rapeseed meal supplementation to the diet. There was a tendency to less sorting without supplementation of rapeseed meal to the diet (Table 14).

Table 14. Intake and sorting by wethers when fed *ad libitum*, affected by supplementation of rapeseed meal. Least square means and standard error of the mean (SEM) when averaged over type of silage (n=16).

	Rapeseed meal			
Silage intake of	Without	With	SEM	*P*-value
DM[1] (kg/day)	2.16	2.29	0.082	0.291
DM (% of LW[2])	2.21	2.28	0.041	0.250
OM[3] (kg/day)	1.58	1.67	0.059	0.310
CP[4] (g/day)	268	280	12.0	0.500
NDF[5] (kg/day)	1.03	1.10	0.042	0.307
NDF (% of LW)	1.06	1.09	0.021	0.275
ADF[6](kg/day)	0.48	0.51	0.021	0.357
ADL[7] (kg/day)	0.048	0.051	0.0037	0.552
Feed sorting (g/kg DM)[8]	2.52	-9.15	3.930	0.081

[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
[7]ADL = acid detergent lignin
[8]Calculated as the difference between the NDF content of feed (silage+rapeseed meal) and the NDF content of the refusals. A negative value shows sorting.

In vivo digestibility

The early harvested silages, GE and WCBH, had the highest DM digestibility (Table 15). Digestibility of OM was highest for the GE diet followed by the WCBH, WCBM and the GL diets, which differed in OM digestibility. Digestibility of CP, NDF and ADF were highest for the GE diet and lowest for the WCBM diet with the GL and WCBH diets in between. (Table 15).

Table 15. Effect of whole crop barley silage harvested at heading (WCBH) and at milk stage (WCBM) and grass silage harvested early (GE) and late (GL) on the *in vivo* digestibility by wethers fed 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed meal supplementation (n=8).

	Experimental diets					
Digestibility of	WCBH	WCBM	GE	GL	SEM	*P*-value
DM[1] (%)	68.3[a]	65.2[b]	70.0[a]	63.3[b]	0.76	<0.001
OM[2] (%)	69.6[b]	66.8[c]	73.6[a]	64.5[d]	0.71	<0.001
CP[3] (%)	86.0[b]	84.1[c]	88.2[a]	86.3[b]	0.44	<0.001
NDF[4] (%)	57.7[b]	47.0[c]	72.1[a]	58.5[b]	1.01	<0.001
ADF[5] (%)	58.2[b]	45.4[c]	73.5[a]	57.7[b]	1.14	<0.001

[a-d]Least square means within a row differ significantly ($P < 0.05$).
[1]DM = dry matter
[2]OM = organic matter
[3]CP = crude protein
[4]NDF = neutral detergent fibre
[5]ADF = acid detergent fibre

When the diets were supplemented with rapeseed meal the digestibility of CP was significantly higher compared to unsupplemented diets. Digestibilites of DM, OM, NDF and ADF were not affected by protein supplementation (Table 16).

Table 16. Effect of supplementation of rapeseed meal on *in vivo* digestibility by wethers fed 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over type of silage (n=16).

	Rapeseed meal			
Digestibility of	Without	With	SEM	*P*-value
DM[1] (%)	67.1	66.3	0.73	0.460
OM[2] (%)	68.9	68.4	0.70	0.577
CP[3] (%)	85.4	86.9	0.33	0.016
NDF[4] (%)	59.5	58.1	0.97	0.336
ADF[5](%)	60.2	57.2	1.17	0.123

[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

Total N intake was highest for the GE diet, followed by the GL and WCBH diets and lowest for the WCBM diet (Table 17). Excretion of N in faeces in g/day was higher for the GE diet than for the GL, WCBH and WCBM diets, which did not differ. When excretion of N in faeces was expressed in % of N intake the WCBM diet had the highest excretion and the GL and WCBH diets had higher excretions than the GE diet. Excretion of N in urine in g/day was higher for the GE diet than for the other treatments. Also, the GL diet had higher N excretion than the WCBM diet. When expressed in % of N intake, the excretion of N in urine was higher for the GE diet compared to both whole crop barley silage diets. The retention of N in g/day did not differ significantly between diets. When N retention was expressed in % of N intake there was a tendency to significantly higher N retention for the barley silage diets compared to the GE diet (Table 17).

Table 17. Effect of whole crop barley silage harvested at heading (WCBH) and at milk stage (WCBM) and grass silage harvested early (GE) and late (GL) on total N intake, excretion of N in faeces and urine and N retention by wethers fed at 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed meal supplementation (n=8).

	Experimental diet					
	WCBH	WCBM	GE	GL	SEM	*P*-value
Total N intake g/day	31.4^{b}	24.7^{c}	47.8^{a}	30.8^{b}	1.54	<0.001
N in						
faeces (g/day)	4.84^{b}	4.38^{b}	5.96^{a}	4.57^{b}	0.180	<0.001
urine (g/day)	16.4^{bc}	12.4^{c}	31.7^{a}	17.6^{b}	1.40	<0.001
faeces (% of N intake)	15.4^{b}	17.8^{a}	12.6^{c}	14.9^{b}	0.48	<0.001
urine (% of N intake)	52.6^{b}	50.2^{b}	66.1^{a}	56.5^{ab}	2.96	0.004
N retention (g/day)	10.2	7.8	10.1	8.6	1.08	0.247
N retention (% of N intake)	32.0$^{(a)}$	32.0$^{(a)}$	21.3$^{(b)}$	28.6$^{(ab)}$	3.22	0.071

[a-c]Least square means within a row differ significantly ($P < 0.05$)

There was no effect on total N intake when rapeseed meal was added to the diets (Table 18). Excretion of N in faeces expressed in g/day tended to be higher when rapeseed meal was added to the diets. There was no effect of rapeseed meal on N excretion in faeces, when

expressed in % of N intake. Excretion of N in urine was higher, both in g/day and in % of N intake, when the diets were supplemented with rapeseed meal compared to no rapeseed meal. Retention of N in g/day tended to be lower when rapeseed meal was added and this decrease was significant. when expressed in % of N intake (Table 18).

Table 18. Effect of supplementation of rapeseed meal on total N intake, excretion of N in faeces and urine and N retention from feed by wethers fed at 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over type of silage (n=16).

	Rapeseed meal			
	Without	With	SEM	*P*-value
Total N intake g/day	32.8	34.5	1.49	0.439
N in				
faeces (g/day)	4.65	5.23	0.173	0.054
urine (g/day)	17.3	21.7	1.27	0.050
faeces (% of N intake)	14.6	15.7	0.40	0.106
urine (% of N intake)	50.4	62.3	2.38	0.012
N retention (g/day)	10.8	7.6	1.00	0.062
N retention (% of N intake)	35.0	22.0	2.68	0.014

Excretion of nitrogen compounds in urine

Excretion of urea and urea-N in urine in g/day was highest for GE diet and there were no significant differences between the other diets (Table 19). Also, total-N excretion was highest for the GE diet followed by the GL diet, which differed from the WCBM diet. There were no differences among the silage diets for excretion of creatinine and uric acid, expressed in g/day, mmol/day or creatinine expressed in mg/kg LW. Allantoin and PD excretions tended to be higher for the WCBH diet than for the WCBM diet when expressed in g/day and in mmol/day. Hippuric acid excretion was higher for the GE diet than for the WCBM and GL diets, when expressed both in g/day and in mmol/day (Table 19).

Table 19. Effect of whole crop barley silage harvested at heading (WCBH) and at milk stage (WCBM) and grass silage harvested early (GE) and late (GL) on the excretion of different N compound in urine by wethers fed at 80% of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over rapeseed meal supplementation (n=8).

	Experimental diet					
Excretion of	WCBH	WCBM	GE	GL	SEM	*P*-value
g/day						
Urea	24.6^{b}	18.1^{b}	51.9^{a}	27.3^{b}	3.32	<0.001
Urea-N	11.4^{b}	8.4^{b}	24.2^{a}	12.7^{b}	1.55	<0.001
Tot-N	16.4^{bc}	12.5^{c}	31.7^{a}	17.6^{b}	1.40	<0.001
Creatinine	1.31	0.80	0.94	1.24	0.25	0.376
Uric acid	0.090	0.083	0.077	0.080	0.019	0.971
Allantoin	2.51$^{(a)}$	1.42$^{(b)}$	1.62$^{(ab)}$	1.77$^{(ab)}$	0.300	0.085
PD[1]	2.60$^{(a)}$	1.50$^{(b)}$	1.68$^{(ab)}$	1.85$^{(ab)}$	0.317	0.100
Hippuric acid	5.09^{ab}	1.97^{b}	7.80^{a}	2.68^{b}	1.336	0.011
mmol/day						
Creatinine	11.61	7.08	8.27	11.00	2.191	0.376
Uric acid	0.53	0.49	0.46	0.48	0.114	0.971
Allantoin	15.86$^{(a)}$	9.01$^{(b)}$	10.25$^{(ab)}$	11.18$^{(ab)}$	1.899	0.085
PD	16.39$^{(a)}$	9.44$^{(b)}$	10.59$^{(ab)}$	11.66$^{(ab)}$	1.998	0.100
Hippuric acid	28.43^{ab}	10.98^{b}	43.54^{a}	14.94^{b}	7.454	0.011
Creatinine (mg/kg LW)	13.28	8.26	9.87	12.56	2.506	0.428

$^{a\text{-}c}$Least square means within a row differ significantly ($P < 0.05$).
[1]PD = purine derivatives

Excretion of total N in urine was higher when rapeseed meal was added to the diet than for the diet without rapeseed meal (Table 20). Excretion of allantoin and PD tended to be higher when the diets were supplemented with rapeseed meal, expressed both in g/day and mmol/day.

Table 20. Effect of supplementation of rapeseed meal on the excretion of different N compounds in urine by wethers fed at 80 % of *ad libitum* DM intake. Least square means and standard error of the mean (SEM) when averaged over type of silage (n= 16).

	Rapeseed meal			
Excretion of	Without	With	SEM	*P*-value
g/day				
Urea	27.3	33.6	2.51	0.126
Urea-N	12.73	15.67	1.173	0.126
Tot-N	17.34	21.74	1.272	0.050
Creatinine	0.87	1.27	0.202	0.212
Uric acid	0.070	0.095	0.0139	0.269
Allantoin	1.56	2.10	0.212	0.084
PD[1]	1.62	2.18	0.224	0.090
Hippuric acid	5.31	3.46	1.215	0.324
mmol/day				
Creatinine	7.73	11.25	1.78	0.212
Uric acid	0.42	0.56	0.083	0.269
Allantoin	9.86	13.28	1.34	0.084
PD	10.26	13.78	1.413	0.090
Hippuric acid	29.62	19.32	6.781	0.324
Creatinine (mg/kg LW)	9.11	12.88	2.031	0.237

[1]PD = purine derivatives

Live weight

Mean LW of wethers was lower for the GE diet than for the other diets at *ad libitum* intake (Table 21). At restricted intake, LW was higher for the WCBH and WCBM diets than for the GE diet. LW gain at *ad libitum* intake was higher for WCBH than for WCBM expressed both in kg and % of LW. There were no differences in LW loss among treatments at restricted intake expressed both in kg and % of LW.

Table 21. Effect of whole crop barley silage harvested at heading (WCBH) and at milk stage (WCBM) and grass silage harvested early (GE) and late (GL) and) on body condition scores (BCS), mean live weight (LW), LW gain and loss by wethers fed *at libitum* (*ad lib*) DM intake and at 80 % of *ad libitum* intake (rest intake). Least square means and standard error of the mean (SEM) when averaged over rapeseed meal supplementation (n=8).

	Experimental diets					
	WCBH	WCBM	GE	GL	SEM	*P* - value
BCS[1]	3.91	3.97	3.87	3.97	0.064	0.213
Mean LW *ad lib* intake (kg)	100.1[a]	100.2[a]	97.6[b]	99.8[a]	2.560	0.014
Mean LW rest intake (kg)	99.0[a]	98.4[a]	95.8[b]	97.8[ab]	2.519	0.007
LW gain *ad lib* intake (kg)	1.31[a]	-0.37[b]	0.62[ab]	0.31[ab]	0.431	0.04
LW loss rest intake (kg)	-3.56	-3.31	-4.25	-4.37	0.642	0.377
LW gain *ad lib* intake (%)[2]	1.26[a]	-0.44[b]	0.53[ab]	0.28[ab]	0.419	0.044
LW loss rest intake (%)[2]	-3.52	-3.25	-4.26	-4.35	0.591	0.328

[a-b] = means within a row differ significantly ($P < 0.05$)

[1]BCS = body condition scores

[2]LW gain as % of LW

No significant differences in BCS, LW, LW gain and LW loss at *ad libitum* or restricted intake were found between unsupplemented diets and diets supplemented with rapeseed meal (Table 22).

Table 22. Effect of supplementation of rapeseed meal on body condition scores (BCS), mean live weight (LW) and LW gain and loss by wethers fed at *ad libitum* (*ad lib*) DM intake and at 80% of *ad libitum* DM intake (rest intake). Least square means and standard error of the mean (SEM) when averaged over type of silage (n=16).

	Rapeseed meal			
	Without	With	SEM	*P* - value
BCS[1]	3.87	3.98	0.079	0.368
Mean LW *ad lib* intake (kg)	97.9	101.0	3.55	0.553
Mean LW rest intake (kg)	96.2	99.2	3.49	0.566
LW gain ad lib intake (kg)	0.06	0.87	0.384	0.186
LW loss rest intake (kg)	-3.31	-4.44	0.676	0.284
LW gain ad lib intake (%)[2]	-0.004	0.82	0.358	0.154
LW loss rest intake (%)	-3.31	-4.38	0.581	0.241

[1]BCS = body condition scores

[2]LW gain as % of LW

Discussion

Feed intake and sorting

Intake of DM did not differ between GE and GL although GE had higher digestibility of OM (*in vivo* and *in vitro*) and of DM. Nadeau *et al*. (2015) reported decreasing DM intake from leaf stage to late heading when GS was fed to ewes in late pregnancy and early lactation, which agree with the findings by Rinne *et al.* (2002) using dairy cows. Jalali *et al.* (2012) reported higher intake from the late harvested GS compared to GS harvested at leaf stage when fed to pregnant ewes. The lack of differences in DM intake for the GS in our study is due to that the wethers only had maintenance requirements, which the GL could fulfill. No differences in DM intake were found between WCBH and WCBM, despite lower *in vivo* digestibility of DM and OM for WCBM, which might be related to sufficient nutrient intake for fulfilling the maintenance requirements of the wethers. Wallsten *et al*. (2009b) have earlier reported higher DM intake when WCBS was harvested at heading instead of early-milk or early dough-stage of maturity and fed to dairy heifers. As in this trial, Rustas *et al*. (2010) did not find any differences in intakes of DM and OM between heading and mid-dough stage when WCBS was fed to growing dairy steers.

Intake of DM in % of LW was higher for WCBH than for WCBM and GL, which could be explained by the higher OM digestibility both *in vitro* (VOS) and *in vivo*. The higher DM intake of WCBH compared to GE could not be explained by higher digestibility of OM. The lower DM concentration and a higher concentration of ammonia-N in GE could be the reasons for the lower DM intake of GE compared to WCBH despite the higher OM digestibility of GE (Huthanen *et al*., 2007).

In this trial intake of CP was highest for the GE which is obvious with the high content of CP in the silage. The CP content in this trial is even higher than others have reported with GS harvested early in the season (Bernes *et al*., 2008; Nadeau *et al*.,2015). The high CP content for the GE could be explained by the early harvest date of 31st of May. The decrease in CP intake from GE to GL is in accordance with the literature, where Nadeau *et al.* (2015) and Bernes *et al.* (2008) reported decreased concentration of CP when harvesting GS at later stages of maturity. Rinne *et al.* (2002) found that N intake by dairy cows decline as GS was harvested at later stages of maturity. Intakes of CP decreased from WCBH to WCBM due to decreased contents of CP in the forage at the later maturity stage. Decreased CP concentration has also been reported by Rustas *et al.* (2008) and Wallsten *et al.* (2009) with WCBS when harvesting at early milk stage instead of heading.

The late harvested GS had the highest intakes of ADF and ADL, which was expected as fibre and lignin contents increase at later stages of maturity (Nadeau *et al.,* 2015). Intake of NDF did not differ between GL and WCBH although GL had higher NDF content than WCBH. The absence of differences in NDF intake could be explained by the higher intakes of DM and OM for the WCBH. Intake of NDF was higher for GL than for GE despite the similar DM intake. The higher NDF intake of GL is related to the higher content of NDF in GL compared to GE. Jalili *et al*. (2012) showed higher NDF intake of late harvested GS fed to pregnant ewes, which could be related to the higher silage NDF content and the higher DM intake of the late harvested GS. However, Nadeau *et al*. (2015) did not report any differences in NDF intake between early and late harvested GS although the DM intake was higher for the early harvested GS. Differences in NDF intake between studies depends on different physiological status between the wethers and the ewes. The wethers in our study only needed to cover their maintenance requirements, which was fulfilled by intake of GL. The intake of pregnant ewes

also was regulated by the energy requirements of the animals, which were maintenance and pregnancy, whereas the intake of lactating ewes was regulated by rumen fill when fed the late harvested grass silage (Allen, 1996). Intake of NDF decreased for the WCBS from heading to milk stage, which is explained by decreased concentration of NDF in the silage. This is related to increasing head:leaf and head:stem ratios with advancing maturity (Khorasani *et al*., 1997). Likewise, Wallsten *et al*. (2009) observed decreased content of NDF in WCBS at milk stage compared to heading stage.

The wethers sorted the feed to some extent when fed *ad libitum*, except when fed the GE where no sorting occurred. Bernes *et al*. (2008) showed that lambs sorted against NDF content of GS, which is in agreement with the findings in this trial where wethers fed the GL sorted against NDF, which was not shown in wethers fed the GE diet.

In vivo digestibility

When digestibility was estimated *in vivo* the OM digestibility was highest for the GE diet followed by WCBH, WCBM and GL. When estimating the digestibility of OM *in vitro* (VOS) the order of the silages was the same, which indicates that there is a positive correlation between the two methods. Cherney *et al*. (1990) also showed a correlation between DM digestibility determined *in vitro* and *in vivo.* When digestibility was estimated *in vivo* the wethers were fed 80 % of intake at *ad libitum* feeding which minimized the risk for feed sorting that could affect the digestibility. The *in vivo* digestibility is close to the true digestibility in this trial because the absence of feed sorting at restricted intake.

The GE diet showed the highest *in vitro* OM digestibility and GL showed the lowest. Harvesting GS at later stages of maturity is associated with decreased OM digestibility, which is in agreement with previous studies (Bernes *et al*., 2008; Kuoppala *et al*., 2009). At the later harvest date the GS contains higher concentrations of NDF and ADL which has a high impact on OM digestibility (Südekum *et al*., 1995). The pattern for digestibility of OM in WCBS is not the same as for GS at later stages of maturity. At later stages of maturity (dough stage) of the WCBS concentration of starch increases as kernels are formed. Due to that digestibility of OM is rather unchanged even if the digestibility of NDF decreases, because digestibility of starch is high (Nadeau, 2007; Rustas et al., 2011). WCBH had higher OM digestibility than the WCBM. Starch was not analysed of the WCBS in this study as we know from previous studies (Rustas *et al*., 2011) that its content is zero at heading stage and insignificant at the milk stage of maturity when the WCBS is harvested as round bales because of loss of kernels during mowing and baling. Digestibility of NDF and ADF decreased with advanced maturity of both WCBS and GS, which is related to an increased lignification of the cellulose and hemicellulose, making the NDF more indigestible (Jung and Allen, 1995). The GS had higher digestibility of NDF and ADF than the WCBS, which is in line with previous trials (Rustas et al., 2011; Saarsoo, 2015).

Protein digestibility *in vitro* indicates how fast the protein can be degraded by the microbes in rumen (McDonald *et al*., 2002) while digestibility of CP *in vivo* also includes intestinal breakdown of protein. In this trial the *in vivo* CP digestibility was highest for the GE whereas the WCBM had the lowest CP digestibility. The CP digestibility was high compared to the earlier studies for both GS (Kuoppala *et al*., 2009) and WCBS (Rustas *et al*. 2011). The lower digestibility of CP for GL compared to GE is in agreement with others (Kuoppala *et al*., 2009; Rinne *et al*. 2002). Rustas *et al*. (2011) showed a decrease in *in vivo* CP digestibility from heading to milk stage when WCBS was harvested in Skara but when harvested in Uppsala the decrease in CP digestibility occurred at dough-stage. When rapeseed meal was added to the

diet digestibility of CP increased but none of the other intake parameters were affected. The increased CP digestibility when rapeseed meal was added indicates that structure and physical form of the protein in rapeseed meal differ from protein in forage (McDonald *et al*., 2002).

Protein utilization

As already mentioned regarding the CP intake, the highest total N intake was recorded for the GE diet and the lowest total N intake was recorded for the WCBM diet with WCBH and GL in between. Excretion of N in urine in g per day was highest for the GE diet which was expected as that silage had the highest intake of N. The GE diet had high concentration of CP that had a high digestibility which earlier has been shown to give higher concentrations of N excreted in urine as most of the CP digestion occurs in the rumen (Carro *et al*., 2012). Ahvenjärvi *et al*. (2006) associated decreased concentration of N in urine with lower concentrations of CP in the silage, when proportion of WCBS increased in a GS-based diet. When N excretion in urine was expressed in percentage of N intake, the GE diet did not differ from the GL diet but it was higher than for the WCBS diets. This resulted in a tendency to a higher retention of N in wethers fed WCBS than for wethers fed the GE diet, when the N retention was expressed in percentage of N intake. This has been shown earlier by Nadeau *et al.* (2007) that efficiency of N utilisation decreases when concentration of dietary CP increases. In the GE diet, both the content and degradability of CP were high, which increased the risk for excess ammonia in rumen. Excess ammonia will end up in the liver where a large part will be converted to urea and excreted in the urine (McDonald *et al*.,2002). Rinne *et al*. (1997) also reported an inefficient use of N when grass silage from early stages of maturity were fed to young bulls and heifers of dairy breed. The higher excretion of urea, urea-N and total-N in the urine indicates that the use of N from GE was inefficient even in this trial.

Intake of N and excretion of N in the faeces was not affected by supplementation of rapeseed meal to the diet, but excretion of N in the urine increased by rapeseed supplementation. The decreased N retention in % of N intake when the diet was supplemented with rapeseed meal indicates excess protein in the diet and that protein concentration in the forages was enough to cover the need of protein for the wethers.

Excretion of PD in urine tended to be higher for wethers fed WCBH than for wethers fed WCBM but there were no differences between WCBS and GS. This indicates that the ruminal flow of microbial N decreased when WCBS were harvested at later stages of maturity. The case of no differences between WCBS and GS has also been reported by Ahvenjärvi *et al*. (2006) while Owens *et al*. (2009) reported a higher N flow and more efficient microbial N synthesis when WCWS was fed instead of GS. Excretion of allantoin in this trial for all four silages was higher than the findings of Puchala *et al.* (1992). The WCBH diet tended to have higher excretion of allantoin compared to the WCBM diet but not compared to the GS diet. Puchala *et al*. (1992) associated higher excretion of allantoin in urine with increased concentration of both protein and energy in the diet. When WCBH and WCBM were compared, the intake of CP was higher for the WCBH but there were no differences in DM intake and only slightly lower concentration of ME in the WCBM, which could be the reason for the tendency to higher excretion of allantoin for the WCBH. Excretion of uric acid did not differ among the four silages and the concentrations were lower than what Puchala *et al*. (1992) have reported earlier.

Excretion of total N in urine, as mentioned, earlier was higher when rapeseed meal was added to the diets, also excretion of PD and allantoin tended to be higher with protein supplementation. This shows that there was no strong increase in microbial protein synthesis

in rumen when rapeseed meal was supplemented to the diet. Together with lack of effect on silage intake and OM digestibility, the increased urinary N excretion and only an indication of increased microbial protein synthesis shows that the need of protein was fulfilled with the silages for the wethers that only had maintenance requirements.

Live weight

At *ad libitum* intake the wethers gained on average 0.7% of their LW except for the WCBM, but it was only a significant difference between WCBH and WCBM. This could be related to the lower CP intake and to the lower *in vivo* digestibility of DM, OM, CP, NDF and ADF in the WCBM than in WCBH. At restricted feeding, the wethers lost on average 3.8% of their LW and there were no differences between type of silage. As the wethers were overweight (99 kg) and had a high body condition score of 3.9 there was excess body fat that was used during the restricted feeding. Overall, the differences in LW were small and involves some error due to the amount of digesta in the rumen. The wethers were weighed at the same time of the day but there is still some error involved in the recordings.

Conclusions

- Intake of DM in % of LW was highest for wethers fed the WCBH silage, while wethers fed the GE silage had the highest intake of CP. Wethers fed silages of WCBH and GL had the highest intake of NDF in % of LW. Lack of differences in DM intake between GE and GL silages shows that the NDF content of the grass silage did not affect the DM intake of the wethers, which only had maintenance requirements. Intake parameters were not affected by protein supplementation.

- *In vivo* OM digestibility was highest for GE, followed by WCBH, WCBM and GL diets. *In vivo* NDF digestibility was highest for the GE diet and lowest for the WCBM diet. *In vivo* digestibility of DM, OM, CP, NDF and ADF decreased with later maturity stage of both whole-crop barley and grass. *In vivo* CP digestibility increased when protein was supplemented to the diet but the *in vivo* digestibility of OM and NDF were not affected by protein supplementation with rapeseed meal.

- The highest CP intake as well as the highest excretion of total-N and urea-N in urine was recorded for the GE diet. Nitrogen retention expressed as % of N intake tended to be higher in wethers fed whole-crop barley silage diets compared to wethers fed the GE diet but did not differ from the GL diet. When rapeseed meal was supplemented to the diets excretion of N increased and retention of N in % of N intake decreased, when averaged over silages.

- At *ad libitum* intake the wethers gained on average 0.7% of their LW except the wethers fed the WCBM diet, which lost 0.4% of their LW. This could be related to the lower CP intake and to the lower *in vivo* digestibility of DM, OM, CP, NDF and ADF in the WCBM than in WCBH. Supplementation of protein did not have any effect on LW gain.

Implications

Grass silage harvested at an early stage of maturity has a high feed value with a high digestibility but also high excretion of total-N and urea-N in urine. Whole-crop barley silage harvested at heading gives high feed intake with rather high digestibility and a tendency to higher N retention then the early harvested grass silage. For both grass silage and whole-crop barley silage harvested at different stages of maturity the physical and nutritional traits will affect feed intake, digestibility and utilisation of protein. To get high feed conversion the silages could be complemented with other forages, concentrate and grain in rations for ruminants at different physiological states. Both grass silage and whole-crop barley silage harvested at later stages of maturity are suitable forages for example beef cows in early pregnancy when the requirements are low.

Further research

While wethers can be used as models for small and large ruminants further research should focus on lowering N emissions from farm animals. By combining forages from different stages of maturity with right level of concentrate in the ration the use of N can be more efficient. Harvesting grass silage at later stages of maturity seems to lower N emissions.

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