Proceedings of the 4th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

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Foreword

The 1st Nordic Feed Science Conference took place in 2010 and received a positive response by the participants with a total of 44 contributions included in the proceedings. In 2011, it was arranged parallel to the 24th NJF Congress and attracted a somewhat smaller number of participants with a total of 21 submitted papers. In the following year, we decided to arrange the 3rd conference immediately prior to the XVI International Silage Conference in Finland. This attracted a number of overseas visitors and 31 papers were presented even though silage presentations were excluded from the program.

This year, we have had no special restrictions in topics and 24 scientists have delivered their contributions. Our aim this year has been to attract more advisors to participate in the meeting. The conference is organized to include all horse and feed conservation/processing presentations on Day 1 and the methodology and ruminant presentations on Day 2. We have targeted the evening session for forage utilization in dairy cow rations with emphasis on protein utilization and NorFor evaluations of high-roughage rations.

We wish to thank all contributors to this conference and particularly Glen Broderick from the U.S. Dairy Forage Research Center, Madison, Wisconsin for his 3rd (and maybe last) participation in the Nordic Feed Science Conferences. He has given an invaluable support to our conferences over the years and contributed not only scientifically but also with his enthusiasm and humor. However, he has recently retired from his position in Madison and we may see less of him in the future.

Uppsala 2013-06-05

Peter Udén

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Forage energy and nutrient content - possibilities and limitations for the athletic horses

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Introduction

Like lactating brood mares, athletic horses may have energy requirements more than twice their maintenance requirement. However, in contrast to brood mares that are commonly fed pasture-only diets, athletic horses are fed high concentrate diets and not forage-only diets. The reason for this is probably multifactorial. Many of the forage batches sold as horse feed have too low energy density to fully support horses with high energy requirements. Feeding cereals is also a custom that can be traced back to the times when horses were used long days far away from home, and when farmers had poor forage production and storage systems. In those days, high forage-diets were not an alternative to the use of more energy dense cereals. The use of concentrates can also be explained by a strong market for concentrate producers and a comparatively weak one for forage producers which may be supported by a general belief among trainers and horse owners that concentrate is needed for maximal performance. However, there is little scientific support for this. In contrast, we have shown that performance might be improved by a forage-only diet (Jansson and Lindberg, 2012). The use of high forage diets could therefore be a good way to promote both performance and health in athletic horses, since it is well known that high concentrate diets are associated with gastrointestinal problems and stereotypic behavior.

The most common arguments against using high forage diets to athletic horses is usually that horses will not be able to consume enough to maintain body condition and/or that there will be an increase in body weight (BW), because of increased fiber intake and gut fill, and accordingly decreased performance. Other concerns is the low intake of readily available carbohydrates (starch and sugars) which might affect muscle glycogen recovery and an excessive crude protein (CP) intake, which has been suggested to impair performance (Glade *et al*., 1983). Finally the most important and ultimate question is, can they really compete on a forage-only diet? These issues have been addressed recently in a number of studies with the aim to document possible limitations and benefits with forage-only diets to athletic horses. A main hypothesis has been that forage-only diets will *improve* performance in addition to being better from a health perspective. Improved exercise performance has been expected because forage diets will increase availability of volatile fatty acids (mainly acetate) and could cause a metabolic shift in substrate utilization during exercise, that resembles the one that has been documented with fat supplementation in horses (Pagan *et al*., 2002). The aim of the present paper is to give an update on these publications and the current knowledge on forage energy and nutrient content and its possibilities and limitations for athletic horses.

Materials and Methods

Several studies on horses in training on forage-only diets have been performed and the focus has been: 1) Body weight and metabolic response to forage-only diets compared to traditional forage-concentrate diets, both at rest and during exercise (Connysson *et al.,* 2010; Jansson and Lindberg, 2012), 2) The effects on exercise response and fluid balance of forage with high crude protein content (Connysson *et al*. 2006), 3) The effects of diet on muscle glycogen content (Essén-Gustavsson *et al*., 2010; Jansson and Lindberg, 2012), 4) The effect of forage preservation method on fluid balance and exercise response (Muhonen *et al*., 2009a), 5) The

effect of concentrate inclusion and forage composition on the hindgut ecosystem (Muhonen *et al.*, 2008; Muhonen *et al*., 2009b; Willing *et al*., 2009) and 6) The possibility to get young (1.5 years) Standardbred horses into competitive condition at the age of 3 on a forage-only diet (unpublished).

Results and Discussion

In all studies, the forages used have never had estimated metabolizable energy contents lower than 10.2 MJ/kg dry matter and generally, body condition has been maintained stable (exceptions see discussion below). It has earlier been shown that a forage diet might increase BW and heart rate response to exercise compared to traditional concentrate-forage diets (Ellis *et al*., 2002) but until now there has been no studies evaluating forage with very high digestibility, which is needed to support horses with high energy requirements. The studies performed by us show that BW will increase with < 1 % compared to a diet high in concentrates, but that this increase is not significant on a day to day basis or after transportation. In addition, no increase in heart rate during exercise has been documented and therefore it is likely that performance is unaffected. The lack of signs of increased BW is probably because of the high forage digestibility which will result in little gut fill.

However, in order to get high forage digestibility, grasses have to be harvested at an early botanical stage (earlier than commonly done when forage for horses are produced) and at this stage the CP content might exceed the requirements of at least adult horses. Diets with high CP content have not been recommended to exercising horses (Meyer, 1987) and even suggested to reduce performance (Glade *et al*., 1983). In the study by Connysson *et al.* (2006) the response to high intensity exercise was not significantly affected by a forage with 17 % CP compared to a forage with 12.5 % CP, but venous pH was numerically lower on the high CP diet indicating that, if it is a true biological effect, performance might be negatively affected.

The studies show that the metabolic profile is different in horses fed forage-only diets compared to traditional high concentrate diets. On a forage-only diet, the hindgut microbiota is shifted from starch digestion to fibre digestion, the plasma acetate availability is increased and the insulin response decreased. In the study by Jansson and Lindberg (2012) the plasma lactate concentration after exercise was lower on the forage-only diet and there was also a tendency for improved V_{La4} (velocity at plasma lactate concentration 4 mmol/l). Interestingly, the venous pH was also increased on the forage-only diet which, to some extent, may counteract the exercise induced acidosis typical for high intensity exercise. Today we also have data that show that forage-only diets can be used during competition in Standardbred horses (unpublished). We have documented few significant differences in the exercise response due to forage preservation method, but preservation method and CP intake affect fluid balance, which might be of importance to consider during prolonged exercise in hot and humid climates.

Another limitation with forage-only diets could be a reduced availability of substrates for glycogen synthesis (i.e. mainly glucose), which might affect the recovery of muscle glycogen stores post exercise. In the study by Jansson and Lindberg (2012) the resting muscle glycogen content was also slightly reduced on a forage-only diet compared to a high concentrate diet, but the importance of this small reduction (-13 %) remains to be investigated. Glycogen synthesis is stimulated by insulin and in the study by Jansson and Lindberg (2012) the WSC content of the forage was 4-7 % of DM (fructans not included) and

basal insulin levels low. Also in the study by Connysson *et al*. (2010) the post prandial insulin response appeared to be very low on forage with 7 % WSC. Maybe forage WSC content need to be above 7 % per kg dry matter to ensure maximal glycogen resynthesis within 3-4 days. However, horses on high CP forage (17 % CP and 8.5 % WSC) have been shown to have very high muscle glycogen contents (Essén-Gustavsson *et al*., 2010), and also yearlings on a high CP forage-only diet have been shown to have glycogen contents higher or equal to earlier observations from horses in conventional training (Ringmark *et al.*, 2012). These findings are in accordance with observations on rats on high protein diets (Morifuji *et al.,* 2005).

A high voluntary intake of forage is of course crucial for the possibility of increasing the forage allowance to athletic horses. High fiber content might be one limitation (Jansson *et al.*, 2012) and generally also coincides with too low digestible energy content. The lowest *in vitro* digestible organic matter (IVDOM) content we have studied in horses in training have been around 78 % (Table 1; Jansson and Lindberg, 2012; Ragnarsson and Jansson, 2010 (however at low level of training)) and in those studies, body weight and condition was lost in several individuals. This indicates that higher digestibility is required to ensure adequate energy intake, at least in some individuals. However, if horses are offered excessive amounts of forage they might optimize their feed intake by selecting the most digestible parts and thereby achieving a higher energy intake compared to what could be estimated from a feed analysis (Jansson and Lindberg, 2012). In this context, palatability has to be mentioned as a very important factor for high intake. We have conducted a number of studies were the appetite mostly have been good, but at some occasions the intake has been lowered and/or body condition reduced (Table 1).

Table 1 Grass haylage (timothy and meadow fescue) *in vitro* digestible organic matter (IVDOM)[a], chemical and microbial composition and intake by horses

Source[c]	IVDOM	pH	DM	Butyrate	WSC	Yeast	Mould	Clostridia	Enterobact.	Intake[b]
	%		------ g/kg dry matter ------			log colony forming units/g fresh matter				
1, Diet RP	88	4.4	400-500	0.2	69	3.1	1.5	0	0	Good
1, Diet HP	88	5.4	400-500	0	85	2.5	0	0	0	Good
2a	78	5.8	800	-	79	<2.5	<2.0	<2.0	<1.0	Good? [d,e]
2b	-	6	790	-	79	<2.0	<2.0	<2.0	2.4	Good?[f]
3, Exp 1	89	-	820	-	157	0.4	1.1	0.5	1.5	Good
3, Exp 1	90	5.3	450	<0.3	140	0	0	0.5	0	Good
3, Exp 2	88	5.8	680	<0.2	132	3.7	2.2	<2.0	2.7	Not good
3, Exp 2	89	4.8	410	<0.4	106	5.4	<2.0	<2.0	<2.0	Not good
4	86	5.1	680	--	-	2	<2.0	<2.0	<1.0	Not good?

[a]Lindgren, 1979; [b]Good=voluntary intake high enough to maintain body weight within ± 1 %, Not good=voluntary intake causing a body weight decrease of 0.6-2.9 % within 5 days. Definitions are not relevant for project 4 where a general drop in feed intake and body condition was observed. [c]1=Connysson *et al*., 2006. 2a=Jansson & Lindberg, 2012. 2b=Ragnarsson and Jansson, 2010. 3=Muhonen *et al*., 2009. 4=unpublished, growing Standardbred horses in training fed *ad lib* during a period of body weight loss; [d]IVDOM of the leftovers were lower, indicating that the IVDOM of the feed ingested was higher than 78 [e]Selective feed intake observed and two horses lost body condition; [f]Good in Icelandic but not Standardbred horses.

The reason for the reduced appetite and loss of body condition (except for maybe a too low energy content) in individuals that earlier had maintained on forage-only diets is unclear. Possibly, a slightly higher microbial activity in the forage may be one reason (Table 1), although no health disturbances were observed.

Conclusions

Athletic horses can maintain body condition and perform on palatable high-energy forage-only diets. However, the energy content needs to be comparable to oats and maybe surplus CP intake prior to competition should be avoided, but on the other hand it appears that surplus CP could stimulate glycogen synthesis to similar levels as on high starch diets.

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Growth and qualification race performance in 1.5-3 year old Standardbred horses fed a forage-only diet *ad libitum*

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Introduction

In Thoroughbred and Standardbred race horses, training starts early and the goal is generally to compete at the age of 2 or 3 years. These horses should therefore both grow and train moderately or even heavily, and accordingly their energy requirements are high. To meet the high energy requirements young racehorses are fed diets high in concentrates (Redbo *et al*., 1998) although this is a well-documented health risk associated with colic (Tinker *et al*., 1997), rhabdomyolysis (Valberg, 1998), gastric ulcers (Luthersson *et al*., 2009) stereotypic behavior (Redbo *et al*., 1998) and altered cartilage development (Glade and Belling, 1984). With this background, there is a need for diets that support the natural behavior and the digestive and metabolic systems of horses. Recent studies show that the energy requirements in adult Standardbred horses in training can be met by using forage-only diets with a high energy content (≥ 10.4 MJ metabolizable energy (ME)/kg dry matter (DM)) (Connysson *et al*., 2006; Jansson and Lindberg, 2012) and it has been suggested that a forage-only diet may be beneficial for performance compared to a diet rich in starch (Jansson and Lindberg, 2012). For growing horses, *ad libitum* access to a diet containing 60-80% alfalfa/oat straw resulted in an increased growth rate compared to when horses were fed according to recommendations (Cymbaluk *et al.*, 1989). It may therefore be hypothesized that Standardbred horses fed a high energy forage-only diet *ad libitum* will grow well and will be able to reach a conventional performance goal of qualifying for official races at the age of 3 years. As far as we know, there are until now no long-term studies evaluating the effects of feeding a forage-only diet to growing horses in training during controlled conditions. The aim of this study was therefore to document growth and performance in qualification races in 16 Standardbred yearlings fed a forage-only diet from the age of 1.5 until the end of the 3-years season.

Materials and Methods

Horses and management

The study was conducted at the National Centre for Trotting Education (Wången, Sweden) and started in August 2010. Sixteen Standardbred horses born the year before were from the age of 1.5 years until the end of the 3-years season included in the project. The horses came from four breeders and were all kept on pasture at least two months prior to the study. During week days, horses were kept 8-14 h in individual boxes (9 m^2) with sawdust litter, and the rest of the time outside in a paddock depending on weather conditions. During weekends and between June-September, they were kept in a paddock (approximately 20 000 m^2) for 24 h with access to shelters, feed and water.

Feeding and training

During the whole study, horses had free access (both in the stable and in the paddocks) to haylage (50-71 % DM, 10.2-11.7 MJ ME/kg DM and 99-156 g crude protein/kg DM), which was supplemented with a commercial mineral- and vitamin feedstuff and 0.25-1 kg of a pelleted Lucerne product (95 % Lucerne, 5% molasses, Krafft AB, Sweden) to meet the

nutrient requirements (NRC, 2007). The haylage was a mixture of meadow fescue and timothy and was harvested in Enköping, Sweden (Latitud: N 59º 37.8. Longitud: E 17º 04.5) and both 1st and 2nd cuts were used. From the end of May until the beginning of September, horses also had access to some pasture grass. Inside the stable, haylage was provided in a crib placed in one corner of the floor of each box to minimize wastage. In the paddock, haylage was provided from 2-3 feeding stations. New haylage was provided once a day in the box and every 3-4 days in the paddock. In the box, water was offered from two 20 l buckets that were refilled twice a day, and in the paddock water was provided from a big tub.

Training program and performance goals was defined together with a reference group consisting of four professional trainers with international experience. Training started with breaking in the horses in September when they were 1.5 years old, and was gradually increased. As 2- and 3-year olds, horses were exercised 2-3 times a week, both on the race track in heats of 1600/1100 m and intervals of 4-6 times 500-700 m or 4-6 intervals of 600 m in a slope (4.8% incline) and in a slightly hilly terrain of 8-12 km. Speed and number of intervals was gradually increased as the horses got older and more fit. The goal was the same for all horses; to pass a qualification race (2140 m at a speed of least 11.76 m/s) as 3-year olds. This is required for horses to be allowed to participate in official races according to the rules of the Swedish Trotting Association.

Registrations, calculations and statistical analyses

Body weight (BW) was registered on a scale (weight indicator U-137, UNI Systems and Vågspecialisten, Skara, Sweden) once every week. An average weight for every 8 weeks period (except for the last period which consisted of 10 weeks) for each horse was calculated and used for statistical analyses. BW was then analyzed by ANOVA and Tukey's test (Statistical Analysis Systems package 9.2) to define differences between periods. Values are presented as lsmeans ± stderr.

Results and Discussion

Performance

Fifteen horses (94%) passed a qualification race during the 3-years season. This means that the hypothesis, that it is possible to get young horses in a condition to pass a qualification race as 3-year olds without feeding any starch rich feeds, could be accepted. The results may be compared with the results from the cohort of Swedish Standardbred horses born the same year, which was in total 3554 registered horses (Swedish Trotting Association, personal communication, 2013). Out of these, 2114 passed a qualification race which corresponds to 59%. A forage-only diet seems therefore not to limit the capacity for passing a qualification race for 3-year old Standardbred horses.

Growth

Horses increased their average BW with approximately 80 kg during the study, from 424±2 kg to 504±2 kg. From February as 3-year olds, the increase in BW was no longer significant ($P>0.05$) and it is therefore possible that the horses at this point had reached their mature BW. This is also in agreement with BW in fit mature Standardbred horses in earlier studies (492±40 kg (Palmgren-Karlsson *et al.*, 2002), 443-548 kg (Connysson *et al.*, 2006) and 515±21 kg (Jansson and Lindberg, 2012).

Conclusions

This study showed that Standardbred horses fed a forage only diet with high energy content *ad libitum* from the age of 1.5-3 years may 1) reach a conventional goal of qualifying for official races as 3-year olds and 2) grow well and reach their mature body weight in spring as 3-year olds.

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Effects of pasture-associated fat deposition on the expression of adipocytokine, chemokine and insulin signalling pathway genes in Finnhorse mares

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Introduction

Equine metabolic syndrome is a health disorder characterized by obesity, insulin resistance (IR), laminitis, dyslipidemia, hyperleptinemia and altered reproductive cycling (Frank *et al*., 2010). In equids, there is a great health concern about the metabolic effects of obesity and its association with IR, laminitis, and increased inflammatory cytokine expression (Hoffman *et al*., 2003; Vick *et al*., 2007; Frank *et al*., 2010). Diet appears to play an important role in eliciting IR in horses, particularly pasture forages or feeds rich in non-structural carbohydrates (Treiber *et al*., 2005). Diets which change insulin dynamics may trigger laminitis (Asplin *et al*., 2007; Frank *et al*., 2010). Adipose tissue is known to play a fundamental role in metabolism and homeostasis regulation through adipocytokines secretion. Various cytokines are synthesized by adipose tissue or by macrophages in adipose tissue. Abnormal amount of cytokines such as tumor necrosis factor α (TNFα), leptin (LEP), adiponectin (ADIPOQ), retinol binding protein 4 (RBP4), and monocyte chemoattractant protein 1 (MCP-1) may contribute to increased IR. Additionally, lipogenesis related genes (e.g. stearoyl-CoA desaturase, SCD) and insulin signalling pathway genes (e.g. insulin receptor substrate, INSR) can be used as indicators of IR in adipose tissue. In the current study, our objective was to analyse pasture-associated effects on gene expression in adipose tissues of Finnhorse mares. In addition, our aim was to analyse associations between fat deposition and gene expression and use these associations as indicators of IR in adipose tissue.

Materials and Methods

The experimental procedures were conducted under the protocols approved by the National Animal Ethics Committee in Finland. Twenty-two mares were divided into two groups, and grazed either on cultivated grassland (CG) or semi-natural grassland (NG) from the end of May to the beginning of September at MTT Agrifood Research Finland. The area of 4.5 ha was grazed rotationally in three paddocks and had tall fescue (*Festuca arundinacea* Schreb.), timothy (*Phleum pratense* L.), and meadow fescue (*Festuca pratensis* L.) as dominant grass species. Two areas of NG were chosen as trial areas. The first area consisted of a 1.2 ha field and 6.5 ha of meadow/forest and the second area of a 2.2 ha field and a 3.6 ha meadow/forest, respectively. The fields had been established as meadow fescue 7-11 years before and grazed since then. Sixteen of these mares, ranged from 6 to 19 years old, were used for gene expression profiling. Body condition score (BCS), body weight (BW), waist (abdominal) circumference, and depth of subcutaneous adipose tissue (SAT) at neck and tailhead were measured using ultrasound scanning both in May and September (Henneke *et al*., 1983; Carter *et al*., 2009).

Adipose tissue samples from neck and tailhead were collected both in May and September, snap-frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted using RNeasy

Lipid Tissue Kit (Qiagen GmbH, Hilden, Germany). Anchored-oligo $(dT)_{18}$ primers were used to synthesize cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Quantitative real-time PCR was conducted using LightCycler 480 instrument (Roche Diagnostics GmbH, Mannheim, Germany). Mitochondrial-like 39S ribosomal protein L39 (MRPL39) was used as an internal control gene. The following genes were examined: ADIPOQ, adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2), INSR, LEP, MCP-1, RBP4, TNFα and SCD.

Statistical analyses were based on ΔCt values (Livak and Schmittgen, 2001). Data of May and September were analyzed separately using MIXED procedure of SAS including a fixed effect of treatment and a random effect of pair. Both May and September data were analyzed in the same model using MIXED procedure of SAS including fixed effects of treatment, time, interaction between treatment and time, and a random effect of pair.

Results and Discussion

Results of body measurements have been presented by Särkijärvi *et al*. (2012) for the 22 horses in the trial. Results for the sub-group of 16 horses used in gene expression profiling were mostly similar to the results of Särkijärvi *et al*. (2012) (Table 1). However, subcutaneous fat thickness at neck and tailhead was similar between treatment groups. Treatment × time interactions were detected for BCS ($P < 0.05$) and BW ($P < 0.10$), suggesting that fat deposition was greater in CG than in NG. Body condition score of the CG group was 6.8 at the end of grazing season compared to 5.5 in the NG group, suggesting that CG mares might be regarded as overweight if not obese. A time effect ($P < 0.05$) was observed for the fat depth showing increased fat accretion during grazing season in both groups.

Table 1 Effect of two pasture types on the body measurements of Finnhorse mares

Item[a]	Time	Treatment[b]			Statistical significance (P value)[c]		
		NG	CG	SEM	Treatment	Time	trt * Time
BCS	May	5.7	5.4	0.22	0.05	0.01	0.001
	September	5.5	6.8	0.27			
BW, kg	May	545.2	552.4	13.35	0.03	0.001	0.09
	September	570.2	620.7	14.22			
FD at neck, cm	May	0.9	1.0	0.09	0.16	0.02	0.70
	September	1.1	1.2	0.07			
FD at tailhead, cm	May	2.3	1.8	0.16	0.64	0.003	0.21
	September	2.9	3.1	0.38			
Waist, cm	May	207.9	209.5	2.98	0.09	0.01	0.26
	September	212.0	219.2	2.22			

[a]BCS=body condition score, BW=body weight, FD=fat depth. [b]NG= semi-natural grass group, CG=cultivated high yielding grass group, SEM=standard error of mean. [c]trt=treatment.

Results of gene expression analyses at neck and tailhead SAT are presented in Figures 1 and 2. Gene expressions of ADIPOQ, AdipoR2, RBP4 and INSR at both neck and tailhead SAT were not different between treatment groups. A trend for an increase in the mRNA abundance of AdipoR1 at neck SAT was detected in the CG group compared to the NG group. Adiponectin expression is decreased in obesity and inversely correlated with IR, glucose intolerance and dyslipidemia. Adiponectin receptor 1 and 2 are expressed in peripheral tissues, where adiponectin mediates fatty acid metabolism and energy homeostasis (Kadowaki and Yamauchi, 2005). Insulin receptor substrate play an important role in the regulation of glucose homeostasis and binding of insulin to INSR stimulates glucose uptake. Increased

serum RBP4 levels have been detected with obesity, IR and type 2 diabetes in humans (Graham *et al*., 2006). The lack of difference in gene expression of ADIPOQ, RBP4 and INSR between treatment groups indicates that the CG mares had no signs of increased IR. Concordantly, we observed down regulation of RBP4 and INSR, and up regulation of ADIPOQ, AdipoR1 and AdipoR2 in tail head SAT in September compared to May across the groups.

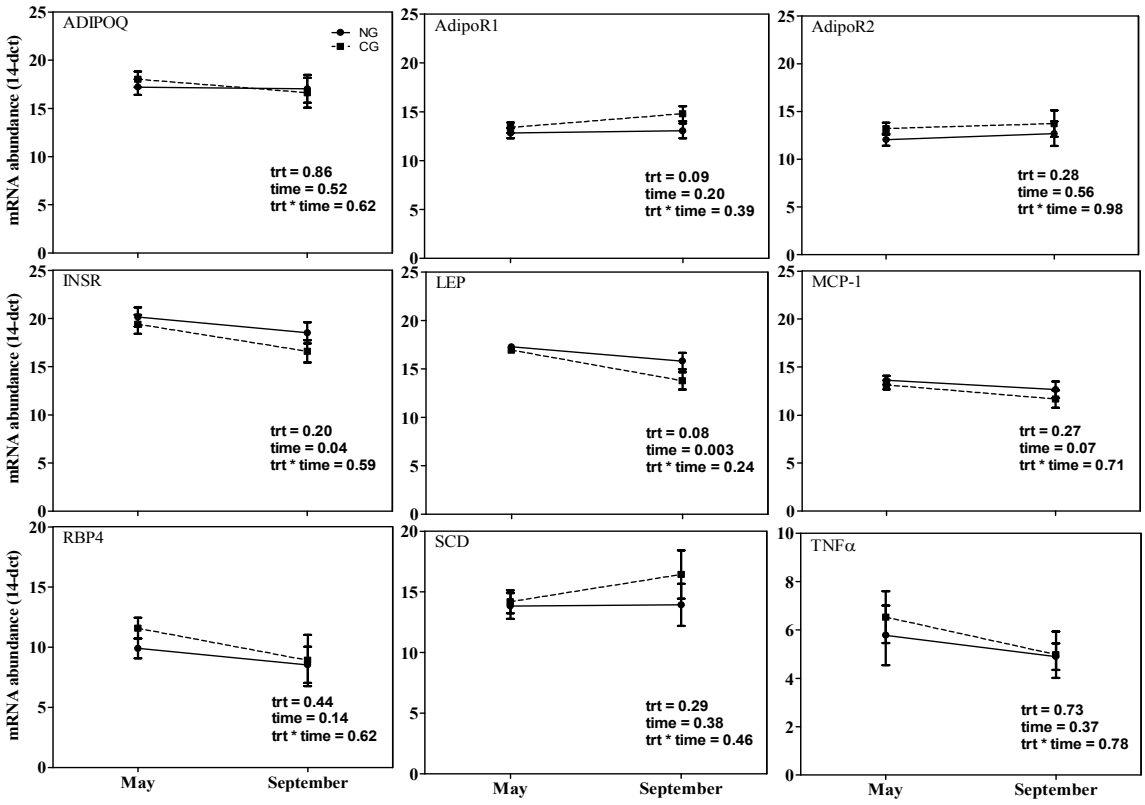

Figure 1 Relative mRNA abundance of neck subcutaneous adipose tissue genes (14-ΔCt) of Finnhorse mares grazed either on cultivated high-yielding pasture (CG) or semi-natural grass (NG) from the end of May to the beginning of September. Values are expressed as mean ± SEM.

We observed a trend for higher LEP mRNA expression at neck SAT in NG compared to CG while no difference at tailhead SAT were present between groups. Leptin is a key endocrine signal of adipose tissue mass and nutritional status (Houseknecht *et al*., 1998). This finding may indicate that horses would have to undergo a greater change in BCS to detect subsequent change in LEP gene expression. Furthermore, the differential pattern in the mRNA expression of LEP and AdipoR1 between neck and tailhead SAT was unexpected, and might be due to adipose tissue location as well as individual differences in adipose tissue distribution. We did not observe any difference in the mRNA expression of SCD between NG and CG. The tailhead SAT exhibited higher expression of SCD than neck SAT, thus lipogenesis may differ among fat depots.

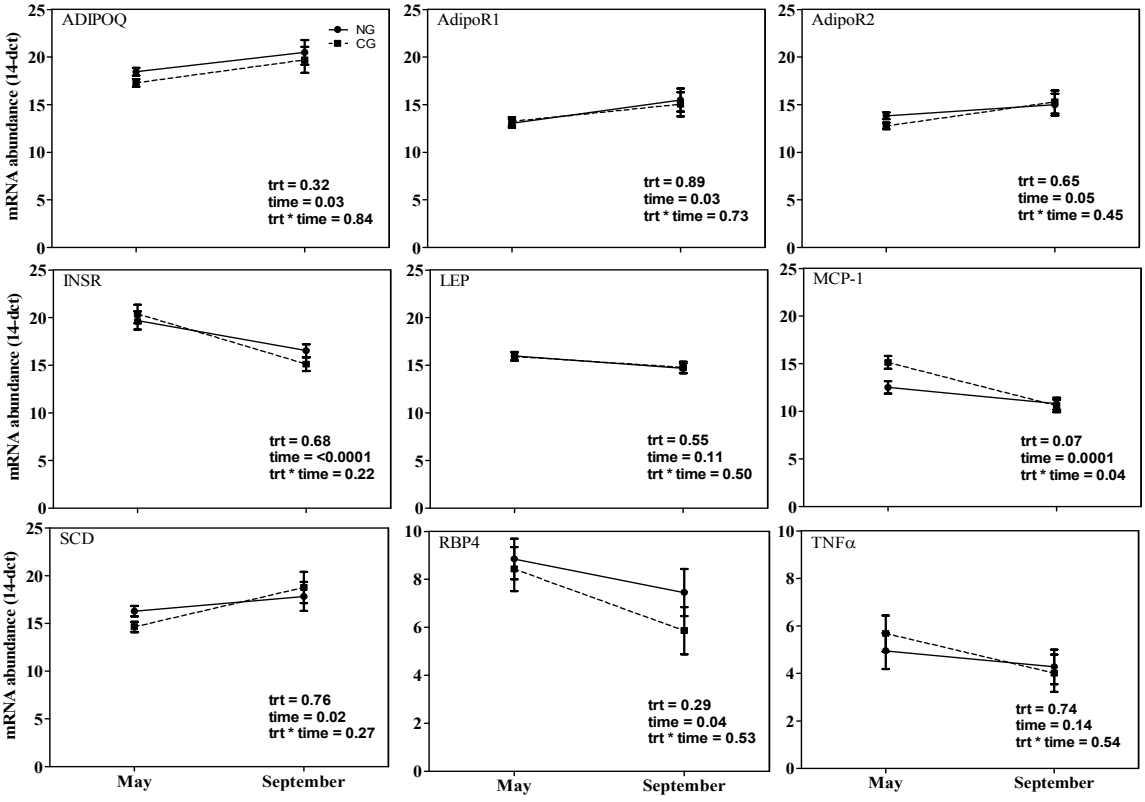

Figure 2 Relative mRNA abundance of tailhead subcutaneous adipose tissue genes (14-ΔCt) of Finnhorse mares grazed either on cultivated high-yielding pasture (CG) or semi-natural grass (NG) from the end of May to the beginning of September. Values are expressed as mean ± SEM.

We did not detect any treatment or time related differences in the mRNA expression of TNFα among groups. However, Vick *et al*. (2007) reported that increased TNFα expression is associated with increased BCS and fat % in horses. A higher mRNA expression of MCP-1 in tailhead SAT was observed in CG compared to NG in May, but this difference disappeared by September. Treatment × time interactions were observed for MCP-1, indicating that MCP-1 expression was lower in the CG group during grazing season. In addition, there was downregulation in MCP-1 mRNA abundance of both neck and tailhead SAT in September compared to May across the groups. In agreement with this, Burns *et al*. (2010) reported that in non-obese or over-conditioned horses, MCP-1 mRNA was not different across adipose depots or altered by insulin sensitivity status.

Conclusions

Based on the SAT gene expression at neck and tailhead, the BCS and weight gain in the current study were not associated with increased IR. The lack of differences in the expression of studied genes between groups may indicate that horses would have to undergo a greater change in BCS to detect subsequent changes in gene expression profiles related to obesity and IR. Changes in the mRNA abundance of the studied genes from May to September in both groups were congruent with increased body weight and fat deposition during the grazing

season. Our results suggest that grazing on cultivated high yielding pasture may not increase the risk for metabolic diseases in healthy Finnhorse mares when BCS is below 7.

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Fecal and cecal pH in horses fed hay only or hay and pelleted barley

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Introduction

Diet effects on the hindgut environment are sometimes evaluated based on a single fecal sample (Berg et al. 2005; Zeyner et al. 2004) as the accessibility of cannulated horses is limited. Whether a fecal sample really mimics the hindgut environment is though questionable. Four cecum cannulated horses were used to study diet effects on pH changes simultaneously in the cecum and in feces over 24 hours, when two different diets were fed in a cross over experiment.

Materials and Methods

The experimental periods consisted of twelve days of adaption to a diet followed by one day of measurements. The horses were housed individually in stalls with wood shavings as bedding material and allowed access to a dirt paddock for approximately six hours a day during the adaption period. The two diets fed in the experiment were a hay only diet (diet 1) or a hay supplemented with concentrate diet (diet 2). The horses were fed 2.7 kg of hay 3 times a day (06:00, 14:00 and 22:00 h) when fed diet 1, and 2.0 kg of hay 3 times a day (06:15, 14:00 and 22:00 h) supplemented with 2.1 kg of pelleted barley (~2 g starch per kg body weight) fed once a day (06:00 h) when fed diet 2. The feeding is illustrated in Figure 1, where 06:00 h = time 0.

The horses were fitted with a permanent cannula at the base of the cecum close to the ileocecal junction, from where samples of cecal fluid could be obtained. Samples of cecal fluid and feces were collected just before the 06:00 h meal and sampling continued for 24 hours. Every hour a sample of approximately 100 ml cecal fluid was taken and pH was measured immediately, and every second hour a fecal grab sample was taken (simultaneously with the cecal sample) and pH was measured immediately after mixing of 15 g of feces with 15 g ionized water.

The data was analyzed as repeated measurements using the MIXED procedure SAS® (Version 9.3, SAS Institute Inc. Cary, North Carolina, USA) where diet, time and the interaction diet x time were included as fixed effects, and horse and period within horse were included as random effects. Mean effects were considered statistically different if $P<0.05$.

Results and Discussion

The pH changes in the cecum and in feces measured over 24 hours are shown in Figure 1. The mean pH of the two diets at each time point and the standard error of the mean (SEM) are presented. The decrease in cecal pH on diet 2 was probably caused by easily fermentable starch reaching the cecum, and as expected there was an effect of the interaction diet x time ($P<0.001$) on pH changes in the cecum. Cecum pH was lower 2 to 9 hours after feeding on diet 2 compared to diet 1. The pH was much more stable when diet 1 was fed, and the three daily meals of hay only caused small fluctuations in cecum pH. The SEM was also much smaller when hay was fed compared to the meal of barley, as can be seen in Figure 1, which

shows that the individual variation was larger when the horses were fed diet 2 compared to diet 1. Chewing intensitiy might have affected the small intestinal digestibility of the starch (Meyer *et al.* 1995) and differences in hay intake time might have influenced the passage rate through the stomach and small intestine (Kienzle 1994).

Fecal pH was lower than cecal pH (Figure 1), and there was no effect of diet (P=0.40) or the interaction diet x time (P=0.50) on fecal pH, however, there was an effect of time (P=0.02). A similar effect of time of sampling was also reported by Jensen *et al.* (2013) when two concentrate meals were compared and pH in feces was evaluated. This illustrates the importance of taking fecal samples at the same time in experiments where only one sample is taken, to obtain comparable results.The mean retention time of feedstuffs is often 24 hours or more but varies depending on several factos (e.g. feedstuffs and feeding level) (Van Weyenberg *et al*. 2006). The effect of the barley meal fed the day before on fecal pH was expected to be seen after the morning meal with a time delay of ~24 hours or more. However, it was not possible to see a clear decrease in fecal pH at any time that could be explained by the single barley meal in diet 2.

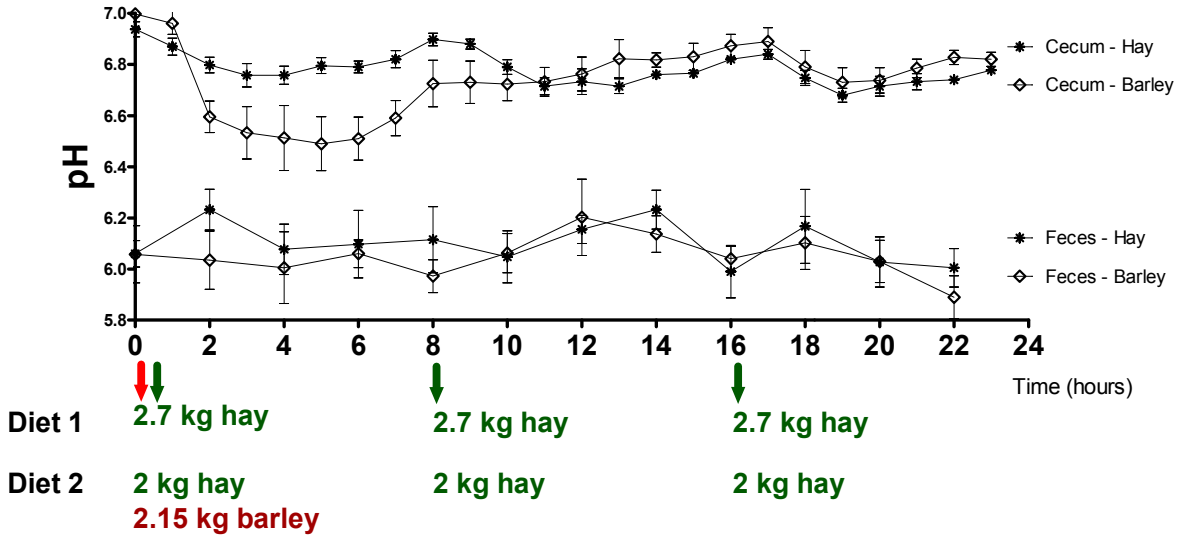

Figure 1 The effect of hay or a hay supplemented with pelleted barley diet on cecal and fecal pH measured over 24 hours (mean ± SEM). The arrows illustrate feeding times and amounts.

The diets might not have been extreme enough to cause an effect of diet on pH in feces. If the level of barley had been higher it could be expected that fecal pH had been lower on a mixed diet compared to a hay only diet.

Conclusions

The effect of diet on pH changes in the cecum were clearly documented, when a diet containing pelleted barley was compared to hay. However, these effects were not found in fecal samples in which only sampling time affected the results when studying diet effects on fecal pH.

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Haylage harvest in late plant maturity – effects on ingestion and digestion in horses

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Introduction

Adult horses, not performing strenuous exercise, have low nutrient requirements (NRC, 2007). To avoid development of stereotypic behaviour (McGreevy *et al*., 1995), behaviour indicating poor welfare (Benhajali *et al.,* 2009) or digestive upsets (Archer and Proudman, 2006), sufficiently long eating time is required. Forage harvested at in late plant maturity is less digestible and therefore provides the horse with less energy and protein compared to plants harvested in early maturity. However, information on how plants harvested in different maturities affect ingestive behaviour in horses is scarce. As mature plants have a higher stem to leaf ratio and rough stems, they may take longer time to ingest compared to immature plants. An experiment was therefore conducted were the effect of plant maturity at harvest on equine ingestion and digestibility was studied.

Materials and Methods

Haylage

Haylage was harvested from primary growth of a grass-dominated sward at the beginning of June, July and August in 2009. The crops were wilted to approximately 570 g dry matter (DM)/kg, and were baled and wrapped in round bales (Müller, 2011). Samples were taken when bales were opened for feed out. Analyses were made for content of DM and neutral detergent fibre ($aNDF_{om}$) exclusive of residual ash according to Van Soest *et al.* (1991), as modified by Chai and Udén (1998). The content of acid detergent fibre (ADF_{om}) was analysed according to AOAC (1990; # 973.18) and reported exclusive of residual ash. Analysis of lignin ($pm_)$ used permanganate oxidation according to Robertson and Van Soest (1981). *In vitro* digestibility of organic matter (IVDOM) was estimated by a 96 h method, using fresh rumen inocula from dairy cows, and content of metabolizable energy for ruminants (ME_r) was calculated from IVDOM-values (Lindgren 1979 as corrected in 1983). Content of metabolizable energy for horses (ME_h) was calculated from ME for ruminants (ME_r) using the equation $ME_h = (1.12\ ME_r) – 1.1$ (Lindberg and Ragnarsson, 2010; Jansson, 2011). The concentration of N was determined by a Kjeldahl method using Cu as a catalyst, and crude protein (CP) content was calculated from total N.

Horses and feeding experiment

The three haylages were fed to twelve horses in a change-over study consisting of three periods, where each period comprised three weeks (two weeks adaptation time and one week sampling time in each period). Horses were fed haylage in amounts corresponding to ca 1.5 their maintenance requirement for metabolizable energy (ME) (NRC, 2007), and the haylage ration was divided on four daily feedings (06:00, 11:00, 16:00 and 20:00 h), with the largest part fed at 20:00 h. Horses also received 0.5 kg molassed sugar beet pulp soaked in water, mineral pellets and NaCl once daily at 20:00 (details in Müller, 2012). On average, horses were fed 1.4 (sd 0.21), 1.5 (sd 0.26) and 1.8 (sd 0.23) kg DM/100 kg body weight of June, July and August haylage, respectively, to keep the diets as isoenergetic as possible.

Ingestion and digestion

Ingestion variables were recorded once daily for each horse during the last week of each period at the evening meal, (20:00 h) and consisted of eating time (kg DM/min), chewing rate (chews/min), swallowing rate (swallowings/min), number of chews/swallowing and number of chews/kg DM. Apparent digestibility was estimated using acid insoluble ash (AIA) as a marker (Van Keulen and Young, 1977; Bergero *et al*., 2009). During the last week in each period, fresh faeces were sampled once daily from each horse, frozen, and then pooled to produce one sample per horse and period before analysis. Faecal samples were then analyzed for content of DM and AIA (Van Keulen and Young, 1977). Content of AIA was also analyzed in duplicate for all feeds according to the 2 N acid method described by Bergero *et al.* (2009).

Results and Discussion

Haylages differed in chemical composition; fibre and lignin content increased and content of CP, ME_h and IVDOM decreased with advancing plant maturity at harvest (Table 1). Ingestion variables showed that haylage harvested in July and August took longer time to ingest compared to when harvested in June (Table 2). The number of chews before swallowing increased with plant maturity at harvest (Table 2). The apparent digestibility of diet DM decreased with plant maturity at harvest (Table 3). Haylage harvested in August had an apparent digestibility close to values previously reported for oat straw (0.48) in ponies (Cuddeford *et al*., 1995) and of late cut timothy haylage (0.46) in Icelandic horses (Ragnarsson and Lindberg, 2008). As horses were fed isoenergetic (ME) diets using only the forages to balance energy intakes, the feeding level differed for the haylages and was highest for August haylage. This may have influenced the resulting apparent digestibility, as increased feeding level have been reported to decrease mean retention time of digesta in horses (Pearson *et al*., 2006) as well as apparent digestibility (Ragnarsson and Lindberg, 2010). However, a high feeding level of forage without increased digestibility or energy intake is an advantage for the horse with low nutrient requirements, as it prolongs eating time.

Table 1 Chemical composition (g/kg DM if not otherwise mentioned) of haylages harvested from the primary growth of the same ley in June, July and August. Means with different superscripts differed at P<0.05

Variable	June	July	August	SEM
Dry matter, g/kg	549	573	583	10.8
Neutral detergent fibre	522[a]	610[b]	637[c]	9.0
Acid detergent fibre	300[a]	385[b]	422[c]	4.0
Lignin (pm)	56[a]	85[b]	100[c]	2.4
Crude protein	130[a]	93[b]	80[c]	3.6
In vitro digestible organic matter	871[a]	687[b]	613[c]	5.3
Estimated metabolizable energy for horses, ME_h/kg DM	12.4[a]	9.1[b]	7.7[c]	0.10
Acid insoluble ash	14	21	24	3.6

Table 2 Ingestion variables in horses fed haylage harvested from the primary growth of the same ley in June, July and August, means and standard deviation (stddev). Means with different superscripts differed at P<0.001

Variable	June		July		August	
	Mean	stddev	Mean	stddev	Mean	stddev
Eating time, min/kg dry matter	29[a]	4.4	37[b]	7.4	36[b]	9.8
Chewing rate, chews/min	84[a]	5.9	78[b]	6.3	77[b]	7.4
Swallowing rate, swallowings/min	1.9[a]	0.46	1.3[b]	0.48	1.2[b]	0.43
No. of chews/swallowing	51[a]	11.7	65[b]	22.7	81[c]	34.4
No. of chews/kg DM	2472[a]	388.0	2947[b]	546.5	2969[b]	660.1

Table 3 Content of dry matter (DM) and acid insoluble ash (AIA) in faeces, and apparent DM digestibility of diets estimated by AIA in feeds and in faeces from horses fed haylage harvested from the primary growth of the same ley in June, July and August. Means with different superscripts differed at P<0.05

Variable	June	July	August	SEM
Dry matter, g/kg faeces	203	195	210	4.0
Acid insoluble ash, g/kg DM faeces	58[a]	50[b]	42[c]	2.5
Apparent digestibility of diet dry matter	0.75[a]	0.59[b]	0.44[c]	0.020

Conclusions

Feeding haylage harvested late (July and August) resulted in longer ingestion time and lower apparent dry matter digestibility in horses, compared to feeding haylage harvested in June. This harvest strategy may therefore be an interesting alternative for the feeding of horses with low nutrient requirements.

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BLGG AgroXpertus has been the leading horticultural and agricultural analytical laboratory in The Netherlands for over 80 years. With it`s sampling, analytical and advisory activities BLGG AgroXpertus plays a supportive role in agricultural and horticultural procedures, thereby improving production and lowering production costs. BLGG AgroXpertus offers analyses and advice on nutrient content and quality of soil, manure, water, substrate, crops and cow feed to almost every Dutch farmer and crop grower. The last 12 years, BLGG AgroXpertus, expanded the activities to Europe. 2011 BLGG started up forage analyses in Hörby, Sweden as well.

The company has its own accredited sample collection and transport system, ensuring that samples are collected according to predefined guidelines and ensuring fast overnight transport to the different laboratories. Samples can be transported refrigerated if needed. In total, more than 500.000 samples/year are analyzed in accredited and highly automated BLGG AgroXpertus laboratories.

BLGG AgroXpertus did more than 100 000 analyses of forage 2011. BLGG AgroXpertus has developed a model based on local calibration, that calculates a large number of parameters with very high reliability. Over the years, BLGG AgroXpertus has collected a large number of calibration sets containing data from samples that are analyzed by both NIR and reference methods. BLGG AgroXpertus has separate databases for all common roughage, raw materials and Total Mixed Ration (TMR) types. Standard NIRS calculation models can miscalculate the parameter values when the roughage under analysis is contaminated with soil. Therefore, BLGG AgroXpertus, always determines the crude ash content in roughage in the traditional way. Thanks to a partnership with Masterlab, BLGG AgroXpertus has calibration sets for raw materials as well.

A novel sterilization technique with application to silage research and inoculant evaluation

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Introduction

Despite elaborate knowledge in biochemistry and microbiology of ensiling process, a large proportion of variation often seen in ensiling results cannot be explained in most circumstances. One likely source for this variability could be changes of field floral composition caused by environmental factors such as humidity, temperature and ultraviolet radiation (Schreiber et al., 2004). To test this effect, it is necessary to systematically manipulate microfloral composition on the crop. However, with extant sterilization techniques, this is rather infeasible as these methods are either expensive and in many cases impractical (*i.e.* gamma-irradiation; Heron et al., 1986) or result in extensive losses of substrates (*i.e.* autoclaving; Graham et al., 1985). Therefore, this study aimed to evaluate an effective, practical and inexpensive sterilization technique.

Materials and Methods

Samples used were grass or red clover harvested by hand with minimal soil contamination from fields nearby the Kungsängen Research Centre, Uppsala, Sweden. Each sterilization treatment was tested on two crop samples (2-3 kg each) with samples being harvested from two different spots on the field either on one day or two consecutive days except otherwise stated. The trials were as follows.

Ethanol (Trial 1)

From an intact mixed grass sample, bundles of approximately 200 g were formed and subjected to one of the following treatments: dipping in 700 mL/L ethanol (EtOH700) for 1) 30 seconds, 2) 30 seconds followed by 30 seconds rinse with distilled water, 3) 60 seconds or 4) 60 seconds followed by 30 seconds rinse with distilled water. Thereafter, treated samples were aerated in a ventilation chamber for 30 minutes at 20±2°C and aseptically chopped (3-5 cm length) and sub-sampled (30 g) for microbial analysis.

Ethanol /sodium hypochlorite/ethanol (Trial 2)

A bundle of 200 g was tied from an intact matured timothy sample and dipped in EtOH700 for 60 seconds. Thereafter, the bundle was dipped in sodium hypochlorite (NaClO) containing 60-140 g/L of active chlorine for 60 seconds followed by dipping in concentrated ethanol (960 mL/L) for 30 seconds and then in sterile water for 60 seconds. The sample was then chopped (3-5 cm length) aseptically, aerated at 40°C for 20 minutes and sub-sampled (30 g) for microbial analysis.

Ethanol /sodium hypochlorite, and sodium hypochlorite (Trial 3)

From an intact matured red clover sample, two bundles of 200 g were tied and subjected to one of the following treatments: 1) the bundle was placed in a polypropylene cylinder (9 cm diameter, 36.5 cm height) equipped with a valve at the bottom. Then, EtOH700 was poured into the cylinder covering the sample for 60 seconds before draining off the ethanol. Likewise, the sample was treated with NaClO (60-140 g/L active chlorine) for 120 seconds

and then twice with sterile water for 30 seconds. Finally, it was aerated at 35°C for 25 minutes and aseptically chopped (3-5 cm length) and sub-sampled (20 g) for microbial analysis. Or 2) same as in 1) except the EtOH700 step was eliminated and treatment with NaClO was prolonged to 180 seconds.

Neutral detergent solution (Trial 4)

From an intact mixed grass sample, bundles of 200 g were tied and subjected to one of the following treatments: 1) dipping twice in sterile water for 30 seconds (ND0), 2) dipping in 40°C neutral detergent (ND; Van Soest et al., 1991) solution (30 mL saturated ND/L) for 30 seconds followed by dipping twice in sterile water for 30 seconds (ND30) or 3) same as in 2) but concentration of ND solution was increased to 50 mL/L (ND50). The treated samples were then aerated at 40°C for 15 minutes and aseptically chopped (3-5 cm length) and sub-sampled (20 g) for microbial analysis.

Cold shock (Trial 5)

A matured timothy sample was chopped to a length of 3-5 cm and representative sub-samples (30 g each) were subjected to one of the following treatments: 1) freezing at -20°C for 24 hours, 2) freezing at -20°C and then at -60°C for 3 hours at each temperature and finally at -80°C for 18 hours or 3) freezing at -80°C for 24 hours. The samples were then thawed and subjected to microbial analysis.

Moist and dry heat (Trial 6)

A mixed grass sample of 3 kg was wilted for 1 hour at 40°C in a force drought oven to reach a dry matter (DM) concentration from approximately 260 g/kg to 400 g/kg. The crop was then chopped to a length of 3-5 cm and divided into two batches. Each crop replicate was then freeze dried to a DM concentration of 950 g/kg and milled to pass a 1-mm screen in a hammer mill. Thereafter, 10-20 g of ground sample was weighed in the sterile glass beakers covered with sterile aluminum foil and subjected to either: moist heating at 121°C and 320 kPa for 20 minutes (autoclaving) or dry heating at 121°C in a forced drought oven for 20 (Heat I), 30 (Heat II) or 45 minutes (Heat III). The unheated (control), moist and dry heated samples were then reconstituted to the same DM concentration as the wilted crop by adding sterile distilled water. An amount of 5-10 g of the reconstituted materials was then subjected to microbial analysis.

Microbial analysis

Microbial analysis was carried out according to Seale et al. (1986). Sterile 0.25-strenght Ringer solution (Merck KGaA, Darmstadt, Germany) was added to the samples at a proportion of 9:1 (v/w) before macerating for 2 minutes in a stomacher (Seward 3500, Seward Ltd, Worthing, UK). Thereafter, serial 10-fold dilutions were prepared from the aqueous extract using the same Ringer solution as the diluent. The diluted microbial cultures were then tested in duplicate for viable counts of lactic acid bacteria (LAB) and enterobacteria by the pour-plate method, and viable counts of yeasts and moulds by the spread-plate method. In the pour-plate technique, 1 mL of microbial culture was pipetted into sterile Petri plates before pouring molten agar medium and swirling the plates. In the spread-plate technique, 0.1 mL of microbial culture was pipetted onto agar Petri plates and spread evenly using a sterile glass spreader. The culturing media used to enumerate LAB, enterobacteria and yeast/moulds were Rogosa agar, crystal-violet neutral-red bile glucose agar and malt extract agar, respectively. All media were obtained from Merck KGaA,

Darmstadt, Germany. LAB were incubated anaerobically for 3 days at 30°C. Enterobacteria were incubated aerobically for 2 days at 37°C. Yeasts and moulds cultured on the same Petri plates were incubated aerobically at 25°C and counted after 3 and 5 days, respectively.

Chemical analysis

Concentration of DM was estimated after drying at 103°C over night in a forced draught oven. Ash was analyzed by incineration at 550°C for 3 hours in a muffle furnace. Concentration of water soluble carbohydrates was estimated by the Fourier transform mid-infrared transmission spectroscopy technique after hot water extraction as described by Udén (2010). On similar extracts, pH was also measured by a pH-meter (Metrohm 654, Metrohm AG, Herisau, Switzerland). Nitrogen concentration was determined by the Kjeldahl method on a 2400 Kjeltec analyzer unit with Cu as a catalyst. Buffer soluble N (BSN) was measured according to Udén and Eriksson (2012) after 60-minute extraction and the centrifugation-passive filtration separation method. The N fraction in residues after acid detergent extraction (Van Soest, 1973) was also determined (ADIN).

Results and Discussion

The sterilization trials were performed in the form of series during the course of the summer 2012 in an exploratory manner and could therefore not be analyzed statistically. Effects of all sterilization attempts on LAB, enterobacteria, yeasts and mold counts are shown in Table 1.

In Trial 1, 2 and 3, the viable counts of LAB were lowered below the detection limit by EtOH, NaClO and by the combination of the two. Enterobacteria, on the other hand, were noticeably affected only when the exposure to NaClO was extended (Trial 3). Neither yeasts nor moulds were affected by EtOH alone (Trial 1) but seemed more susceptible when NaClO was included or applied solely (Trial 2 and 3). A hypothesis tested in Trial 4 was to physically remove epiphytic microbes by the use of water or detergents. The results, however, showed that this technique might only be effective in reducing LAB counts. Similar results were also obtained when the crop was subjected to freezing temperature (Trial 5). These two techniques could therefore be of a potential use in inoculant studies focusing on LAB strains in relation to other groups of epiphytic microbes. In Trial 6, the heat treatment was applied on dried crop to minimize the magnitude of Maillard reaction, as this heat-induced reaction is water-dependent (Van Soest, 1994). Samples subjected to heat III and II were only analyzed for enterobacteria as the shorter heating time (heat I) had already eliminated other microbes.

From the results it was evident that LAB are the most sensitive group of microbes. Enterobacteria, on the other hand, appeared as the most resistant group, with yeasts and moulds being intermediate in this respect.

Among the tested methods, autoclaving and dry heating appeared as the most effective techniques. Chemical analyses of autoclaved and dry heated samples were therefore made (Table 2). The ADIN fraction increased five times by autoclaving. Autoclaving also resulted in the greatest reduction of WSC (33%) and BSN (56%). In contrast, samples treated by Heat I remained almost unchanged and only small chemical changes were induced by Heat II and III.

Conclusions

Dry heating at 121°C of previously dried plants seems a promising sterilization method by eliminating all the epiphytic microbes and rendering only a minor chemical change. However, the procedure should be further developed and tested on a greater variety of crops. Ensiling of reconstituted crops inoculated with original field floras should be the next step to validate the efficacy of the methodology.

Table 1 Effect of performed sterilization tests on epiphytic microbial counts of grass and red clover samples

Trials[a]	Treatment	LAB[b]	Enterobacteria	Yeasts	Molds
			Log CFU/g FM		
1	Control	3.6[c]	5.4	5.4	4.8
	30 sec	<0.7[d]	4.8	3.6	3.4
	30 sec/rinse	2.0	4.0	3.6	1.9
	60 sec	<0.7	4.2	3.3	2.8
	60 sec/ rinse	<0.7	4.4	3.8	3.3
2	Control	1.1	2.7	4.5	2.3
	EtOH/ NaClO/ EtOH	<0.7	2.1	2.5	<1.7[d]
3	Control	2.7	5.3	4.4	5.7
	EtOH/ NaClO	<0.7	1.2	<1.7	<1.7
	NaClO	<0.7	1.9	<1.7	<1.7
4	Control	3.6	3.6	3.8	4.1
	ND0	2.1	4.1	3.9	4.1
	ND30	1.9	3.2	3.9	4.1
	ND50	1.6	3.7	3.7	3.9
5	Control	1.2	2.5	3.9	3.0
	Cold I	<0.7	2.5	4.0	3.0
	Cold II	<0.7	2.0	4.0	2.9
	Cold III	<0.7	2.5	3.9	2.8
6	Control	2.1	3.5	4.1	4.1
	Autoclaving	<0.7	<0.7	<1.7	<1.7
	Heat I	<0.7	1.1	<1.7	<1.7
	Heat II	-	1.1	-	-
	Heat III	-	<0.7	-	-

[a]1= sterilization property of ethanol (EtOH) at two exposure lengths (30 sec or 60 sec) with or without rinsing with water; 2=combined sterilization property of EtOH and sodium hypochlorite (NaClO); 3=combined sterilization property of EtOH and NaClO or sole NaClO; 4=physical removal of epiphytic microbes by water or neutral detergent (ND) solutions; 5=sterilization property of freezing temperature; 6=sterilization property of moist heat (autoclaving) or dry heat treatments. [b]LAB=lactic acid bacteria; CFU=colony forming units; FM=fresh matter. [c]Values are the mean of two independent replicates. [d]Detection limits. For detailed information on experimental procedures see materials and methods.

Table 2 Chemical composition of a grass sample subjected to autoclaving and dry heating

Treatments[a]	pH[b]	Ash	WSC	ADIN	BSN
		% DM			
Control	6.1[c]	11.4	12.0	0.1	0.9
Autoclaving	5.5	11.7	8.0	0.5	0.4
Heat I	6.1	11.5	11.7	0.1	0.7
Heat II	6.0	11.5	9.8	0.1	0.6
Heat III	5.9	11.4	9.9	0.1	0.5

[a]Autoclaving=moist heating at 121°C and 320 kPa for 20 minutes; Heat I=dry heating at 121°C for 20 minutes; Heat II=dry heating at 121°C for 30 minutes; Heat III=dry heating at 121°C for 45 minutes. [b]WSC=water soluble carbohydrates; ADIN=acid detergent insoluble N; BSN=buffer soluble N; DM=dry matter. [c]Figures are the mean of two independent replicates.

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Effects of delayed sealing during silo filling – Experiments with lab-scale silos

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Introduction

Swedish farmers have generally a good understanding of the recommendations for silage making provided by advisory services. However, few farmers apply the recommendation to seal the silo temporarily when the filling process is interrupted (e.g. overnight break or machinery failure). We believe this might be due to a prevailing uncertainty about the negative effects of delayed sealing. The objective of this study was to evaluate effects of delayed sealing on silage quality.

Materials and Methods

To perform studies in full-scale farm silos is difficult since environmental conditions (e.g. degree of anaerobiosis, moisture content, microbial flora, density and porosity) vary between different places in the silo. A certain treatment applied to a silo will therefor manifest itself in different ways, which usually makes the interpretation of results difficult. This study was therefore performed under controlled environmental conditions in small-scale lab silos.

Picture 1. Glass jar silo with water-filled siphon on lid.

Three lab-scale ensiling trials with forage composed of chopped grass-clover mixtures (approx. 15-30% clover) and with DM contents between 25 to 30% were conducted within a period of two years. Glass jars (1.7 L) with metal lids were used as silos (Picture 1). Water-filled plastic siphons (water locks) mounted on the lids allowed fermentation gases to escape without the ingress of air. Water columns indicated if a silo was badly sealed (i.e. water columns in level). Silos were filled to forage densities ranging from 117 to 134 kg DM/m^3. After filling silos were exposed to air for 0, 5, 10 and 24 hours (aerobic period) before lids were screwed on to seal silos. Silos were brought to a temperature controlled room (20±2°C), put in horizontal position and were covered with 45 mm thick glass wool mats to minimize the loss of respiration heat. Forage temperature was monitored during the aerobic period with 2 small, wireless temperature sensors (Kooltrak logger 214002, www.kooltrak.com) inserted into centre of each silo. Forage samples were collected at the start and end of each aerobic period for analysis of chemical composition (DM, WSC) and lactic acid bacteria (LAB). To follow the course of acidification, 2 replicate silos per aerobic period were sampled after 3, 6 and 18 days to determine silage pH. The three remaining silo replicates were stored for 90 days for a more extensive quality analysis including fermentation products (organic acids, alcohols, ammonia), aerobic stability and pH. Aerobic stability was determined by monitoring the increase of silage temperature during at least 7 days of aerobic exposure at 20°C ambient temperature.

Results were analysed statistically with a one-factorial (aerobic period) completely randomized model. Calculations were made with the PC package SAS 9.1 (SAS Institute Inc., Cary, USA) and the Proc GLM procedure was applied. A significance level of 0.05 (5%) was used for comparisons between aerobic periods.

Results and Discussion

Forage temperature in open silos increased during the aerobic period in relation to the length of the aerobic period. After 24 hours forage temperatures varied between 30° to 37°C (Table 1). The temperature increase was highest in the driest and lowest in the wettest forage, which was expected.

Table 1. Mean forage temperature (°C) in open silos at the end of the aerobic period. Ambient temperature was 20°C.

	Delayed sealing (hours)		
Trial	5 h	10 h	24 h
1	24	26	30
2	28	31	35
3	25	30	37

Ensilability coefficient (FC) of the fresh forage, expressed as a function of buffering capacity (BC), DM and WSC contents (Weissbach 1996), deteriorated as the aerobic period increased. FC values in Table 2 indicate that delayed sealing for more than 10 hours will increase the risk of producing silages with lower fermentation quality. This is in agreement with increasing BC, pH and ammonia-N values and decreasing WSC values during the aerobic period.

Fermentation products typical for badly fermented silages such as butyric acid, 2.3-butanediol tended to increase and lactic acid and WSC levels tended to decrease towards the longest aerobic period (Table 3). This shows that delayed sealing gradually decreased the fermentation quality of silages.

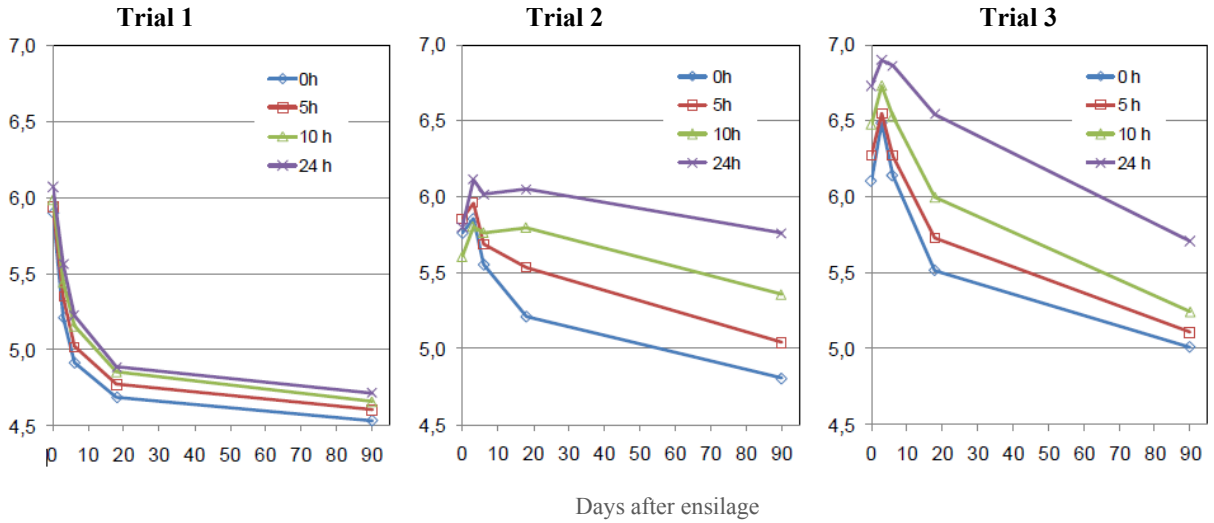

Table 2. Forage composition at the end of the aerobic period.

	Trial 1				Trial 2				Trial 3			
Parameters[1]	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h
DM, %	24.7	25	25.4	24.9	28.3	28.2	28.2	28.1	29.7	29.7	30	29.3
WSC	10.4^{a}	10.0^{ab}	9.6^{b}	8.4^{c}	11.7^{a}	11.0^{b}	10.3^{c}	7.5^{d}	3.3^{a}	2.8^{b}	2.2^{c}	1.5^{d}
BC	7.35^{a}	7.27^{a}	7.44^{a}	7.93^{b}	6.61^{a}	7.09^{bc}	6.98^{b}	7.34^{c}	7.63^{a}	8.11^{b}	8.46^{c}	9.59^{d}
FC	36^{a}	36^{a}	36^{a}	33^{b}	42^{a}	41^{ab}	40^{b}	36^{c}	33^{a}	32^{ab}	32^{ab}	31^{b}
pH	5.92^{a}	5.94^{a}	5.99^{b}	6.07^{c}	5.76^{a}	5.86^{b}	5.61^{c}	5.80^{a}	6.10^{a}	6.27^{b}	6.48^{c}	6.73^{d}
NH_3-N[2]	1.10^{a}	1.11^{a}	1.17^{b}	1.70^{c}	0.66^{a}	1.00^{b}	1.06^{b}	1.41^{c}	1.49^{a}	1.86^{b}	2.74^{c}	4.50^{d}

^{a,b,c,d}Different superscript letters within parameter and trial indicate significant differences at $p<0.05$; [1]BC = buffering capacity in g lactate/100 g forage DM; FC = fermentability coefficient = DM + (8 x WSC / BC) acc. to Weissbach (1996); [2]% of N.

Table 3 Silage composition after 90 days as affected by delayed sealing. Values in % of DM if not indicated otherwise. Values are means of 3 silo replicates

	Trial 1				Trial 2				Trial 3			
Parameters	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h
DM, %	25	24.6	24.5	24.4	27	26.8	26.7	26.4	28.2	29.1	29	28.9
pH	4.53^{a}	4.60^{b}	4.66^{c}	4.72^{d}	4.81^{a}	5.04^{b}	5.36^{c}	5.76^{d}	5.01^{a}	5.11^{b}	5.24^{c}	5.71^{c}
NH_3-N[1]	7.3^{a}	8.0^{ab}	8.6^{bc}	9.0^{c}	12.6	11.9	13.6	13.7	14.7	14.2	14.9	15.6
Lactate	6.6^{a}	6.9^{a}	6.8^{a}	5.5^{b}	6.6^{a}	5.5^{b}	4.0^{c}	2.5^{d}	6.2^{a}	5.7^{b}	5.2^{c}	3.8^{d}
Acetate	1.5	1.6	1.7	1.9	1.4	1.4	1.3	1.5	2.6^{a}	2.6^{a}	2.5^{a}	2.4^{b}
Butyrate	0.03^{a}	0.09^{b}	0.10^{b}	0.10^{b}	0.06^{a}	0.16^{ab}	0.29^{bc}	0.36^{c}	<0.03	<0.03	<0.03	<0.03
2.3-butanediol	0.6^{a}	0.8^{b}	1.0^{c}	0.9^{b}	3.0^{a}	3.3^{bc}	3.4^{c}	3.2^{b}	0.2^{a}	0.3^{b}	0.4^{c}	0.5^{d}
Ethanol	1.5^{a}	2.1^{b}	2.2^{b}	1.8^{ab}	2.3^{a}	3.2^{ab}	3.4^{b}	3.5^{b}	0.1	0.1	<0.1	<0.1
WSC	1.9^{a}	1.2^{b}	0.9^{c}	0.7^{c}	0.21^{a}	0.14^{ab}	0.07^{b}	0.07^{b}	0.12	0.18	0.06	0.16
NDF	46.9^{a}	48.2^{b}	48.4^{b}	50.1^{c}	49.3^{a}	49.2^{a}	49.6^{a}	51.2^{b}	46.6^{a}	46.9^{a}	47.2^{a}	48.6^{b}
iNDF[2]	23.1^{a}	23.2^{a}	24.7^{ab}	26.4^{b}	18.4^{a}	20.2^{b}	19.9^{b}	23.0^{c}	11.0^{a}	12.5^{b}	14.3^{c}	17.0^{d}

^{a,b,c,d}Different superscript letters within parameter and trial indicate significant differences at $p<0.05$; [1]% of N; [2]% of NDF.

Fermentation characteristics were negatively affected in relation to the length of the aerobic period. The pH drop during ensilage was slower and final pH values higher in silages that were exposed to the longest aerobic periods (Figure 1). After 90 days all pH values were significantly different from each other. We have no good explanation for the pH increase at day 3 in trial 2 and 3.

The aerobic stability of silages after 90 days of storage was not affected by delayed sealing. All silages were completely stable over the entire test period of 7 days and silage temperatures increased with only 1-3°C (Table 4). It is probable that the low fermentation quality of our silages contributed to their high aerobic stability.

Table 4. Aerobic stability of silages after 90 days storage as affected by delayed sealing. Values represent the highest silage temperatures detected during 7 days of aerobic exposure (means of 3 silo replicates)

	Delayed sealing (hours)			
Trials	0 h	5 h	10 h	24 h
1	19.2[a]	19.5[a]	21.6[b]	19.5[a]
2	21.3[a]	20.8[a]	20.7[a]	22.9[a]
3	21.3[a]	19.8[a]	20.8[a]	21.1[a]

Conclusions

Negative effects on fermentation quality of silages emerged clearer the longer the sealing of silos was postponed. However, the negative effect varied considerably among the 3 trials as illustrated in the graphs showing the pH drop after aerobic periods of 5, 10 or 24 hours (Picture 2). Conditions such as high ambient temperatures and long transports stimulating plant respiration might amplify the loss of WSC and increases the risk of producing badly fermented silages (increased concentration of butyric acid and reduced lactic acid) particularly when the fresh forage has a low sugar concentration already at the time of cut. The results of this study support the recommendation to minimize aerobic exposure of fresh forage during silo filling.

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Heat treatment increases the protein value in Faba beans and Lupins

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Introduction

Organic milk production constitutes about 10% of the milk production in Denmark. Focus has been on increasing the proportion of home-grown feeds, including protein supplements. One of the major challenges in increasing the amount of home-grown protein is to cover the requirements for amino acids absorbed in the small intestine (AAT). Heat treatment of home-grown protein feed has the potential to decrease rumen degradability of protein and increase the supply of AAT. The aim of the study was to test the effect of heat treatment of home-grown faba beans and lupins with on-farm equipment, and to estimate the optimal treatment temperature and time to maximize the AAT concentration in faba beans and lupins.

Materials and Methods

Testing was conducted on an organic farm. One batch of faba beans from different fields as well as one batch of lupins were used in the test. Both faba beans and lupins were dried at harvest to obtain storage stability. On-farm toasting equipment from Dilts-Wetzel Manufacturing Co was used. The toaster utilized a heated oil chamber outside an auger and continuously produced cooked beans. The beans were cooked at a temperature between 99 to 140°C for 1 hour, then entered a steeping chamber and continued to be cooked by steam for an additional ½ hour, exiting the steeping chamber at 70 to 110°C. The temperature was controlled via the temperature in the oil and the treatment time was controlled via the velocity of the auger. Four different temperatures (oil temperature 120, 140 160 and 180°C) were tested in combination with 3 different treatment times controlled by the velocity of the auger (L=24, M=30 and H=36 rpm, corresponding to a capacity ranging from 1000 kg to 2500 kg/d). The treatment temperature inside the auger was measured at the different oil temperatures and velocities. The 12 heat treated and control samples of lupins and faba beans were analysed for dry matter according to the EC No. 152/2009, crude protein was analysed by the Dumas method and soluble crude protein using NorFor 2005-03-15. NDF was analysed only in the control sample using the ANKOM fiber bag method according to the AOAC 2002.04. All analyses

Table 1 Chemical composition of the control sample of faba beans and lupins

	Faba beans	Lupins
Dry matter, g/kg	889	908
Ash, g/kg DM	45	39
Crude protein, g/kg DM	330	331
Buffer soluble protein, g/kg protein	713	673
NDF, g/kg DM	154	233

were carried out in a commercial laboratory. Chemical composition of the faba beans and lupins is shown in Table 1.

One sample of faba beans and lupins was analysed for rumen degradability in fistulated cows. The samples were selected based on the results from the analysis of soluble crude protein. The samples that was treated at 160°C was chosen because there was a significant reduction in soluble protein compared with samples treated at 120 and 140°C, whereas there was no further reduction in soluble crude protein in the samples treated at 180°C. It is also well-known that high temperature increases the risk of heat damage to the protein with reduced intestinal digestibility as a result. The rumen protein degradability was determined using the nylon bag technique (Åkerlind et al., 2005). Bags were incubated in the rumen of three dry cows for 0, 2, 4, 8, 16, 24, 48 h and nitrogen residues were determined using the Kjeldahl procedure. For each sample, the disappearance from the bags were corrected for particles loss. Total tract digestibility and degradation rate were determined using the mobile nylon bag technique (Weisbjerg and Hvelplund, 2005). Intestinal digestibility of rumen undegraded dietary protein was determined in accordance with Hvelplund et al. (1992). Water soluble protein (a), potentially degradable protein (b) and degradation rate of potentially degradable protein (c, /h) were estimated based on a non-linear regression (Ørskov and McDonald, 1979). AAT and PBV values were calculated using the NorFor model (Åkerlind and Volden, 2011).

Results and Discussion

The untreated faba beans and lupins were characterized by having a high (713 and 673 g/kg protein) content of soluble protein. Overall heat treatment reduced the proportion of soluble protein in both faba beans and lupins. But the effect was depending on both temperature and, to some extent, also on treatment time. Results are shown in Figure 1 and 2. For faba beans, soluble protein was halved already at a temperature of 110°C at either a low velocity of the auger or a higher oil temperature whereas lupins required a higher temperature before the same reduction in soluble protein was achieved. For faba beans, no further reduction in soluble protein was seen when the treatment temperature was 120°C or higher independent of treatment time. Lupins, however, required higher temperature to achieve the same reduction in soluble protein.

Heat treatment effectively reduced the degradation rate of protein in both faba beans (0.106 to 0.039 h^{-1}) and lupins (0.101 to 0.024 h^{-1}) and thereby reduced effective rumen degradability of protein from 78.0 to 53.2% in faba beans and from 74.6 to 50.3% in lupins. Despite the pronounced effect on rumen protein degradability, heat treatment had only a minor effect on the total tract digestibility of protein; results are shown in Table 2. In Table 3, the calculated values of AAT and PBV are shown. The results show that heat treatment of faba beans increased the AAT value from 112 to 197 g AAT/kg dry matter as well as reduced the PBV value from 155 to 52 g/kg dry matter. For lupins, the AAT value almost doubled from 117 to 222 g AAT/kg dry matter and the PBV was reduced from 173 to 49 g.

Conclusions

The study concludes that controlled heat treatment with on-farm toasting equipment can lower the degradability of protein in the rumen without sacrificing digestibility in the intestine. Therefore the examined method is an effective method to raise the levels of AAT in home-grown faba beans and lupins.

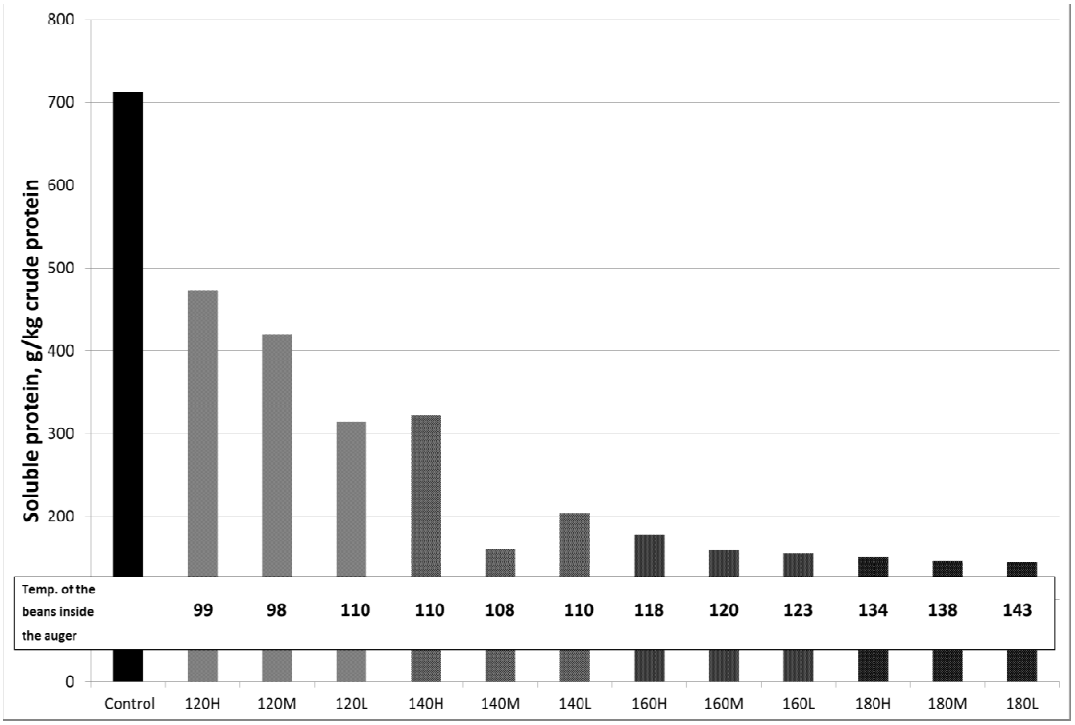

Figure 1 Soluble protein in faba beans. (120, 140, 160, 180 = temperature of the oil; H, M, L = velocity of the auger). Inserted table values are in °C.

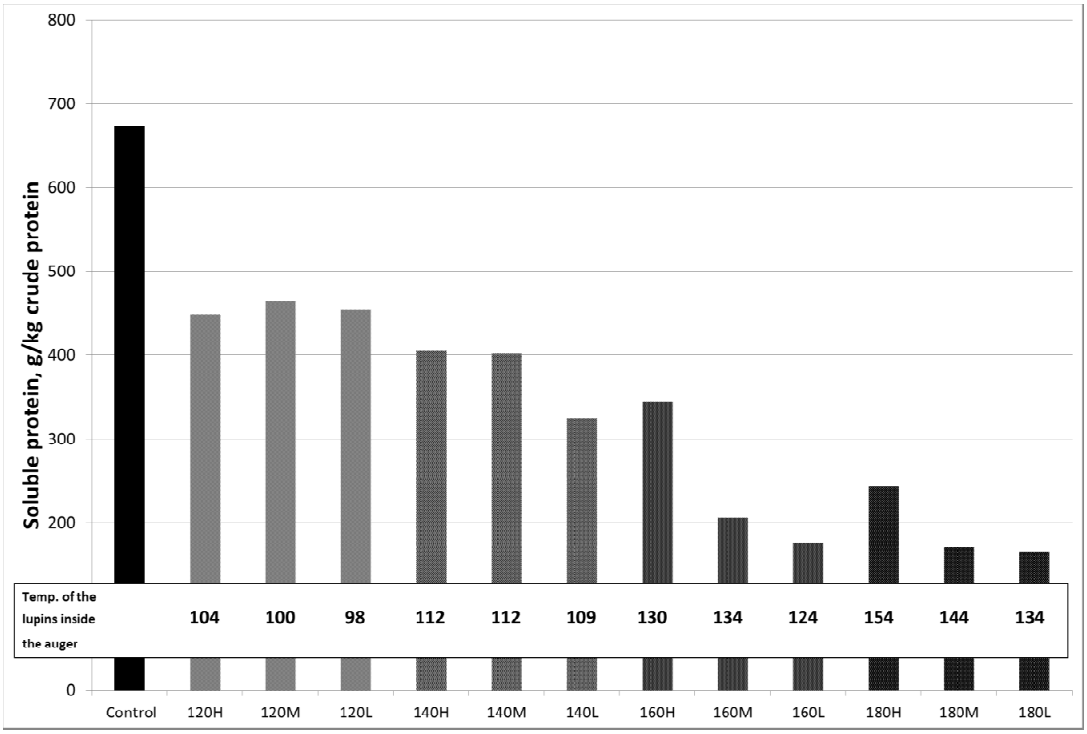

Figure 2 Soluble protein in lupins. (120, 140, 160, 180 = temperature of the oil; H, M, L = velocity of the auger). Inserted table values are in °C.

Table 2 Protein value of untreated and heat treated faba beans and lupins.

	Faba beans Control	Faba beans Heat treated	Lupins Control	Lupins Heat treated
Treatment temperature[a], °C		120		134
Treatment time, min.		60		60
Crude protein, g/kg DM	331	329	340	347
Soluble protein, g/kg protein[b]	713	159	673	206
a[c]	0.541	0.278	0.387	0.298
b[c]	0.479	0.862	0.631	1.00
c[c], h^{-1}	10.6	3.89	10.12	2.39
EPD[d]	78.0	53.2	74.6	50.3
TPD[e]	97.2	96.6	96.9	95.0
dUDP[f]	87.2	92.6	87.7	89.9

[a]Temperature of faba beans or lupins inside the auger; [b]Buffer soluble protein; [c]Water soluble fraction (a), potential degradable fraction (b) and degradation rate (c); [d]Effective rumen degradability using a fractional passage rate of 0,05 h^{-1} corrected for particle loss; [e]Total tract digestibility using mobile nylon bags; [f]Intestinal digestibility of rumen undegraded feed protein

Table 3 Calculated protein value of untreated and heat treated faba beans and lupins.

	Faba beans Control	Faba beans Heat treated	Lupins Control	Lupins Heat treated
Treatment temperatur[a], °C		120		134
Treatment time, min.		60		60
Crude protein, g/kg	315	315	338	338
Soluble protein, g/kg protein[b]	713	159	673	206
Indigestible protein, g/kg protein	28	34	31	50
Degradation rate, h^{-1}	10.6	3.89	10.12	2.39
AAT[c], g/kg dry matter	112	197	117	222
PBV[c], g/kg dry matter	155	52	173	49

[a]Temperature of the faba beans or lupins inside the auger; [b]Buffer soluble protein; [c]AAT/PBV 20 kg dry matter, calculated according to NorFor (Åkerlind and Volden, 2011).

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Rumen degradability of protein in field beans after heat treatment or ensiling

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Introduction

The production of organic milk is increasing in Sweden. In 2012 it made up 12.7% of total milk delivered. One constraining factor in the production is the scarcity of protein- rich feedstuffs. The price of protein- rich concentrates certified as organic is high, creating a growing interest in home-grown protein- rich feeds. Field beans, *Vicia faba*, is an attractive crop for organic cultivation and should have the potential to add valuable protein to diets for ruminants. Being a legume, field beans can fix atmospherics nitrogen and are also appreciated as a preceding crop in the crop rotation system due to its deep root system. However, rumen solubility of the protein fraction in field beans is high, as in most other home-grown crops. When feeding a diet based on grass-clover silage and concentrates based on wheat, barley or oats, all feeds consist of easily degrade protein. A protein supplement with higher resistance towards rumen degradation combined with a high total digestibility would be desirable.

Heat treatment has been used to reduce solubility of the protein fraction of other feeds, e.g. rape seed meal. This is used commercially in the production of ExPro® (AAK Ltd, SE-374 82 Karslhamn, Sweden), where effective protein degradability (EPD) is depressed from 72% to 35% by treating rapeseed meal with heat and steam (Spörndly, 2003A). Treating field beans with heat was proposed by Lund et al (2004). It decreased protein solubility but maintained total digestibility resulting in an increased metabolizable protein, calculated as amino acids absorbed in duodenum (AAT). The primary aim of the present study was to estimate rumen degradability of the protein fraction in whole, dry field beans after treatment in a farm-based roaster at three different temperatures.

Field beans require a comparatively long growing season. In some years, this can result in low DM content at harvest. As an alternative to drying the beans after harvest, crimping and ensiling is sometimes practiced for safe preservation and storage. The beans are then crushed at approximately 60-70% DM and packed into airtight plastic tubes where an ensiling process takes place. This process will have an effect on protein degradability and a pilot study was also performed to evaluate the effect ensiling on rumen protein degradability.

Materials and Methods

Field beans harvested 2011 were dried and stored at a commercial feed factory. In the autumn of 2012, these intact beans were treated in a farm based toaster at 165, 185 and 205°C for 5.5 minutes. The toaster (Roastech; Roastech Ltd, Bloemfontein,South Africa) used a forced convection roasting technique where the beans maintained in a rotating tunnel with electrically heated walls for 5.5 minutes at selected preset temperature. At unloading, the beans were subjected to moderate cooling by forced air ventilation at ambient temperature. The capacity of the machine was 100 kg per hour.

Parallel to this study, field beans harvested in 2012 were ensiled with the crimping technique. Propionic acid, 6-7 L per tonne, was used as silage additive (Perstorp AB, SE-284 80 Perstorp, Sweden).

After heat treatment the beans were milled through a 1.5 mm sieve and rumen degradable protein was analysed by the rumen *in situ* technique. Ensiled beans were pre-dried at 60°C before milling. The degradability was estimated using three dry cows according to Åkerlind et al. (2011). The standard diet of cows contained 12% crude protein (CP) and consisted of 67% hay and 33% concentrate during an adaptation period of 21 days before the trial started.

The two batches of field beans for heat treatment and crimping were of comparable nutrient quality. The content of dry matter (DM), CP, neutral detergent fiber (NDF), starch, ether extract and ash are shown in Table 1, analyzed by conventional wet chemistry methods. Table 1 also shows the fermentation products and microbial composition in the silage.

Table 1 Basic nutrient composition of field beans used for roasting and crimping respectively and fermentation products for crimped beans. Number of samples per crop =1

	Dry matter, g/kg	CP, g/kg DM	NDF, g/kg DM	Starch, g/kg DM	Ether extract, g/kg DM	Ash, g/kg DM
Field beans for roasting	851	307	114	425	15	34
Field beans for crimping	637	303	117	419	17	37

		% of DM							Log CFU/g	
	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Formic acid	2,3-butane-diole	Ethanol	yeast	mold
Ensiled crimped field beans	5.0	1.26	0.2	0.22	<0.02	<0.02	<0.02	0.08	6.2	0

Results and Discussion

Results show that the roasting had the intended effect. The effective protein degradability (EPD) decreased with increasing temperature (Table 2) and EPD for the highest temperature was 10% lower than for the untreated control. Under the assumption that duodenal digestibility is unchanged, , this difference would give 13% more AAT per kg DM in the field beans treated at 205°C. The EPD, AAT and PBV values in Table 2 are calculated according to the classical method described by Madsen et al (1995) and Spörndly (2003B) using a passage rate of 8% h^{-1} and 80% duodenal degradability of rumen undegraded feed protein. The ensiling process of the crimped field beans had the opposite effect on protein degradability resulting in 7% higher EPD value compared to the dried beans.

The protein degradation fitted to an exponential curve, as practiced in NorFor (Åkerlind et al, 2011) is illustrated in Figure 1. The two field beans batches differed in protein solubility; after 2 h in the rumen, 65% was degraded in the untreated batch designated for heat treatment while 72% was degraded in the untreated batch for ensiling. In both treatments, the impact on EPD was

Table 2 The effect of increasing temperatures or ensiling of field beans on in situ measured efficient protein degradability (EPD). Metabolizable protein (AAT) and rumen protein balance (PBV) are calculated using the classical method used in Sweden (Spörndly, 2003B)

		Heat treated			Untreated	Ensiled
	Untreated		Heat treated	Heat treated		
Temperature, °C		165	185	205		
Crude protein, g/kg	303	303	303	303	307	307
EPD[a],%	79	76	74	72	84	87
AAT[b], g /kg dry matter	95	101	104	108	86	80
PBV[b], g /kg dry matter	121	113	108	102	135	143

[a]Calculated according to Madsen et al (1995) using 8% h^{-1} passage rate and 80% duodenal digestibility; [b]Amino acids absorbed in the duodenum (AAT) and protein balance in rumen (PBV) calculated as described by Spörndly (2003B).

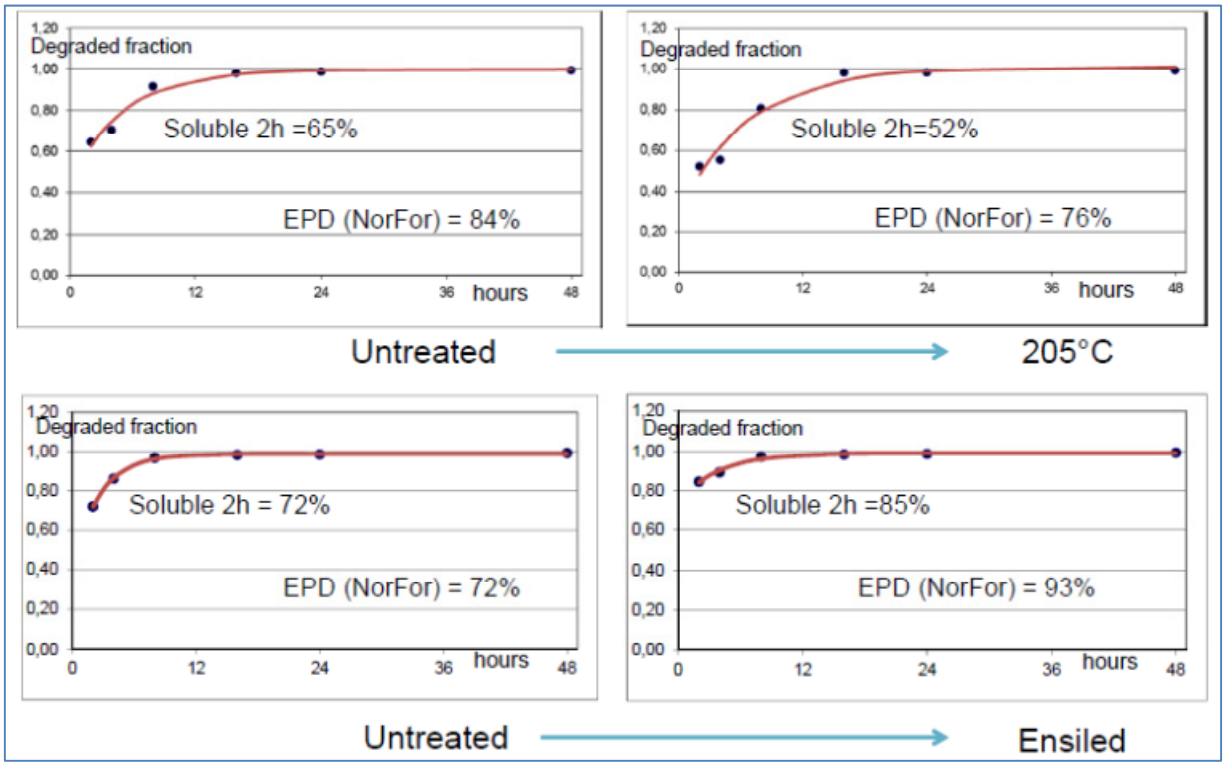

Figure 1 The effect on protein degradability when subjecting field beans to heat or ensiling. Exponential function fitted to degradation measured at 2, 4, 8, 16, 24 and 48 hours. Efficient protein degradability (EPD) calculated at 8% passage rate (Åkerlind et al, 2011).

most marked at the initial phase, decreasing and increasing the EPD at heat treatment and ensiling, respectively. At 24 h, close to 100% degradability was obtained in all treatments. This also indicates that the total protein digestibility was not affected. In Table 3, the parameters used in the Nordic feed evaluation system NorFor (Åkerlind et al, 2011), computed from the exponential degradation curve, are presented. By inserting the parameters in the ration formulation program 'IndividRAM', AAT and PBV used in that system were calculated. A uniform content of CP, NDF and starch was used in order to equalize the comparison.

Table 3 Calculated protein value of untreated and heat treated and untreated or ensiled field beans according to the NorFor system (Åkerlind et al, 2011)

	Field beans untreated	Filed beans Heat treated, 205°C	Field beans untreated	Field beans Ensiled
Crude protein, g/kg	303	303	303	303
Soluble protein, g/kg protein[b]	447	303	410	736
Indigestible protein, g/kg protein	1	0	11	11
Degradation rate,% h^{-1}	19.5	14.7	38,7	26,7
AAT[a], g /kg dry matter	121	135	109	107
PBV[a], g /kg dry matter	139	122	153	155

[a]Metabolizable protein (AAT) and rumen protein balance (PBV) calculated at a standard diet at 20 kg DMI using NorFor application IndividRAM 5.8b (Svensk Mjölk AB, Stockholm, Sverige) 2012).

The NorFor system generally estimates a higher metabolizable protein content than AAT by the classical Swedish system does. The increase due to the heat treatment though, is of the same magnitude in the two systems. The decrease in AAT caused by ensiling is however of a lower magnitude when calculated in the NorFor system compared with the classical system.

Conclusions

Heat treatment of field beans (*Vicia faba*) with the farm based bean roaster using a rotating tunnel with electrically heated walls made the protein fraction less rapidly degraded in the rumen of dairy cows. Roasting the beans at 205°C for 5.5 minutes resulted in an increase in AAT of 11-13%. On the contrary, ensiling crimped beans at a DM content of 63% with propionic acid as additive increased rumen degradability and resulted in a slight decrease in AAT.

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Methods for examining fungal prevalence in haylage

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Introduction

Haylage, also known as baled forage with a dry matter (DM) content between 500 g DM kg^{-1} and 800 g DM kg^{-1} is commonly fed to horses in Sweden (Holmquist and Müller, 2002), Finland (Saastamoinen and Hellämäki, 2012) and Norway (Vik and Farstad, 2012). Haylage is characterized by restricted lactic acid fermentation due to the high DM content and low water activity. These conditions may be advantageous for increased growth of fungi (O'Brien *et al*., 2008). Since some fungal species produce mycotoxins and/or fungal spores that are associated with respiratory diseases in *e.g*. horses and humans, fungal growth in haylage should be avoided. Hygienic quality of feedstuffs is often examined visually, however, not all fungi are dangerous for animals, and it is also difficult to perform fungal identification and enumeration in the field. Therefore, it is important to use objective methods for detection of fungal species in haylage.

There are several methods for detection of fungi in feed; visual examination, direct plating, and dilution plating are three common methods. In visual examination, the sampling is selective by taking fungal colonies observed on the feed and put them on plates containing growth substrate media. In direct plating, the sample is representative, often taken by core samplers, where the bale is drilled from the surface to the middle, and small particles of the forage are placed on plates. In dilution plating, the sample is taken in the same way but instead of placing particles on plates the sample is homogenized and diluted in standard ten-fold series before being spread on plates. After the cultivation, colony-forming units (CFU) of the fungal species are counted. The dilution plating method can be used for quantitative determination as well as identification of species while the visual examination and direct plating merely are methods for identification of species only (Samson *et al*., 2010). Furthermore, it is important to consider that different fungal species have different cultivation requirements. General substrates are therefore important to use if the sample contain unknown fungal species.

The aim of this project was to evaluate and compare three sampling methods for detection of fungi in haylage; direct visible plating (i), direct core plating (ii), and dilution core plating (iii).

Materials and methods

Sample collection

In 2010, samples from 150 haylage bales were collected at 50 farms in Sweden between 26th of April and 17th of July. In 2011, samples from 150 haylage bales at 50 farms in Sweden and 75 bales from 25 farms in Norway were collected between 28th of February and 20th of April and 2nd of May and 7th of June, respectively. At each farm, three bales from the same harvest batch were sampled. One of the three bales was opened and visible fungal colonies were collected by a

sterile cotton swab if spores were visible, or by cutting small fragments of mycelia. Thereafter, haylage samples were collected at eight locations on each bale by using steel core sampler (0.65 m × 40 mm) connected to an electric drill. For fungal analysis, 150-200 g of sample was collected and kept at 4°C for up to 24 hours (up to 48 hours for Norwegian samples) during sample transport before analysis.

Fungal culturing and identification

Three methods were used for analysis of fungi: i) direct visible plating; ii) direct core plating; and iii) dilution core plating. In procedure i), visible fungal colonies on the bale were inoculated and grown on malt extract agar (MEA) (Merck, KGaA, Darmstadt, Germany) plates for ten days at 25°C. In procedure ii), 6-8 pieces of plant material from the core sample were inoculated on MEA and dichloran 18 % Glycerol agar (DG18) (Merck, KGaA, Darmstadt, Germany) for ten days at 25°C and 37°C (Seale *et al.*, 1986). In procedure iii), dilution core plating was made by mixing 50 g sample with 450 mL autoclaved (15 minutes at 121°C) quarter-strength Ringer solution (Merck KGaA, Darmstadt, Germany) including 0.5 mL/l Tween® 80 (Sigma-Aldrich, Saint Louis, USA). Ten-fold dilution series was made from the extracted solution and each dilution was cultured on MEA and DG18 plates for ten days at temperatures 25°C and 37°C. Mould colonies were further identified.

Identification of *Aspergillus* spp. isolates and *Penicillium* spp. isolates was performed on macro- and microstructures according to identification keys described by Klich (2002) and Pitt (2000), respectively. These identifications were verified by molecular identification using PCR and samples of mycelia from pure-cultured isolates for DNA extraction, according to Stewart and Via (1993). *Fusarium* spp. isolates were amplified in the translation elongation factor (EF) 1α coding region (O´Donnell *et al.,* 1998), *Aspergillus* spp. and *Penicillium* spp. were amplified in the β-tubulin gene (Glass and Donaldson, 1995), and isolates of unknown species identity and *Mucor* spp. were amplified in the internal transcribed spacer (ITS) region (White *et al.,* 1990; Gardes and Bruns, 1993). Sequences from GenBank data base (NCBI´s webpage, http://www.ncbi.nlm.nih.gov/BLAST) were used for comparing the fungi isolate sequences using the BLASTN algorithm (Altschul *et al.,* 1997).

Results and Discussion

Fungal contamination on bales should be avoided, however some fungal species are potentially more harmful than others. One of the most predominant fungal species detected by use of the three methods was *P. roqueforti.* This species may produce the mycotoxin roquefortine C, which has been found in silage with growth of *P. roqueforti* (Auerbach *et al.,* 1998). It is therefore important to use sampling methods that could detect potentially hazardous fungi.

In total, there was fungal contamination on 110 of 124 farms (one excluded) from the three methods; direct visible plating (i), direct core plating (ii) and dilution core plating resulting in 64, 98 and 69 farms, respectively. Combining direct visible plating (i) and dilution core plating (iii), fungi was detected at 93 farms, and from direct core plating (ii) and dilution core plating (iii) fungi was detected at 103 farms. Altogether, 52 fungal species were detected, direct visible plating (i) method (17 species), direct core plating (ii) method (46 species) and the dilution core

plating (iii) method (26 species). Combining results from the three methods, the five most commonly isolated species appeared in the following order: *Arthrinium spp* (68 farms). *Penicillium roqueforti* (59 farms)*, Sordaria fimicola* (38)*, Eurotium herbariorum* (28 farms) and *Aspergillus fumigatus* (24 farms).

The most common species in the direct visible plating method (i) was *P. roqueforti* (35 farms), followed by *Arthrinium* spp. (22 farms), and *A. fumigatus, S. fimicola* and *Fusarium poae* which were detected at seven farms. In the direct core plating method (ii), *Arthrinium* spp was the most common species, detected at 58 farms. Other common species were *P. roqueforti* (35 farms), *S. fimicola* (31 farms) and *E. herbariorum* (20 farms). The most common species detected in the direct core dilution (iii) method was *P. roqueforti* (35 farms), followed by *Arthrinium* spp. (19 farms). Other common species were *E. herbariorum* and *A. fumigatus,* detected at nine farms (Figure 1).

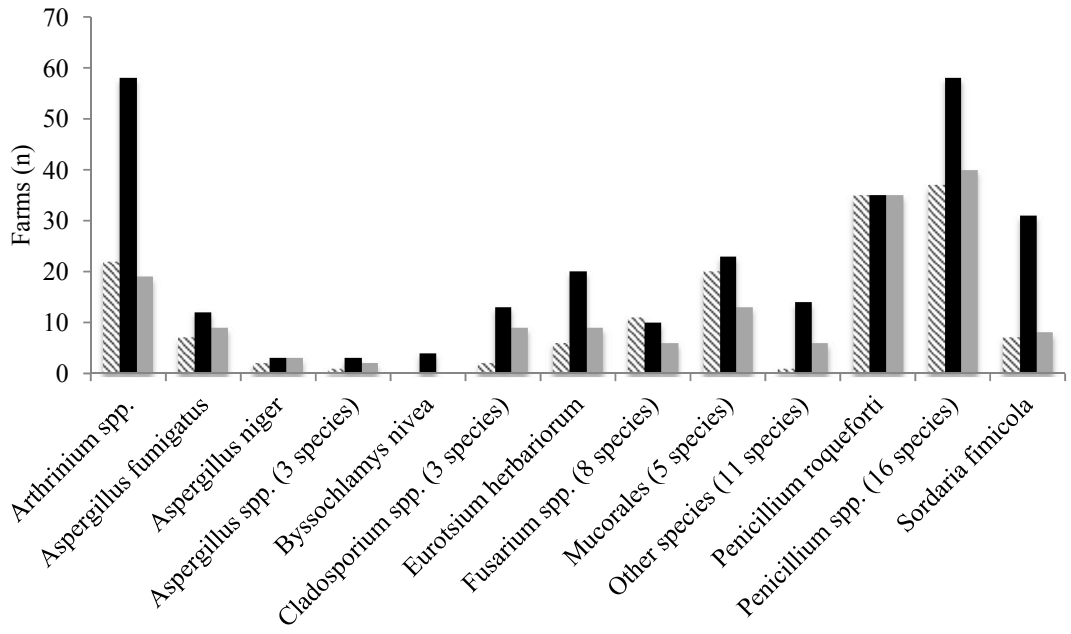

Figure 1. Number of farms (Y-axis) were fungal species (X-axis) were detected using i) direct visible plating (▨), ii) direct core plating (■) and iii) dilution core plating (▩).

Almost all *Penicillium* species (except one) were found by using the direct plating method (15 *Penicillum* species). However, the *Penicillium* species were only isolated at one to three farms with *P. roqueforti* as exception, isolated on 35 farms. It is important to consider that even though *P. roqueforti* was isolated at 35 farms regardless of method, it was only found on 10 farms simultaneously by all the three methods and on 59 farms if the methods where treated

separately. In direct visible plating (i) and direct core plating (ii) together, *P. roqueforti* was isolated on 17 farms, direct visible plating (i) and dilution core plating (iii), and direct core plating (ii) and dilution core plating (iii) resulted in 19 farms, respectively.

All the three methods detected *Arthrinium* spp. on four farms and *S. fimicola*, *E. herbariorum* and *A. fumigatus* on zero farms. Direct plating method (i) and direct core plating method (ii) detected *Arthrinium* spp. on 16 farms, *S. fimicola* on three farms, *E. herbariorum* on two farms and *A. fumigatus* on one farm. Direct plating method (i) and dilution core plating method (iii), detected *Arthrinium* spp. on three farms and *S. fimicola*, *E. herbariorum* and *A. fumigatus* on one farm each. Direct core plating method (ii) and dilution core plating method (iii) detected *Arthrinium* spp. on 14 farms, *S. fimicola* on three farms, *E. herbariorum* on three farms and *A. fumigatus* on two farms.

Depending on choice of method, different results on sampled fungi were achieved, indicating the importance of selecting the right methods. In the core plating methods (ii and iii), there was a risks of variation in the direct core plating method (ii) compared to the dilution core plating method (iii), where the sample before cultivation of fungi is homogenized and consequently more representative. Furthermore, fast growing fungi will probably be overrepresented in the direct core plating method (ii) compared to the dilution core plating method (iii), where fungal spore/mycelia is diluted in ten-fold series, leading to less fungal colonies per plate at higher dilutions. However, in the dilution core plating method (iii), there is a risk that fungal spore/mycelia are lost in dilutions. The direct visible method (i) is important since it shows fungi that are actually growing in the feed. It is important to consider that there could be other fungal species growing inside the haylage bale compared to the direct visible method (i).

Conclusions

Results from this study show that fungal species detected by direct visible plating method (i) do not correspond with fungal species cultivated from the core sampling methods; direct core plating (ii) and dilution core plating (iii). Additionally, the core sampling methods (ii and iii) do not correspond to each other either, indicating the importance of method and/or methods selection which will depend on purpose of the study.

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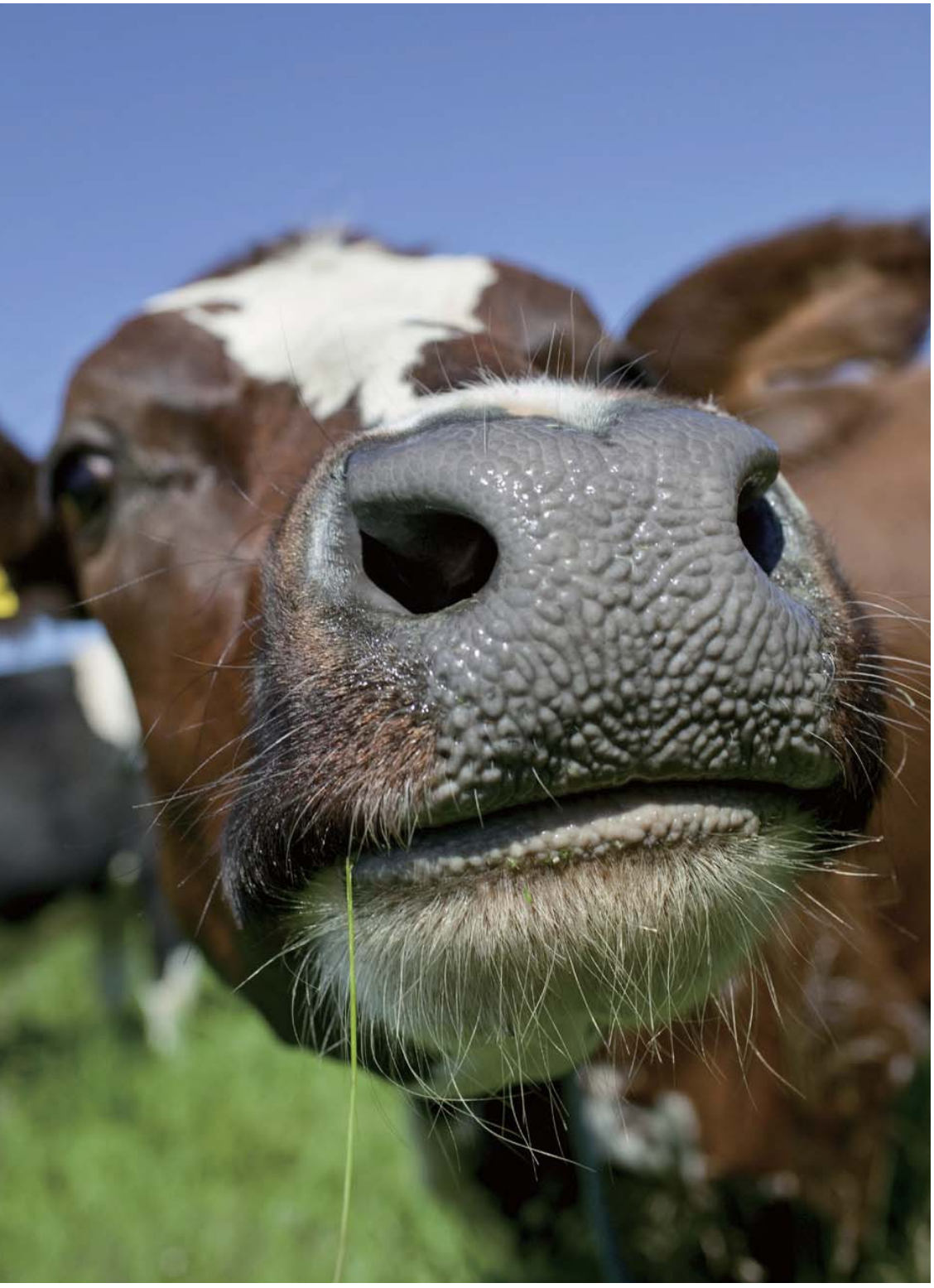

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Milk production based on grass/clover silage and cereal feeding

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Introduction

When producing milk according to the rules for organic production, one of the problems is the supply of protein. There is a general shortage of organically produced protein rich feeds and the prices for these feeds are high. Therefore, it is interesting for these producers to evaluate the effect of offering the cows a diet based on home grown feeds only. In most cases, cereals in combination with high quality grass silage makes up a diet that can be produced entirely on Swedish farms. Milk production for cows on such a diet would be lower compared with a diet where a protein rich supplement also is included but there is a lack of information about how large the production drop would be. With this knowledge, it would be easy for the producers to utilize current prices for organically produced milk and feeds to calculate if it would be economically beneficial to exclude protein rich supplement from the cows' diet. High prices for concentrate feeds, in combination with comparatively low milk prices during the last year, have made the following question highly relevant: Which diet is economically most favourable?

The aim with the present experiment was to study the effect of exclusion of protein concentrates in the diet for dairy cows using silage with high or low crude protein contents. The response was evaluated in terms of yield and composition of milk and feed intake. Economic calculations of milk income minus feed cost were made using the result of the experiment and current prices of feed and milk in spring 2013.

Materials and Methods

In a production experiment, a diet with only cereals and grass/clover silage to dairy cows was compared to a diet where protein rich supplements including soybean and rapeseed products, were also included. These two diets were combined with two grass/clover silages with different contents of crude protein (130 and 170 g kg^{-1} DM) in a factorial design with 37 cows of the Swedish Red (SRB) breed during 20 experimental weeks. The silages, offered *ad libitum*, were of first cut and to achieve the higher protein content additional pure red clover silage was added (32% of DM) in a mixer wagon prior to feeding. The low protein silage was to 95 % dominated by timothy (*Phleum pretense)* and meadow fescue (*Festuca pratensis*) . Concentrates were fed according to yield with the assumption of a silage DM intake of 15 kg DM day^{-1} and adjusted regularly.

The four treatments summarized:

1. Silage170 + cereals and protein concentrate
2. Silage170 + cereals
3. Silage130 + cereals and protein concentrate
4. Silage130 + cereals

The data was statistically analyzed with SAS, version 9.1 (SAS Institute Inc., Cary, N.C., USA). Means of the milk production data (kg milk, kg energy corrected milk (ECM) and milk composition), feed intake and nutrient intake throughout the period were analyzed by analysis of variance (Proc GLM, SAS). The fixed factors: silage type, concentrate type and days in milk were treated as independent variables. The interaction between silage type and concentrate type was tested but found to be non-significant and was therefore omitted in the final model. The milk yield at start of the experiment was used as covariate when milk yield was analyzed. Milk fat and milk protein were used as covariates in the same way when they were analyzed. The effect of lactation number was found non-significant and was therefore omitted from the model. When analyzing the data of milk cell counts, logarithmic values were used.

Table 1 Composition of feeds used. Cereals consisted of 36% barley, 34% wheat and 25% oats. Protein concentrate consisted of soy expeller 47%, rapes seed cake 16%, oats 15%, rapeseed 11%. Both cereals and protein concentrate were pelleted and contained binding material, minerals and vitamins. Means with standard deviation within brackets

Item[1]	Cereals	Protein concentrate	Silage170	Silage130
DM, %	89.4 (1.50)	92.0 (1.79)	35.0 (1.20)	36.4 (1.76)
ME, MJ/ kg DM	13.0	15.5	11.3 (0.21)	11.6 (0.11)
AAT, g/kg DM	84	160	72	73
PBV, g/kg DM	-17	99	44	6
CP, g kg DM	125 (17.7)	328 (6.2)	169 (4.3)	132 (3.7)
EE, g/kg DM	34	130	NA	NA
Ash, g/kg DM,	58 (2.0)	76 (3.4)	86 (4.7)	75 (5.9)
Starch, g/kg DM	559	99	NA	NA
NDF, g/kg DM	205	183	414 (19.9)	471 (13.9)

[1)] AAT = metabolizable protein; PBV = protein balance in the rumen, both calculated according to Spörndly (2003); CP = crude protein; EE = ether extract; NDF = neutral detergent fiber.

Results and Discussion

Feed intake data is presented in Table 2 and response in milk production is presented in Table 3. No effects of concentrate type on silage intake were detected ($P>0.05$). The higher intake of Silage 130 as affected to silage type was partly due to one malfunctioning feeding trough, resulting in 1.75 kg DM of Silage170 consumed by the cows assigned for Silage130. Reducing the intake of Silage130 with this quantity, to 14.7 kg DM, erased the difference in intake between the silages ($P>0.05$).

The production results showed that milk yield without protein supplement gave a lower milk production but a higher milk fat content ($P<0.01$), resulting in 30.9 kg ECM and 35.3 kg ECM, respectively. Studying cows over 70 days in milk separately resulted in a slightly lower effect of concentrate type; 3.8 kg ECM less milk instead of 4.5 kg less ECM for cows fed cereals only. There was, however, no effect of silage type on the production parameters ($P>0.05$), with the exception of a tendency to lower live weight gains when Silage130 was fed ($P<0.10$). The diet

without protein supplement gave an increase in nitrogen efficiency by 20% compared with the diet with the protein supplement.

When designing the experiments, it was believed by some that the higher protein content of Silage170 (compared with Silage130) would result in a higher milk production for the group fed silage170 when only cereals were fed. The higher crude protein content in Silage170 resulted in an excess of soluble protein in the rumen (higher PBV value) but similar estimated amounts of AAT were achieved (1549 and 1625 g AAT for cows fed cereals with Silage170 and Silage130, respectively). As no additional metabolizable protein or energy was added with Silage170, there was no production response (Table 3).

Table 2 Feed intake data. Least square means (LSM) with standard error in brackets

	Effect of concentrate N=37			Effect of silage N=37		
Intake per day	Cereals/conc	Cereals	Sign[1]	Silage170	Silage130	Sign[1]
Silage, kg DM	15.8 (0.58)	14.4 (0.58)	NS	13.8 (0.57)	16.4 (0.57)	(**)[2]
Cereals, kg	1.9 (0.47)	7.1 (0.48)	***	4.8 (0.47)	4.2 (0.48)	NS
Protein conc, kg	4.0 (0.29)	0.0 (0.30)	***	2.0 (0.29)	1.9 (0.30)	NS
Total DM, kg	21.2 (0.74)	20.4 (0.74)	NS	19.7 (0.73)	22.0 (0.74)	NS
ME, MJ	257 (9.7)	246 (10.0)	NS	239 (9.7)	264 (10.0)	Tend
AAT, g	1854 (72.7)	1572 (74.6)	*	1641 (72.7)	1788 (74.6)	NS
PBV, g	777 (37.4)	514 (38.4)	***	876 (37.4)	415 (38.4)	***
Crude protein, g	3887 (153.0)	3096 (157.1)	**	3588 (153.0)	3396 (157.1)	NS
NDF, g	7943 (256.8)	7642 (263.7)	NS	6874 (256.8)	8710 (263.7)	***
Starch, g	1281 (234.4)	3455 (240.7)	***	2526 (234.4)	2209 (240.7)	NS

[1] $P<0.10$ = Tendency; $P<0.05$ = *; $P<0.01$ = **; $P<0.001$ = *** NS= Not significant

[2] The significantly higher silage intake of Silage 130 was partly due to a technical error. One malfunctioning feeding trough made it possible for cows assigned for Silage 170 to steal Silage 130. The quantity of stolen fodder was 1.75 kg DM and made up 11% of the total silage intake. Deducting this amount, the silage intake was reduced to 14.7 kg DM in the Silage 130 group and the difference to Silage 170 was no longer statistically significant.

Table 3 Energy corrected milk (ECM), live weight gain (LWG), body condition score (BCS) and N efficiency. Least square means and standard error in parentheses

	Effect of concentrate N=37			Effect of silage N=37		
	Cereals/conc	Cereals	Sign[1]	Silage170	Silage130	Sign[1]
Kg milk	35.7 (0.97)	30.0 (1.00)	***	32.9 (0.96)	32.8 (0.99)	NS
Kg ECM	35.3 (0.86)	30.9 (0.89)	**	33.6 (0.85)	32.7 (0.87)	NS
Fat, %	4.01 (0.10)	4.40 (0.10)	**	4.21 (0.10)	4.21 (0.10)	NS
Protein, %	3.16 (0.05)	3.25 (0.05)	NS	3.24 (0.05)	3.17 (0.05)	NS
Lactose, %	4.80 (0.02)	4.77 (0.02)	NS	4.80 (0.02)	4.77 (0.02)	NS
Cell counts, log	1.57 (0.10)	1.61 (0.11)	NS	1.55 (0.10)	1.63 (0.11)	NS
LWG, kg	36.8 (7.02)	25.2 (7.02)	NS	40.6 (7.02)	21.4 (7.02)	Tend
BCS	0.28 (0.148)	0.34 (0.125)	NS	0.30 (0.128)	0.31 (0.145)	NS
N efficiency[2], %	28.0 (1.14)	33.6 (1.17)	**	30.6 (1.14)	31.0 (1.17)	NS

[1] $P<0.10$ = Tendency; $P<0.05$ = *; $P<0.01$ = **; $P<0.001$ = ***; NS= $P>0.05$

[2] Calculated as 100*nitrogen in milk /nitrogen intake

From milk production and feed intake data obtained from the experiments, the milk revenue minus feed costs for these diets were calculated using current feed prices in Sweden (February 2013). For organic milk, the price was 3.76 SEK/kg ECM and for cereals and protein supplement mix and grass silage the cost was 3.40 SEK/kg, 6.06 SEK/kg and 1.30 SEK/kg dry matter respectively. This resulted in milk revenues of SEK 2.31 per kg ECM for the protein concentrate treatment and SEK 2.37 for the silage-cereals treatment. As cows on the silage-cereal diet yielded less, they showed a SEK 8.20 lower profit when expressed per cow and day. With prices from the previous year, the results came out differently, demonstrating the importance of always applying the prices relevant for the actual situation to draw the correct conclusion.

Conclusions

Feeding grass/clover silage and cereals only, without protein concentrate, can be expected to decrease the milk production with about 13 %. Increased silage crude protein content above 130 g per kg DM did not increase milk production when fed with concentrate consisting of cereals only.

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NIR analyses in forages: quality assurance in compliance with ISO 12099:2010

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Introduction

Near-infrared (NIR) spectroscopy is widely used for the determination of feedstuff composition. NIR spectroscopy is an indirect method (secondary technique), which means that a calibration is needed before it can be applied to unknown samples. The samples used for the calibration must have a known composition determined using recognized chemical reference methods (primary techniques). Compared to traditional chemical methods, NIR analysis provides rapid, low-cost, non-destructive and multiparametric measurements.

ISO standard for the application of NIR spectroscopy to forage samples

In 2010, the international standard ISO 12099:2010 "Animal feeding stuffs, cereals and milled cereal products - Guidelines for the application of near infrared spectrometry" was published. This standard focuses on the validation of calibration models using independent test sets, statistics for performance measurements and running performance checking of calibration. It also includes a glossary with comprehensive explanations of terms and definitions.
The standard states that samples used for calibration must be representative and should for example cover variations in composition as well as seasonal, geographic and genetic differences. For a solid validation of the calibration, at least 20 independent samples are required. The test set should be representative of the same population to be analyzed.

Statistics for performance measurement

The performance of the calibration can be assessed by calculating the bias between the methods (systematic error) and the random error, SEP (standard error of prediction), which expresses the accuracy of the NIR results. Bias can occur due to drift of the instrument or drift in wet chemistry, changes in the sample preparation or due to new sample types not included in the calibration. The bias is defined as the mean difference between the reference values (chemical method) and the predicted values (NIR method). The significance of the bias can be checked by a simple t-test. The random error, SEP, is defined as the standard deviation of the differences between the reference values and the predicted values. Using an F-test, the SEP of the validation set can be related to the SEC (standard error of calibration) to check the validity of the calibration model for the selected validation set.

Visualization of calibration performance

Validation results should also be visualized by plotting the reference values against NIR predictions (Figure 1). Next, the slope can be calculated by linear regression and obvious outliers identified. As for the bias, a t-test can be used to check the hypothesis that the slope, b, equals 1.

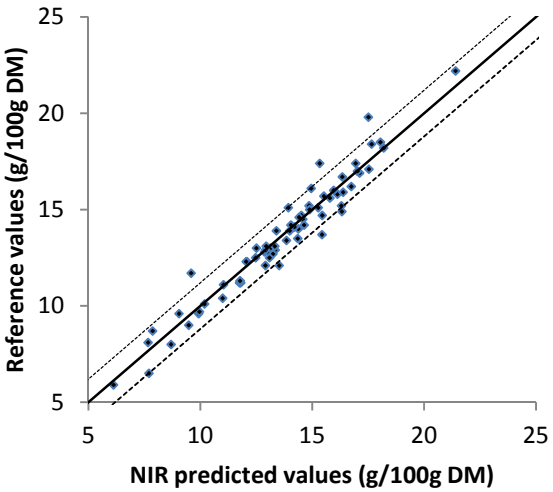

Figure 1 Determination of crude protein in grass silage. Results obtained from an independent test set consisting of 69 Swedish grass silage samples using a calibration equation validated by Eurofins Food & Agro Testing Sweden AB. For these 69 samples; SEP=0.74; bias=-0.06 and slope b=1.02. The solid line represents the ideal line with slope b=1 (y=x) and the dotted lines show the warning limits, ±2SEP (SEP of the validation test set).

Measurement uncertainty

Defining the measurement uncertainty of an indirect method is not always straightforward. According to the ISO standard, the test report should contain the current SEP and bias (if statistically significant). However, continuously updating this information on the test reports is not practical. Other practices are therefore needed. One way is to use data from independent test sets and express the measurement uncertainty as two times the SEP or two times the root mean square error of prediction, RMSEP, which includes the random error and the systematic error, as well as the error of the reference method. The extended measurement uncertainty expresses the variation within a 95 % confidence interval, which means that 95 % of the samples are expected to vary within this range (±). If a larger set of samples have been analyzed as a part of the control program (see below), these data could also be used for determining the measurement uncertainty.

Running performance check

At least one representative and stable control sample should be measured daily to check the stability and function of the NIR instrument (Verner et al. 2012). Also, the calibration equation must be monitored over time to ensure that its accuracy is maintained. The running validation should be performed on randomly selected samples from the pool of routinely analyzed samples. Control charts plotting the differences between results obtained by the reference and NIR methods against the running sample numbers are assessed continuously (Figure 2). The control charts should be checked for systematic patterns and excessive variation of results. Warning and action limits can be set and used as tools for identifying problems.

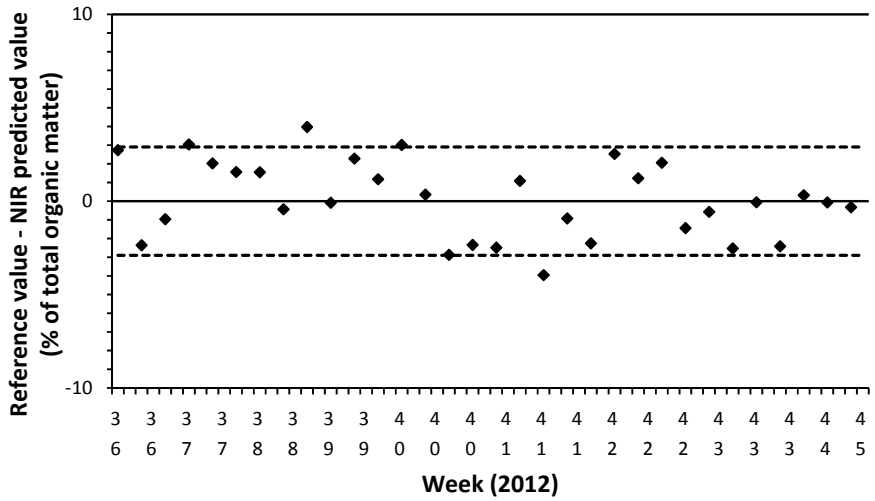

Figure 2 Control chart for the forage parameter IVOMD, in vitro organic matter digestibility (IVOS, in vitro vomvätskelöslig organisk substans), in grass silage. Results obtained from 32 Swedish grass silage samples used for the running performance check using a calibration equation validated by Eurofins Food & Agro Testing Sweden AB. For these 32 samples; SEP=1.12 and bias=0.09. The dotted lines represent the warning limits, ±2SEP (SEP of the validation test set).

NIR calibrations used for forage analysis at Eurofins in Sweden

The NIR calibrations used for forage analysis in Sweden have been developed in collaboration between Eurofins companies in Sweden, Norway and Denmark. Today, NIR calibrations for five different sample types are in use; fresh grass, grass silage, fresh maize, maize silage and whole crop silage. NIR calibrations for parameters such as crude protein, crude fiber, crude ash, BSN (buffer soluble nitrogen), in vitro organic matter digestibility IVOMD (IVOS, in vitro vomvätskelöslig organisk substans), enzyme digestible organic matter EFOS (enzymfordøjlig organisk substans), neutral detergent fiber NDF, iNDF (insoluble NDF), sugar and starch are available to the Swedish customers, as well as quality indicating parameters such as pH, lactic acid, acetic acid and ammonium nitrogen. The calibrations have been developed using a large set of samples originating from all three countries taking part in the Nordic NIR network within Eurofins. By continuously updating these calibrations using relevant samples, we are able to serve the Swedish farmers with robust and reliable forage analyses accredited by the Swedish accreditation body Swedac (Swedish Board for Accreditation and Conformity Assessment).

Conclusion

NIR spectroscopy has revolutionized the nutritional characterization of forages enabling rapid evaluation and efficient utilization of forages. This non-invasive and high-throughput technique is not only rapid and cost effective; the low environmental impact of NIR spectroscopy makes it even more favorable compared to traditional chemical methods and can thus reduce the analytical variation. The guidelines presented in the ISO standard provide the cornerstone in the continuous quality assurance work in the field of forage analysis using NIR technology. The

accuracy of an NIR method can by definition never exceed the accuracy of the reference method on which it is based. On the other hand, NIR spectroscopy is often more precise (repeatable) compared to chemical methods. However, it is also important to keep in mind that the sampling uncertainty is most often the dominating factor in the final uncertainty of the results.

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Methods for iNDF determination

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Introduction

Indigestible NDF (iNDF) is one of the most influencing feed characteristics in 'state of the art' ration evaluation systems for ruminants to assess potential NDF degradability. The reference method for iNDF in NorFor (Åkerlind et al., 2011) is in situ using 288-h rumen incubations in synthetic monolayer bags with pore size 11-15 µm and replicated in a minimum 2 cows. In practice, in the Nordic countries, iNDF is estimated using NIR, and NIR calibrations for iNDF have up to now been based on direct calibrations on in situ values. However, this is not satisfying in the long run due to both costs and delay in calibration control when calibration samples have to be run outside the NIR laboratory.

iNDF is related to several chemical and availability characteristics of the feedstuffs. However, simple or multiple regression equations seem not to be universally satisfactory across feed types (Krämer et al., 2012), although used in e.g. the CNCPS system (Fox et al., 2004). Further, reproducibility of in situ iNDF determination could be improved and in a ring test, Eriksson et al. (2012) found considerable variation among laboratories, especially for rapeseed meal. Part of the intra-laboratory variation was probably due to milling and thereby particle size of the incubated samples. A major concern about the iNDF in situ method is, therefore, that particles could be lost from the bag both immediately or during incubation, a risk which seems to be very feed type specific (Krämer et al., 2013). This calls for research in reproducible and less resource demanding alternatives to the in situ method.

Therefore, the aim of the present study was to examine whether long time in vitro filter bag incubations could result in NDF residues comparable to the in situ 288 h determination, and further to study the effect of incubation time, milling and bag type on some typical feed and faeces samples.

Materials and methods

The study had a 5x2x2x4 factorial design. Five sample types, 2 grind sizes, 2 bag pore sizes and 4 incubation methods. Feed samples consisted of maize silage, low digestibility grass-clover silage, high digestibility grass-clover silage, rape seed meal, and faeces (Table 1). Samples were dried at 60^{o}C, and then milled on a 1.5 mm screen (M1.5) in a cutter mill (Pulverisette 15, Fritsch). Half of 1.5 mm milled material was then milled on a 1 mm screen (M1) in a Retsch ZM 200 mill. 0.5 g sample was weighed out in 2 bag types (ANKOM F57 (ANKOM) or 12 µm Dacron (Dacron)), heat sealed, and incubated using 4 different incubation methods (288 h rumen in situ (R288), or either 48 h (D48), 96 h (D96), or 2x96 h (D2x96) using the Daisy (ANKOM) methodology). For the D2x96 treatment, the rumen fluid-buffer was exchanged after 96 h and fermentation continued for another 96 h. When exchanged, a slight amount of the old fluid was retained to assure bags were kept wet during exchange. All incubations were both repeated and replicated twice, ending with a total of 320 bags. For rumen in situ repetitions 2 bags were incubated simultaneous in the same cow, and replicated in a second cow. In Daisy repetitions

incubations were in two different flasks at the same time using similar rumen fluid, replicates were new runs with new rumen fluid.

Beside above mentioned changes, ANKOM recommended procedures were followed for both Daisy incubations and NDF (ANKOM 220) boiling (ANKOM, 2013).

Reference iNDF values were determined for the 5 samples times and 2 millings using the standard NorFor procedure as in Åkerlind et al. (2011) in 3 cows, and NDF concentrations in the 5 samples determined by both Fibertec (glass filter crucibles) and by the two ANKOM (bag) methods (ANKOM and DACRON).

All NDF determinations were according to Mertens (2002) using sodium sulphite and heat stable amylase and were reported as ash free.

Rumen fluid for Daisy incubations was obtained from the same 3 dry cows as used for all in situ incubations, cows were fed at maintenance level with 1/3 concentrate and 2/3 hay + straw. Fluid was harvested as proposed by ANKOM (2013), half direct from the rumen and half from blended fluid + particles, and both fractions strained though double layer gauze.

Results and discussion

NDF concentration

NDF concentration for the 5 types of samples and the 2 millings were chemically analysed using 3 different methods, the 'golden standard' using glass filter porosity 2 crucibles in a Fibertec, the ANKOM method using prescribed filter bags and the ANKOM method using Dacron bags (Table 1). A statistical analysis showed effects of sample and method ($P<0.0001$), and tendency for interaction between method and milling ($P=0.1$), whereas there was no main effect of milling ($P=0.3$). NDF values (Lsmeans for NDF in DM) were higher in ANKOM (40.4) than in Fibertec (37.6), with Dacron (37.7) very similar to Fibertec. Contradictory to this, Ferreira & Mertens (2007) found similar NDF values when comparing crucibles and filter bags.

For 3 sample types (Grass/clover sil. low, rapeseed meal, faeces), M1 gave higher NDF values than M1.5 for the ANKOM filterbag method, which was surprising.

iNDF concentration

The Norfor standard method was used to estimate 'golden standard' values for the samples, using both millings (Table 2). A statistical analysis of iNDF in DM showed generally significant ($P=0.0.007$) lower (0.5% of DM) iNDF values for smaller screen milling, but also a significant ($P=0.004$) sample x milling interaction due to an opposite effect of milling for rapeseed.

Table 1 NDF concentration (% of DM) measured in original samples by different milling, equipment and bag type

Equipment	Fibertec		ANKOM			
Filtration	Glass crucible		Dacron bag		ANKOM filterbag	
Milling	M1.5	M1	M1.5	M1	M1.5	M1
Maize silage	40.1	38.6	39.2	37.4	43.3	40.9
Grass/clover sil. low	41.8	40.6	41.9	40.0	41.1	44.0
Grass/clover sil. high	37.6	36.4	37.8	35.6	40.0	39.5
Rapeseed meal	26.9	26.6	25.3	24.5	25.7	28.7
Faeces	44.3	44.3	47.7	46.3	49.4	51.3

Stat. model: NDF = Sample Method Milling Bag x Milling; P<0.0001 for Sample and Method, P=0.3 for Milling, P = 0.1 for Method x Milling

Table 2 iNDF concentration in original samples measured by standard method but at two millings

	% iNDF in DM		% iNDF in NDF	
Milling	M1.5	M1	M1.5	M1
Maize silage	8.2	7.5	20.4	19.3
Grass/clover sil. low	5.7	5.2	13.6	12.8
Grass/clover sil. high	3.6	3.2	9.5	8.8
Rapeseed meal	12.9	13.7	47.8	51.4
Faeces	24.7	22.9	55.1	51.0

Model: % iNDF in DM = Sample Milling Cow Sample x Milling; P = 0.0001 for Sample, P = 0.007 for Milling, P = 0.004 for Sample x Milling, P = 0.02 for cow

Effect of incubation method

Only main results across samples are reported here (Table 3). Increased incubation time in the Daisy from D48to D96, and further reincubation (D2x96) reduced the NDF residue, but not down to the rumen incubation R288 level, although the D2x96 approached R288. This indicate that even with prolonged and reinoculated Daisy incubations, the long term R288 rumen incubation results in lower residues, and the NDF residues obtained by the 3 Daisy methods all overestimate iNDF.

Effect of bag type

Only main results across samples are reported here (Table 3). Generally, all 2 way interactions were highly significant except bag x milling. Dacron bags resulted in lower NDF residues than ANKOM bags. This difference was affected by Method, and decreased from D48 over D96, D2x96 to R288, the same as the ranking for NDF residues. However, the difference was only to a minor degree affected by milling, which could indicate that the difference is more an effect of better conditions for NDF degradation in the Dacron bags than a result of increased particle loss from Dacron bags, compared to ANKOM bags.

Effect of milling

Only main results across samples are reported here (Table 3). Lower particle size by finer milling reduced the NDF residue, a difference which as for bag type interacted with method.

Methods giving lower residues also diminished the difference. The difference was slightly less for Dacron than for ANKOM bags, which indicates that the milling effect was more due to increased fermentation efficiency than to increased particle loss from the Dacron bags compared to ANKOM bags. This is contradictory to Bossen et al. (2008) who did not find any major or consistent effects of particle size (milling) on NDF degradation pattern.

Table 3 Main effects of milling, method and bag type on NDF residue as % of incubated DM

	Milling	M1				M1.5			
Bag	Method	D48	D96	D2x96	R288	D48	D96	D2x96	R288
ANKOM		22.6	18.5	14.9	12.7	26.4	21.3	16.9	13.6
Dacron		15.4	14.5	13.0	12.3	18.2	16.9	14.2	12.7

Model: NDF residue as % of incubated DM = Sample | Method | Milling | Bag, with Replicate*Sample*Method*Milling*Bag as random. All main effects and 2 ways interactions highly significant ($P<0.0001$) except interactions including bag

Table 4 Main effects of milling, method and bag type on NDF residue as % of NDF, where original NDF was determined with similar methods and bags as used for residues

	Milling	M1				M1.5			
Bag	Method	D48	D96	D2x96	R288	D48	D96	D2x96	R288
ANKOM		50.5	41.2	33.2	28.5	60.3	48.7	39.0	31.8
Dacron		38.9	37.0	33.2	31.8	44.0	40.9	34.6	30.4

Model: NDF residue as % of incubated NDF = Sample | Method | Milling | Bag, with Replicate*Sample*Method*Milling*Bag as random. All main effects and 2 ways interactions highly significant ($P<0.0001$)

Variance components for repeats between (Replicate*Sample*Milling*Bag) and within (residual) runs were estimated as covariance parameter estimates within each of the four methods, and were 1.8 and 1.7 for D48, 0.2 and 2.3 for D96, 0.1 and 1.0 for D2x96, and 1.0 and 0.8 for R288, respectively, showing a decrease in variance as incubation time was prolonged and residues decreased.

Conclusions

Increasing incubation time, and increased incubation time combined with reinocculation reduced residues from Daisy incubations, but the most efficient Daisy incubation assessed still did not reach rumen in situ residues. Lower residues obtained for finer milling and for Dacron compared to ANKOM bags were probably a result of better conditions for NDF degradation and not due to increased particle loss.

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Effects of amount and source of dietary protein on hydrogen sulphide concentration in the rumen headspace gas of dairy cows.

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Introduction

Hydrogen sulphide (H_2S) is produced by rumen microbes from organic and inorganic sulphuric compounds (Dewhurst et al, 2007). Most feed sulphur comes from cysteine and methionine. Hydrogen sulphide is mainly produced from cysteine which gives a fast response upon incubation as pure amino acid *in vitro* (Bird, 1972) and *in vivo* (Dewhurst et al, 2007). An increase in H_2S production is reflected in an enhanced rumen head space gas concentration. The quick response upon substrate being available (Dewhurst et al, 2007) and relatively short half-life in rumen head space gas (Dewhurst et al., 2001) makes H_2S a candidate marker for protein degradation, as proposed by Fonseca et al. (2013). The authors compared protein feeds with varying degradation characteristics and suggested H_2S to be useful especially when studying the degradation of soluble protein. However, Dewhurst et al. (2007) reported considerable variation in rumen head space gas concentration of H_2S due to animal, diet and feed characteristics.

The aim of this experiment was to evaluate H_2S as a marker for protein degradation *in vivo*. Two common protein sources for Swedish for dairy cows, field beans, with a high proportion of soluble protein, and rape seed meal, with a low proportion of soluble protein, were examined at two levels of inclusion.

Materials and Methods

Four ruminally cannulated dairy cows in mid-lactation (Swedish Red breed) were used in a 4 x 4 Latin square design experiment with four treatments and four periods of one day each. The same basal ration was fed to all cows at individually fixed levels throughout the experiment according to Eriksson and Rustas (2013). The ration consisted of silage, a commercial concentrate (SOLID 620, Lantmännen, Stockholm, Sweden) and urea (AB Johan Hansson, Uppsala, Sweden) in the proportions 39.3:60.0:0.7 on a dry matter basis. Silage was fed with two equal meals at 10:00 and 16:45 h each day, and concentrates were fed in a separate through with three equal meals at 09:30, 13:00 and 17:00 h, respectively. The 09:00 and 13:00 h concentrate meals were fortified with urea that was manually mixed into the concentrates at feeding. Average total feed intake was 20.2 kg DM per day with a CP concentration of 16.5%.

Experimental treatments were field beans and rapeseed meal, ground through a 2.0 mm screen in a Wiley mill, given at two levels to supply 0.34 or 0.68 kg of crude protein from each feed. Amino acid supply and composition was calculated from the NorFor feed table (Norfor, 2013). On each experimental day, protein feed was administered into the rumen through the rumen cannula and was then mixed by hand into the rumen content. The first cow was fed at 06:00 h followed by the other cows at 5 min intervals.

Hydrogen sulphide concentration of rumen gas was measured with a hand held gas instrument designed for CH_4, CO_2, O_2, CO and H_2S measurements (GA2000, Geotech, Lemington Spa, UK). A tube with a nylon cloth covering the opening to avoid clogging was entered through a ball valve mounted on the lid of the rumen cannula. Gas was monitored for 1-2 min. until stable values were reached starting directly before feed was given. After 20 min., gas composition was measured again and then in 20 min. intervals until 200 min. after each cow was fed. Directly after each gas measurement, rumen liquid was collected through the ball valve by a tube connected to a peristaltic pump. Ruminal liquid samples were analyzed for αa-N (Moore, 1968; Broderick and Kang 1980).

At all gas measurements, different proportion of oxygen was detected and to standardize rumen gas concentrations, H_2S values were adjusted to zero oxygen concentration. Hydrogen sulphide concentrations at 60 min. after administration of experimental feeds were compared but values were also averaged over all sampling points (area under curve) for comparison of gas produced.

Data from gas measurements was analyzed with the GLM procedure of Minitab 16 (Minitab Inc., State College PA, USA) with cow and period as random and treatment as fixed factors in the model.

Results and Discussion

Amounts of added feeds, their sulphur content and calculated amino acid composition are presented in Table 1. Although similar amounts of CP was supplied from field beans and rapeseed meal, the sulphur supply differed largely between treatments which resulted in large differences in H_2S concentrations, both in spot samples and in the averaged values (Table 1). Nevertheless, at the measurement point 60 min. after adding protein into the rumen, H_2S in rumen gas was linearly related to S supply (H_2S (ppm) = 27.7 S(g) - 22.5, $p<0.001$, $R^2=0.82$). This was despite that H_2S did not seem to have reached its peak value on the rapeseed treatment which, on the other hand, the field bean treatment seemed to have passed (Figure 1). Dewhurst et al. (2007) found different temporal patterns in rumen gas H_2S concentrations with increasing levels of cysteine addition. They suggested 8 g of cysteine to be a level where transformation to H_2S was saturated and therefore H_2S concentrations remained higher for a longer time. This is supported by Figure 2 where it seems that a greater inclusion of rapeseed meal results in prolonged and elevated H_2S concentrations. It can be compared with the curves from the field bean treatment where the level of H_2S concentration differ but the time with elevated concentration are similar between inclusion levels, suggesting no limiting effects on H_2S production due to substrate level. The different patterns of the curves from rapeseed and field bean might also have been caused by differences in S form (Table 1). Dewhurst et al. (2007) found faster release of H_2S from amino acid S than from inorganic sulphur. This might explain why rapeseed meal, with only about half of its S coming from amino acids (Table 1), started slower compared with field bean with practically all of its S from amino acids. The different temporal pattern might also reflect differences in protein degradation characteristics, as suggested by Fonseca et al. (2013). The field bean treatment reached its peak value at about the same time as that of α–amino N in the rumen liquid which also seemed to be the case for the rapeseed treatment.

Table 1 Treatment feed, sulphur and amino acid content and hydrogen sulphide in rumen gas.

Item	Field beans		Rapeseed meal			Effect (*P*-value)		
	Low	High	Low	High	SED	Feed	Level	F×L
Treatment, kg/d	1.3	2.6	1.0	2.0				
S, g/d	1.6	3.1	6.5	13.0				
CP[a], kg/d	0.34	0.68	0.34	0.68				
Cysteine [a]g/d	4.4	8.9	7.5	15.0				
Methionine[a], g/d	2.7	5.4	6.5	12.9				
S from amino acids[a], g/d	1.7	3.5	3.4	6.9				
H_2S								
60 min[b], ppm	24	45	182	330	26.7	<0.001	0.019	0.055
AUC[c], min×ppm	2944	4443	25180	42246	1832	<0.001	0.002	0.005

[a]Calculated from NorFor feed tables (Norfor, 2013)
[b]Values adjusted to zero oxygen content, N=16
[c]Area under curve, values averaged over all measurement points, N=16.

Conclusions

Hydrogen sulphur concentration in rumen gas is a promising marker for protein degradation *in vivo* and *in vitro*. Several questions, e.g. availability of S in relation to protein degradation characteristics and S source (inorganic vs. organic and amino acid composition), need to be further investigated.

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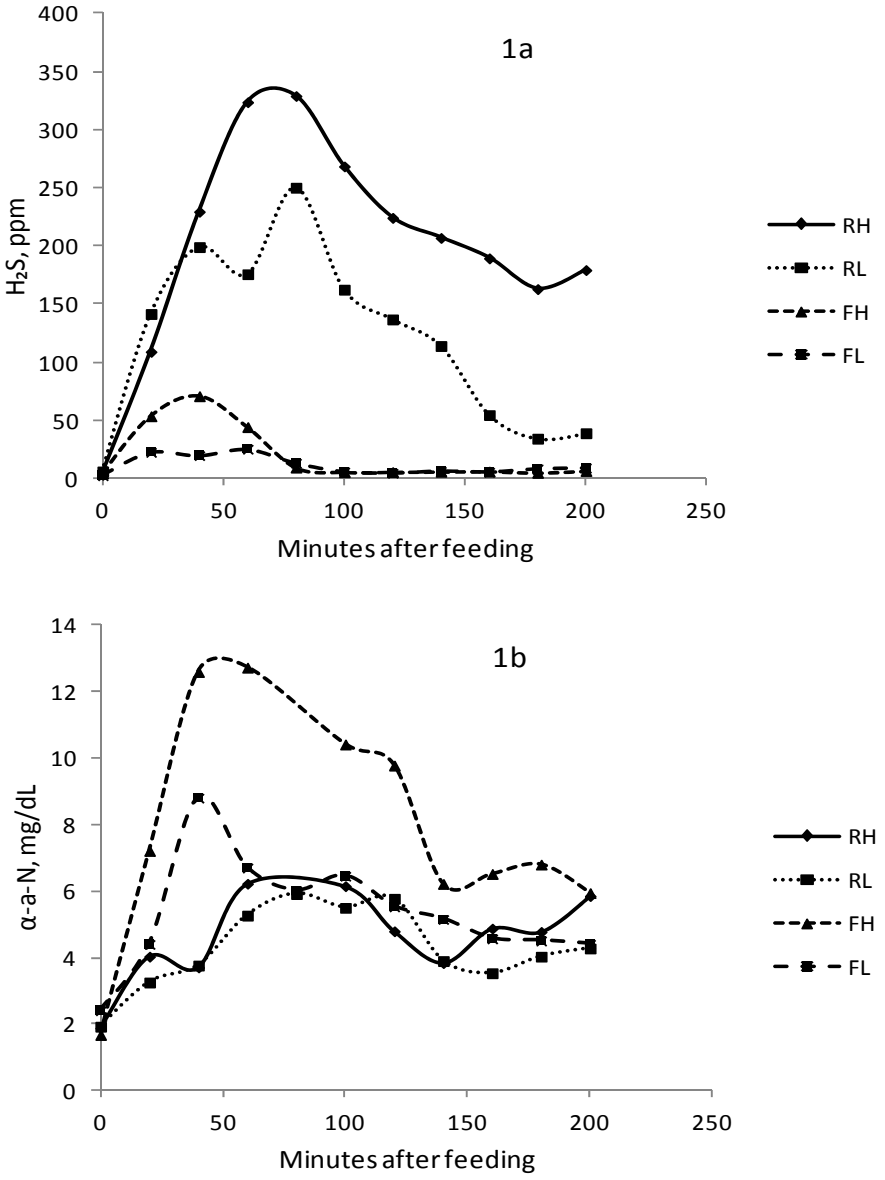

Figure 1a and b. Concentration of hydrogen sulphide (H_2S) in rumen headspace gas at different time points after addition of high (H) and low (L) doses of rape seed meal (R) and field bean (F) in the rumen (1a). Concentration of α-amino N (aa-N) in rumen liquid sampled immediately after H_2S measurements.

New recommendations of the ruminal in situ determination of indigestible neutral detergent fiber with special reference to near infrared reflectance spectroscopy

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Introduction

Indigestible neutral detergent fiber (iNDF) predicts forage digestibility accurately and precisely when determined by a 288-h ruminal in situ incubation in dairy cows (Huhtanen et al., 2006; Krizsan et al., 2012). Near infrared reflectance spectroscopy (NIRS) is applied in routine analysis of farm samples in all Nordic countries. An NIRS calibration is continuously under development by adding spectral information from new samples. New reference samples are usually collected by the farm service laboratory. A NIRS instrument requires a fine grind size of the sample of approximately 0.8 mm, while a grind size of up to 2 mm is often used for the sample in the ruminal in situ determination of iNDF. In this project we aim to compare the incubation results of samples displaying a range in fiber concentration and composition for the two different grind sizes mentioned above and incubated in nylon bags of four different qualities. Further, due to losses during ensiling the project will examine if predictions of iNDF by NIRS would benefit from separate calibrations for herbage and silage samples. This was accomplished by analyzing a set of cut herbage and corresponding silage with known in vivo digestibility.

Materials and methods

Experiment 1
Samples of oat (*Avena sativa*) and rapeseed (*Brassica napus*) meal were provided by the feed manufacturer AB Västerbottens Fodercentral in Umeå, Sweden. A sample of dried alfalfa (*Medicago sativa*) was taken from a bag of the commercial feed Krafft Lusern (Krafft AB, Falkenberg, Sweden). Samples taken from two grass silages that were harvested on June 10 and on July 19 in 2012 from a timothy (*Phleum pratense*) dominated sward at Röbäcksdalen research farm in Umeå (63°45´N, 20°17´E) were included in this experiment. Samples of barley (*Hordeum vulgare*) straw and crimped barley were also from feeds harvested at Röbäcksdalen research farm. A hay sample from a timothy dominated sward harvested in July 2010 was included in the study. Additionally, fecal samples from a dry and a lactating cow were collected at Röbäcksdalen research farm. All experimental samples were dried at 60°C for 48 h and were ground through a 2.0-mm screen for in situ incubations or a 1.0-mm screen for chemical analysis, using the same cutting mill (Retsch SM 2000; Retsch GmbH, Haan, Germany). The fecal samples were ground with mortar and pestle to pass the same screen sizes. A fine grind size representative of that needed for scanning in a NIRS system was achieved by grinding the samples at the research laboratory at Valio Ltd. in Helsinki, Finland. The samples were there ground to pass a 0.8 mm screen with a Laboratory Mill 3100 (Danfoss AB, Linköping, Sweden).

The iNDF concentration of the 0.8- and the 2-mm grind size of each sample was determined following in situ incubations of 288 h in the rumen using three cows. Two dry and one lactating

Methodology

Swedish Red cows were used for the ruminal in situ incubations. The lactating cow was fed a diet consisting of 60% grass silage and 40% concentrate on dry matter (DM) basis with amounts regulated to meet production requirements. Samples of 2 g were weighed into polyester bags with a pore size of 6, 11, 12 and 17 µm and pore areas equal to 5, 5, 6 and 9%, respectively, of the total surface area. The 12-µm bags were made of the cloth Saatifil PES 12/6 from Saatitech S.p.A., Veniano, Como, Italy, which is classified as the Nordic standard per today (Åkerlind et al., 2011). The other bags were made of 07-6/5, 07-11/5 and 07-17/9 Sefar Petex from Sefar AG, Heiden, Switzerland. The internal dimensions of the nylon bags and the sample size were adjusted to give a sample size to surface area ratio of 10 mg/cm^2. All samples were incubated in each bag type in each of the three cows. Incubation residues were treated and analyzed for neutral detergent fiber free of residual ash (NDFom) as described by Krizsan and Huhtanen (2013).

Residual moisture of all feed samples was determined by oven drying for 16 h at 105°C. Ash concentration was determined by ignition of the dried sample at 500°C for 4 h. The samples were analyzed for NDFom using heat stable α-amylase (aNDFom) in an ANKOM^{200} Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Concentrations of crude protein (CP) were determined from Kjeldahl digestion in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA).

Experiment 2

Samples of herbage and corresponding grass silages used in digestibility trials were collected during several years at MTT Agrifood Research Finland in Jokioinen (60°48´N, 23°29´E), Finland. The samples have been used to evaluate different laboratory methods for predicting in vivo organic matter digestibility (OMD) in forages with the aim to develop rapid and reliable farm service analyses. In vivo digestibility of all the silages was determined in sheep fed at a maintenance level of feeding. More information about the silage samples, chemical analyses and digestibility trials can be found in the article by Huhtanen et al. (2006).

In this study, a total of 34 primary and regrowth silages of timothy (*Phleum pratense)* and meadow fescue (*Festuca pratensis*) grass mixtures were used. The grass silages were harvested in five different years and the corresponding herbage sample of each of the silages was collected on the day of ensiling. The primary growth grass (n = 16) was harvested at different growth stages between June 5 and July 3 across years; the growth time from previous harvest of the regrowth (n = 16) ranged between 41 and 81 days with an average of 60 days. The growth times from previous harvest of the third cut samples (n = 2) were 37 and 40 days, respectively. The silages were ensiled direct cut and preserved with formic acid. Silage samples were collected during weighing of the daily rations and composited within the experimental period of the digestibility trials. The ranges in DM, aNDFom and CP for the grass and silage samples were from 128 to 299 and 151 to 290 g/kg, from 500 to 687 and 447 to 666 g/kg of DM, and from 104 to 211 and 107 to 207 g/kg of DM, respectively. The OMD of the silages ranged between 610 and 823 g/kg. The samples were oven-dried for 24 h at 60°C, equilibrated to room humidity and ground through a 1-mm screen using a cutting mill (Sakomylly KT-3100, Koneteollisuus Ltd., Helsinki, Finland). The silage samples were analysed as a pooled sample across all periods

within each diet in all trials. The in situ determination of iNDF was conducted in two consecutive 288-h incubations using the same three cows as described above. Due to the smaller grind size, the samples were incubated using the 6-µm polyester bags (07-6/5 Sefar Petex, Sefar AG, Heiden, Switzerland). Else, the same procedures as described in Experiment 1 were followed.

Statistical analysis

The data were analyzed using the General linear model of SAS (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC) by applying a model correcting for the effect of cow, sample, bag type, grind size, the interaction between sample and bag, and the interaction between sample and grind size. The interactions between bag type and grind size, and between sample, bag type and grind size were not significant ($P \geq 0.27$) and therefore excluded from the final model. In Experiment 2 the model included main effects of cow, sample, forage type (grass or silage), and the interaction between sample and forage type. Analysis of variance was further used to produce simple regressions of in vivo OMD on iNDF concentration of grass or silage samples. Least square means are reported and mean separation was done by least significant difference to test differences between treatments.

Results and discussion

Experiment 1

The experimental samples displayed a wide variation in chemical composition (Table 1). The sample iNDF concentrations are presented in Table 2. The significant interactions between sample and bag type ($P < 0.01$) indicated that iNDF concentration determined in the 12-µm polyester bags was greater for all samples except for the rapeseed meal, the crimped barley and the oat. The average significant difference in iNDF between feed samples incubated in 12-um bags and the other bags were 22 g/kg DM. The iNDF concentrations determined in 17-µm polyester bags in feces from the dry cow and in the barley straw were lower than the values achieved using the 6- and 11-µm polyester bags ($P < 0.01$), which indicated particle losses during the analysis. Else, there were no differences in iNDF concentrations between the Sefar Petex bags within experimental sample ($P \geq 0.17$). It is difficult to explain the reason behind the on average greater concentrations of iNDF when samples were incubated in the 12-µm polyester bags compared to the Sefar Petex bags. Preliminary results from pictures taken using an electron microscope indicate that the shapes of the pore sizes in the 12-µm Saatifil PES cloth are much more irregular compared to the three different Sefar Petex cloths. We also observed a slower water flow through the 12-µm Saatifil PES bags compared with the other bags made of Sefar Petex cloth. The pore size and open surface area of bags used for in situ studies should allow for removal of degradation end products from substrate fermentation; thereby equating the internal environment within the bag with that of the rumen. Krizsan and Huhtanen (2013) showed that conditions affecting the ruminal micro flora did to a varying degree affect feed samples incubated in situ despite the long incubation time used in the determination of iNDF. Either microbial access or activity could have been impaired in the micro environment of the 12-µm Saatifil PES bags compared with the bags made of Sefar Petex cloth. Several studies have shown that particle-associated enzyme activities have been much higher when measured from rumen

digesta than from feed particles recovered from incubated in situ bags (e.g. see Krizsan et al., 2013), but the incubations were much shorter than for iNDF.

The significant interactions between sample and grind size ($P < 0.01$) indicated that particles were lost during the incubation of samples ground through the 0.8-mm screen, except for the barley straw, rapeseed meal, first cut grass silage and the crimped barley sample. There were no particle losses between any feed samples except for alfalfa ground through the two different screen sizes when the samples were incubated in the 6-µm polyester bags ($P \geq 0.21$). It is well-known that there are particles losses in situ of the fiber fraction, initial as well as secondary losses have been suggested, and that the magnitude of the losses are dependent on the characteristics of the feed (e.g. see Krizsan et al., 2013). However, also cloth type has been shown to affect the recovery of the indigestible residue (Huthanen et al., 1994; Valente et al., 2011).

Table 1 Chemical composition of experimental feeds in Experiment 1

	DM	OM	NDF	CP
	g/kg		g/kg DM	
Feces dry cow	160	842	557	122
Feces lactating cow	157	868	505	207
Barley straw	839	869	781	29
Alfalfa	883	918	452	182
Rapeseed meal	831	906	275	336
Grass silage[a] 1	214	913	547	190
Grass silage[a] 2	213	920	565	144
Crimped barley	729	972	248	128
Grass hay	864	945	582	60
Oat	882	968	333	111

[a]The number 1 and 2 indicate first and second cut grass silage, respectively.
DM, dry matter; OM, organic matter; aNDFom, neutral detergent fiber free of residual ash determined using heat stable α-amylase; CP, crude protein.

Experiment 2

The main effect of sample, and the interaction between sample and forage type were significant ($P < 0.01$). The standard error of mean was 2.0 for all samples. The significant interaction between the main effects differentiated grass vs. silage concentrations of iNDF in four pairs of all experimental samples. In three of these four pairwise comparisons silage concentration of iNDF was on average 9 g/kg of DM greater than in the grass ($P \leq 0.01$), while it was 7 g/kg of DM lower for the silage in the last comparison ($P < 0.01$). The greater concentration of iNDF in the three silages compared to the corresponding grass samples does not seem to be a consequence of the conservation, more likely bad replicate values for some of the samples. Lactic and acetic acid concentrations ranged between 25 and 44, and 8 and 13 g/kg DM in these three silages, and butyric acid concentration was 0.2 g/kg DM in all three silages. The regression of OMD on iNDF in grass or in silage samples respectively were:

OMD (g/kg) = 862(±8.0) – 1.83(±0.091) × iNDF (g/kg DM) [$R^2 = 0.93$ and RMSE = 15.4 g/kg]

OMD (g/kg) = 860(±6.2) – 1.79(±0.068) × iNDF (g/kg DM) [R^2 = 0.95 and RMSE = 12.1 g/kg], where R^2 is the coefficient of determination and RMSE is the root mean square error. Neither intercepts nor did regression coefficient estimates differ between the two equations ($P > 0.05$). The fit of both equations (R^2) were good and the RMSE low.

Conclusions

The determinations of the concentration of iNDF using the 12-µm Saatifil PES bags were generally greater compared to iNDF determined in the Sefar Petex bags. To avoid potential errors from particle losses or impaired microbial activity we would recommend a grind size of 2.0 mm and bags made from Sefar Petex 07-11/5 to be used in ruminal in situ determination of iNDF. The majority of the silages in Experiment 2 were well-fermented silages with small losses of DM and the formic acid preservation did not affect the determination of iNDF. A broader number of samples including less well-fermented silages need to be investigated before it can be concluded that one common calibration dataset is enough for NIRS predictions of iNDF in grass silages.

Table 2 Concentration of indigestible NDF (g/kg of DM) of all experimental samples using four different pore size bags (in µm) combined with two grind sizes (in mm) in Experiment 1[a]

Bag type	6		11		12		17	
Sample[b] Grind size	0.8	2.0	0.8	2.0	0.8	2.0	0.8	2.0
Feces dry cow	299	315	310	316	339	351	290	311
Feces lactating cow	202	235	206	232	234	251	196	228
Barley straw	291	284	274	291	303	307	263	275
Alfalfa	233	250	239	251	255	261	230	253
Rapeseed meal	107	112	105	114	115	109	113	117
Grass silage 1	61	64	60	62	76	83	56	62
Grass silage 2	101	108	96	105	126	132	94	104
Crimped barley	35	41	36	40	39	42	39	44
Grass hay	142	148	141	146	153	170	140	145
Oat	129	134	118	132	117	137	112	138
Mean of column values	160	169	159	169	176	184	153	168

[a]Probability of a significant effect of sample, bag type, grind size, and the interactions sample × bag and sample × grind size were < 0.01. The interaction bag type × grind size and sample × bag type × grind size were not significant ($P \geq 0.27$) and therefore excluded from the final model. [b]The number 1 and 2 indicate first and second cut grass silage, respectively.

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Trying to account for all components in fresh and ensiled forage plants

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Introduction

The analytical assays used for evaluating feeds in NorFor are the basis for the model to function correctly. In NorFor, feeds are divided into ash, neutral detergent fiber (aNDFom), crude protein (CP), starch, fat, fermentation products and a rest fraction (RestCHO). The latter is calculated as the difference between 1000 and the analyzed fractions (g/kg dry matter). However, also water soluble carbohydrates (WSC) is included in the feed description both as a separate entity for calculating rumen load and for estimating metabolizable energy.

Most published papers do not report the proportion of the feeds which can be accounted for. According to NorFor, RestCHO is mainly composed of easily degradable carbohydrates (WSC, pectin and β-glucanes; Volden, 2011). The non-carbohydrate portion of RestCHO is assumed to ferment at a rate of 0.1 /h. This rate is considerably slower than for the WSC portion of the RestCHO, which is assumed to ferment at 1.50 /h. Errors in the proportion of carbohydrates and non-carbohydrates in RestCHO could have a profound effect on NorFor predictions. Active members of the NorFor Scientific Advisory Group have for considerable time been aware of the potentially variable composition of the RestCHO fraction in feeds and that it may impact on services offered to the farmers in terms of diet optimizations. The major potential problems of the rest fraction are likely: i) it can constitute a sizeable fraction of the feed (Table 1), ii) it is composed of widely different substances, iii) errors from analyses of non-RestCHO components will be aggregated in RestCHO.

Table 1 The size of the rest fraction (RestCHO) with (+WSC) and without (-WSC) sugars included, based on NorFor Swedish tables (g/kg DM)

Feed type	No	Mean		Max		Min	
		+WSC	-WSC	+WSC	-WSC	+WSC	-WSC
Grass-clovers	85	238	144	304	294	128	17
Silages	121	150	105	267	253	38	1
Grains	31	36	14	156	142	-26	-59
Oil seed	27	129	68	247	183	-36	-90

One objective of this study was, therefore, to try to account for as much as possible of the total dry matter in fresh and ensiled forages with special emphasis on components which are not part of NorFor analyses. Another objective was to find out effects of ensiling on disappearance of potential substrates.

Materials and Methods

Timothy, red and white clover were harvested at early and late maturities in two cuts. Whole crops of barley, wheat and maize were harvested at early, intermediate and late stages of maturity in the area around Uppsala, Sweden. Plants (5-6 kg) were harvested by hand and leys were wilted in a forced draught oven at 60°C to reach a dry matter (DM) content of approximately 400 g/kg. The whole crops were handled similarly, except that no wilting was done. After chopping, part of the crop was ensiled in duplicate 1.7-L glass jars for 60 days. Of remaining material, 100 g was frozen after adding 100 or 200 g water to enable juice extraction, frozen or dried over night at 60°C (Table 1). Silages samples were handled identically depending on assay. Extracts were obtained by use of a hydraulic press. Analytical methods used are shown in Table 1.

Table 2 Analytical methods and form when analyzed

Source	Type	Form	Analysis	Reference
All	Both	Dried	Lipids	Åkerlind et al (2011)
All	Both	Dried	Pectin	Bucher (1984)
All	Both	Dried	Phenolics	Singelton and Rossi (1965)
All	Both	Fresh	Dry matter	Åkerlind et al (2011)
All	Both	Dried	Ash	Åkerlind et al (2011)
All	Both	Fresh	Crude protein	Åkerlind et al (2011)
All	Both	Dried	aNDFom	Chai och Udén (1998)
All	Both	Fresh	WSC	Udén (2010)
Whole crops	Both	Fresh	Starch	Udén (2010)
All	Crops	Extract	Acids & alcohols	Ericson och André (2010)
All	Silages	Extract	Ammonia	Åkerlind et al (2011)

aNDFom=amylase treated ash-free neutral detergent fiber; WSC=water soluble carbohydrates.

Results and Discussion

A summary of the results are shown in Table 3. Analyses of ash, aNDFom, CP, starch, fat and fermentation products are shown as sums and RestCHO calculated as the difference between 1000 and the analyzed fractions (g/kg dry matter). Recoveries of all analytes ranged from 853–1111 g/kg DM and RestCHO varied from 59-402 g/lg DM. As recoveries are not exactly 100%, proportions of the various RestCHO components did not sum up to 100%. Water soluble carbohydrates, pectins and plant organic acids (organic acids not specified for the analysis of ensiled and un-ensiled crops) accounted for 41, 37 and 29%, respectively in fresh crops. Values for silages were 18, 37 and 29%. Phenolics levels were below 5% in RestCHO. Pectin values are influenced by the assumed proportion of galacturonic acids. In this study, a concentration of 830 g/kg pectin was assumed. Fermentation rate of WSC is high (1.5 /h, according to NorFor) and for pectin, our own studies indicate a rate of approximately 0.40 /h (unpublished). The composition of organic acids are shown in Table 4. Dominating acids were citric, malic and quinic+malonic (could not be separated), with succinate, shikimic and fumarate <5% of total.

The fermentation rate of plant organic acids is unknown, but should be high. Succinate is not part of the NorFor analyses of silage. Values from this study were low in the silages (mean=3 g/kg DM) but our own studies have found levels of 20 g/kg DM.

Table 3 Composition of fresh and ensiled leys and whole crops (g/kg dry matter)

Type	Form		NorFor anal.	Rest-CHO	WSC	Phenol.	Pectin	Organic acids	Sum	Total recov.
			g/kg DM		---------- of RestCHO ----------					g/kg DM
Leys	Fresh	Mean	778	295	30%	1%	40%	23%	94%	976
		Range	668-924	160-402	13-57%	1%	16-68%	15-38%	59-121%	853-1042
Leys	Silage	Mean	813	225	15%	2%	38%	26%	82%	947
		Range	696-946	60-346	1-48%	1-4%	12-59%	11-54%	61-123%	867-1014
WC	Fresh	Mean	930	179	54%	1%	32%	37%	124%	1041
		Range	865-988	60-346	1-98%	0-4%	12-62%	11-101%	57-225%	867-1111
WC	Silage	Mean	923	97	21%	2%	35%	32%	89%	984
		Range	881-960	59-346	1-98%	0-4%	12-62%	15-101%	57-225%	853-1111
All	Fresh	Mean	847	243	41%	1%	37%	29%	108%	1005
		Range	668-988	70-402	13-98%	0-1%	14-68%	15-101%	57-225%	853-1111
All	Silage	Mean	863	167	18%	2%	37%	29%	85%	964
		Range	696-960	59-346	1-52%	1-4%	12-59%	11-55%	57-123%	867-1020
All	All	Mean	855	205	30%	1%	37%	29%	96%	985
		Range	668-988	59-402	1-98%	0-4%	12-68%	11-101%	57-225%	853-1111

WC = whole crops (barley, wheat, maize); NorFor anal = NorFor specified analyses; RestCHO = 1000 - NorFor anal.; WSC = water soluble carbohydrates; Phenol. = soluble phenolics; Organic acids = organic acids not specified by NorFor.

Table 4 Content of organic acids in fresh leys and whole crops (g/kg dry matter)

Type	Citrate	Malic	Quinic & malonic	Succinate	Shikimic	Fumarate	Organic acids
Leys	8.5	10.8	14.9	0.3	0.9	0.4	35.7
Whole crops	4.0	7.8	13.4	0.4	0.2	0.2	21.5
All	5.8	9.0	14.1	0.3	0.5	0.3	27.2

Conclusions

Recovery of all dry matter in crops and silages was reasonably successful in light of the complex nature of the samples and the number of assays required. The RestCHO fraction constituted as much as 402 g/kg DM and consisted mainly of WSC, pectin and organic acids. Of

particular importance to implement in NorFor would be organic acids in fresh crops and pectins in fresh and ensiled crops.

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Comparison of rumen fluid inoculum vs. faecal inoculum on methane production *in vitro*

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Introduction

Methane production from ruminants represents approximately one quarter of total anthropogenic greenhouse gas emissions. Methane is one of the most significant contributors to the greenhouse effect, having effects on climate change and global warming by trapping the heat 20 times more effectively than carbon dioxide. Total dry matter intake and digestibility are among the driving forces affecting CH_4 production in ruminants (Ramin and Huhtanen, 2013). Since *in vivo* studies are very expensive and laborious to conduct, many *in vitro* techniques have been developed to study ruminant nutrition, fermentation processes as well as predicting *in vivo* CH_4 production from kinetic parameters obtained from the *in vitro* gas system (Ramin and Huhtanen, 2012).

In most *in vitro* studies, rumen fluid is used as an inoculum but fresh faeces from ruminants has also been investigated as an alternative to rumen fluid (Cone et al., 2002; Varadyova et al., 2005). Methane is mainly produced in the rumen but also in the hindgut (HG) of ruminants (approximately 5%). Since production of volatile fatty acids (VFA) and gases are stoichiometrically related (Wolin, 1960) and the fermentation pattern in the rumen is similar to the HG (Demeyer, 1991), it can be assumed that stoichiometric calculations will predict a higher amount of CH_4 production from the HG (fresh faeces inoculum) than is actually produced.

The study by Fievez et al. (1999) has indicated that there are acetogenic bacteria in the HG and we hypothesize that in the HG the acetogenesis process is more important as compared to the rumen because H_2 is used mainly by acetogenesis bacteria to produce VFA (mainly acetic acid) and to a smaller extent by methanogens to produce CH_4 (De Graeve and Demeyer, 1990). Therefore, the aim of this study was to compare ruminal and faecal inocula in an automated *in vitro* gas production system for determination of total gas and CH_4 production, and other fermentation variables. The second objective was to compare stoichiometric predictions of CH_4 production based on two different sources of inocula.

Materials and Methods

Five different feeds varying in digestibility were selected as substrates for this study: silage (S), hay (H), a mixture of silage and barley (B) (50:50), pooled samples from digesta particles collected from rumen and reticulum (RR) and faeces (FC) by wet sieving. The reason for selecting RR was to mimic the substrate entering the HG of ruminants and FC to represent a poorly digestible substrate. Rumen fluid was collected from three fistulated Swedish Red cows 2-h after morning feeding. Prior to the incubation, 1 g of each feed was weighed into 250 ml serum bottles and placed in three shaking water baths at 39 °C. Eighteen bottles were filled with 60 ml of buffered rumen fluid (1:5, for rumen fluid and buffer, respectively) and using the same ratio for the other 18 bottles with buffered fresh faeces according to Akhter et al. (1999). Each

feed was incubated in three replicates either with rumen or faecal inoculum in two different 48-h runs with blanks included for each type of inoculum (rumen or faeces).

A logarithmic model of time *versus* CH_4 concentration was developed for each bottle in order to estimate CH_4 production (ml) at time intervals of 0.2 h (the gas system recorded total gas production every 0.2 h). For this, CH_4 concentration at 8, 24 and 48-h was measured for each bottle by injecting 0.2 ml of gas from the headspace to a gas chromatography.

Concentration of total VFA and molar proportions of VFA (not blank corrected) were determined for each feed and inoculum taking samples from the bottles after 48-h of incubation where pH was also measured. Digestibility of neutral detergent fiber assayed with a heat stable amylase and exclusive of residual ash (aNDFom) and true organic matter digestibility (TOMD) was determined on the residues left after 48-h of incubation.

Methane production *in vivo* from both inocula was then predicted based on kinetic parameters obtained from the *in vitro* system and fitting the data to the two pool Gompertz model. Details of the calculations and the modelling procedure were described by Ramin and Huhtanen (2012). Methane production (ml) was also predicted according to VFA stoichiometric equations as described by Wolin (1960). Data for *in vitro* measurements (total gas, CH_4 production, VFA production and digestibility parameters) were analyzed statistically using the GLM procedure of SAS as described by Ramin et al. (2013) considering that Substrate (S), Inoculum (I) and their interaction (SI) was used in the model and the effect of run (R) was used as the random variable in the model as below:

$Y_{ijk} = \mu + S_i + I_j + (SI)_{ij} + R_k + e_{ijk}$,

Results and Discussion

Asymptotic total gas and CH_4 production, and the ratio between CH_4 and total gas production were all higher ($P < 0.01$) when rumen inoculum was used as compared to faecal inoculum for all substrates (Table 1). In agreement with this, earlier studies have reported a lower total gas production from faecal inoculum as compared to rumen inoculum (Varadyova et al., 2005; Fon and Nsahlai, 2012). Demeyer (1991) also reported lower CH_4 production from the caecum of cattle as compared to rumen with ground hay or pure cellobiose as substrate.

The correlations between rumen and faecal inoculum for almost all variables were high, indicating that the ranking of feeds could be well established with both inocula (e.g. $R^2 = 0.99$ for predicted *in vivo* CH_4 production). The predicted *in vivo* CH_4 production was also lower ($P < 0.01$) when the kinetic parameters were estimated from faecal inoculum as compared to rumen inoculum (Table 1).

When CH_4 production was predicted stoichiometrically from VFA (CH_4VFA, Wolin, 1960) after 48-h of incubation with the faecal inoculum, the values were greater as compared to the measured values (Table 1). This may suggest the presence of acetogenesis in the HG as outlined by Fievez et al. (1999). The slope between predicted and observed CH_4 production was 0.59 for the faecal inoculum and 1.01 for the rumen inoculum. This also indicates that there are other H_2 sink reactions in the HG fermentation compared to rumen fermentation, a likely alternative may be acetogenesis from CO_2 following $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ (Demeyer, 1991).

Although differences in molar proportion of acetate were rather small, it is possible that in HG fermentation, acetate was at least partly produced by a different pathway. Acetogenesis results in the production of acetic acid in the HG of ruminants. In our study, there was a tendency of higher acetic acid production (especially from low digestible feeds; RR, FC and H) from the faecal inoculum as compared to the rumen inoculum (Table 2).

Higher concentration of VFA was observed when the feeds were incubated in rumen inoculum as compared to faecal inoculum (Table 2), in agreement with the literature (El-Meadaway et al., 1998; Fon and Nsahlai, 2012). However, it could have been due to difference in blanks for rumen and faecal inoculum. Molar proportion of propionate was higher ($P < 0.01$, Table 2) for all feeds incubated in faecal inoculum, which is consistent with the results obtained in earlier studies (Immig, 1996; Fon and Nsahlai, 2012).

Digestibility of aNDFom was greater ($P < 0.01$) for all substrates when incubated with rumen inoculum as compared to faecal inoculum (Table 3). The same pattern was also observed for TOMD and the difference was greater when less digestible substrates (RR and FC) were used (Table 3).

Conclusions

Total gas, CH_4 production, CH_4/total gas ratio and digestibility were lower with faecal than rumen inoculum. However, there was no difference in total VFA production between the inocula. In general, it is difficult to make any definite conclusion for differences in molar proportions of VFA in production as the blank effect is different in both inocula. The difference between predicted and observed CH_4 production with faecal inoculum suggests the existence of other H_2 sink reactions in HG fermentation such as acetogenesis (the difference in propionate has been already accounted for predicted CH_4 production). Faecal inoculum can be used to mimic the HG fermentation. Ranking of feeds was very consistent between the inocula. It can also be concluded that HG microbial population producing less CH_4 would exist in the rumen if they were competitive in the rumen conditions.

Table 1 The effects of different sources of inoculum (rumen vs. faecal) on total gas production, predicted values of *in vivo* methane production, production of methane predicted based on stoichiometric relationship with volatile fatty acids (VFA) and their kinetic parameters (n = 6).

	1 gr sample (per 60 ml culture)[1]						*P*-value[2]		
Item	RR	FC	S	H	SB	SE[3]	Inoculum	Substrate	I × S
Asymptotic gas, mL/g DM									
Rumen	162	145	250	217	285	4.6	<0.01	<0.01	0.25
Faecal	103	68	183	160	225				
Asymptotic CH_4, mL/g DM									
Rumen	18.5	19.9	38.5	31.1	43.0	0.79	<0.01	<0.01	<0.01
Faecal	7.0	7.4	17.9	16.4	22.3				
Total gas at 48-h/TOMD, mL/g									
Rumen	276	265	286	286	324	5.5	<0.01	<0.01	<0.01
Faecal	233	195	252	262	287				
Predicted *in vivo* CH_4 production, mL/g DM[4]									
Rumen	12.3	14.6	30.3	24.2	36.3	0.53	<0.01	<0.01	<0.01
Faecal	4.9	5.9	14.4	12.5	18.4				
CH_4VFA, mL[5]									
Rumen	13.0	10.2	29.3	18.9	29.5	2.96	0.44	<0.01	0.67
Faecal	9.1	7.8	24.8	22.3	29.5				
CH_4/total gas									
Rumen	0.122	0.141	0.138	0.130	0.130	0.0041	<0.01	<0.01	0.21
Faecal	0.085	0.107	0.086	0.094	0.088				

[1] RR: wet sieved digesta from rumen and reticulum (pooled); FC: faecal particle matter; S: first cut silage; H: hay; SB: 500 mg silage + 500 mg barley, [2] Probability of a significant effect of inoculum, substrate, and interaction between inoculum and substrate (I × S), [3] SE: standard error of mean, [4] Methane was predicted *in vivo* by using a 50-h rumen retention time in the mechanistic rumen model, [5] Methane predicted from VFA (mmol) stoichiometry: 22.4 × (0.5 × acetate – 0.25 × propionate + 0.5 × butyrate).

Table 2 Total volatile fatty acids (VFA) production, concentration and molar proportion of each individual VFA after 48-h incubation from feed samples incubated in rumen inoculum or faecal inoculum in the gas *in vitro* system (n = 6).

		1 gr sample (per 60 ml culture)[1]						*P*-value[2]		
Item		RR	FC	S	H	SB	SE[3]	Inoculum	Substrate	I × S
Total VFA production, mmol[4]										
Rumen		1.96	1.33	4.41	2.89	4.25	0.420	0.63	<0.01	0.60
Faecal		1.44	1.19	4.48	3.60	4.78				
Total VFA concentration, mmol/L[5]										
Rumen		76.6	65.9	117	92.2	115	5.48	<0.01	<0.01	0.42
Faecal		43.2	38.6	93.5	78.8	98.5				
Molar proportion, mmol/mol										
Rumen	Acetate	632	630	619	625	596	4.1	0.13	<0.01	<0.01
	Propionate	192	165	209	205	199	2.0	<0.01	<0.01	<0.01
	Butyrate	115	141	118	114	153	4.1	<0.01	<0.01	<0.01
	Isovalerate	38.4	39.9	29.7	31.7	27.3	1.61	<0.01	<0.01	0.05
	Valerate	22.7	23.6	23.1	23.2	24.2	0.71	<0.01	<0.01	<0.01
Faecal	Acetate	659	635	610	661	559				
	Propionate	217	190	285	245	258				
	Butyrate	65.7	108	71.1	55.2	152				
	Isovalerate	34.9	41.1	21.7	22.0	20.9				
	Valerate	23.1	25.9	12.3	16.6	10.2				
pH										
Rumen		6.57	6.60	6.22	6.36	6.01	0.022	0.10	<0.01	<0.01
Faecal		6.64	6.64	6.18	6.33	6.04				

[1] RR: wet sieved digesta from rumen and reticulum (pooled); FC: faecal particle matter; S: first cut silage; H: hay; SB: 500 mg silage + 500 mg barley, [2] Probability of a significant effect of inoculum, substrate, and interaction between inoculum and substrate (I × S), [3] SE: standard error of mean, [4] Total VFA production corrected for blank, [5] Total VFA concentration and molar proportions are not corrected for blank.

Table 3 Least square means of neutral detergent fiber digestibility (aNDFomD) and true organic matter digestibility (TOMD) from feed samples incubated in rumen inoculum or faecal inoculum in the gas *in vitro* system (n = 6).

	1 gr sample (per 60 ml culture)[1]						*P*-value[2]		
Item	RR	FC	S	H	SB	SE[3]	Inoculum	Substrate	I × S
aNDFomD									
Rumen	0.368	0.351	0.736	0.519	0.686	0.0137	<0.01	<0.01	0.081
Faecal	0.193	0.196	0.554	0.371	0.569				
TOMD									
Rumen	0.468	0.456	0.841	0.706	0.869	0.0146	<0.01	<0.01	0.085
Faecal	0.321	0.325	0.732	0.599	0.773				

[1] RR: wet sieved digesta from rumen and reticulum (pooled); FC: fecal particle matter; S: first cut silage; H: hay; SB: 500 mg silage + 500 mg barley, [2] Probability of a significant effect of inoculum, substrate, and interaction between inoculum and substrate (I × S), [3] SE: standard error of mean.

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Effect of feeding rumen-protected CLA on milk yield and composition

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Introduction

Fat concentration of bovine milk depends on genetics, stage of lactation and nutrition. Diets containing little dietary fiber, and much highly digestible carbohydrates (starch and sugars), and diets supplemented with polyunsaturated oils, may induce milk fat depression. During ruminal lipolysis and biohydrogenesis of linolic and linoleic acids to stearic acid, intermediates, especially C18:1trans10 and C18:2trans10cis12 conjugated linolic acid (CLA) are produced, that inhibit milk fat synthesis. The effect is well documented, and is mediated by a down-regulation of enzymes necessary for mammary de-novo synthesis of short chain fatty acids (≤ C16) such that both milk fat concentration and daily milk fat yield is reduced (Baumgard et al. 2002).

For the farmer, a potential reason for producing low-fat milk could be to increase dairy cow energy balance (EB) during early lactation, thereby improving fertility. If reduced milk fat concentration is accompanied by an increased milk volume, another reason could be the possibility to fill up the milk quota on farms where it otherwise is not possible. Most studies on CLA supplementation are, however, performed with Holstein/Friesians, on diets that differ from common dairy cow diets in Norway. Therefore, a study with Norwegian Red (NRF) dairy cows was performed in 2010.

Materials and methods

The dairy cow study included two continuously fed groups of cows: a control group offered ad libitum of well fermented grass silage and a compound feed, and a CLA-group fed an identical diet supplemented with CLA. The difference between the two diets was obtained by substituting 50 g of protected fat in one kg per cow of the daily compound feed allowance, containing 0.45 g C14:0, 19.82 g C16:0, 2.70 g C18:0, 16.67 g C18:1cis9, 4.05 g C18:2cis9,cis12 and 1.35 g other acids (in total 43.05 g fatty acids), with 50 g of another protected fat source (Lutrell Pure, BASF, Burgbernheim, Germany), containing 4.8 g C18:2trans10cis12 CLA, 4.8 g C18:2cis9trans11 CLA, 0.11 g C14:0, 6.06 g C16:0, 21.29 g C18:0, 4.59 g C18:1cis9, 0.55 g C18:2cis9,cis12, 0.20 g C20:0, 0.20 g C22:0 and 0.48 g other acids (in total 45.05 g fatty acids). The offered dose was as recommended by the producer.

In total 10 primiparous and 15 multiparous cows entered the experiment 3 weeks prior to expected calving date. Cows for the two groups were balanced for expected calving date, and multiparous cows were additionally balanced for maximum daily milk yield, and average milk fat and –protein concentration in the previous lactation. The cows calved between 31 January and 18 April and were let out on pasture on 20 May. The study was finished on 3 June. Daily concentrate levels were: 1 kg prior to calving, increasing with 0.5 kg/d from the day after calving to a maximum of 12 kg (11 kg for primiparous cows). On pasture the daily level was reduced to 7 kg (6 kg for primiparous cows). The fat substitution for the CLA group was kept constant on 50 g/d throughout the study. One cow got coli-mastitis on 19. May, and did never recover, and

was not included in the pasture-part of the study. Cows were held in loose housing, and offered concentrates in several small daily portions in feed stations during the indoor period. Concentrate was offered in two daily portions during the pasture period.

Grass silage was harvested from a first year ley, sown with timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*). The crop was mown at early heading of timothy 4-6 June 2009, wilted under dry conditions to 30% DM, harvested using JF 1100 precision chopper with 16 knives, applied 4 L/t GrasAAT Lacto (78% formic acid and ammonium formate) and ensiled in tower silo. Silage composition: 317 g DM/kg, and, per kg DM: 941 g organic matter (OM), 123 g crude protein (CP), 8 g water-soluble carbohydrates (WSC), 541 g neutral detergent fiber (NDF), 41 g fat, 62 g lactic acid, 13 g acetic acid, 10 g formic acid and 6 g ethanol. Silage pH was 4.11, NH_3-N was 54 g/kg total N, and only traces of butyric acid were detected. Estimated feed value, per kg DM: 690 g digestible organic matter (DOMD), 5.98 MJ net energy lactation (NE_L), 74 g AAT and 13 g PBV. Estimated feed value according to NorFôr, per kg DM: 6.22 MJ NE_{L20}, 85 g AAT_{20}, 8 g PBV_{20}, 0.51 fill value lactation (FVL) and 80 min chewing index (CI). The concentrate (Fiskå TopLac, Fiskå Mølle AS, Tau, Norway) consisted of (g/kg): 330.5 barley, 200 extracted rape seed, 127.2 wheat, 80 maize gluten, 60 molasses, 53 protected fat, 50 oats, 44 heat treated extracted soybean, 5 fish meal, 40 vitamin and mineral premix. Chemical composition: 873 g DM/kg, and, per kg DM: 929 g OM, 219 g CP, 307 g starch, 62 g WSC, 176 g NDF, 71 g fat. Estimated feed value, per kg DM: 8.35 MJ NE_L, 151 g AAT and 6 g PBV. Additionally, a mineral mixture was freely available in the loose housing.

Cows were milked twice daily. Milk sampled 3 days per week was analyzed on FTIR (MilcoScan 6000, Foss Electric, Hillerød, Denmark). Milk samples for analyses of fatty acid composition (Dønnem et al. 2011) were taken from an aliquot morning + evening milk sample at three time points: In early lactation on DIM 22 ± 2; in the last week on indoor feeding on DIM 64 ± 21; and in the last week on pasture on DIM 77 ± 21. Dietary effects on feed intake, body weight (BW) and milk yield traits were analyzed using the GLM procedure in SAS (SAS Institute 2008). The model included diet and age (primiparous or multiparous cow). Days in milk (DIM) was used as covariate where significant.

Results

Feed intake tended to be higher for CLA cows than for control cows prior to calving, but this difference was reduced after calving (Table 1 and 2). Cows fed CLA increased their BW with 138 g/d, whereas control cows decreased their BW with 150 g/d during the, on average, 44-d indoor period. During the same time period, estimated daily EB was 3.8 MJ NE_L for CLA cows and -8.07 MJ NE_L for control cows (Table 2). During the 14-d pasture period, CLA cows lost 44 kg BW whereas control cows lost 47 kg BW (NS, not presented). Those BW differences included changed rumen fill during transition from indoor feeding to grazing. No significant differences in milk yield or composition were found during the indoor period, but cows fed CLA tended to have highest milk concentration of free fatty acids (FFA) (Table 3).

Table 1 Feed intake pre and post calving

	CLA	Control	SEM	*P*
N	13	12		
DIM -15 to -2				
Grass silage, kg DM	13.2	11.6	0.61	0.09
Concentrate, kg DM	0.87	0.81	0.021	0.1
Total, kg DM	14.0	12.5	0.62	0.09
DIM 2 to 15				
Grass silage, kg DM	13.1	12.5	0.42	NS
Concentrate, kg DM	4.80	4.81	0.100	NS
Total, kg DM	18.0	17.2	0.47	NS

Table 2 Feed intake, BW change and energy balance from DIM 20 to 64 (indoor period)

	CLA	Control	SEM	*P*
N	13	12		
Grass silage, kg DM	12.1	11.8	0.42	NS
Concentrate, kg DM	10.2	9.7	0.114	0.01
Total, kg DM	22.3	21.5	0.49	NS
DM, g/kg BW	36.1	35.1	0.99	NS
NDF, g/kg BW	13.4	13.1	0.42	NS
Daily BW change, g	138	-150	92.0	0.04
Daily energy balance, MJ NE_L	3.80	-8.07	3.62	0.03

In the following pasture period, cows fed CLA had lowest milk fat yield and tended to have lowest yield of ECM, partly due to lower milk yield and partly to lower milk fat concentration. Protein yield and concentration also tended to be lowest for CLA-fed cows, and milk FFA concentration was highest (Table 3).

The effect of CLA-feeding on milk fatty acid composition was not influenced by stage of lactation (DIM 22 or 64), or indoor versus pasture feeding (DIM 64 or 77). Therefore, average values for the three sampling times are presented in Table 4. CLA fed cows had slightly higher milk fat proportions of C18:0, C18:2trans10cis12 CLA and C20:0 than control cows, otherwise, no differences in single fatty acid proportions were found. The CLA supplement did neither influence the proportion of saturated, monounsaturated or polyunsaturated acids, nor the n6/n3 proportion, the proportion de-novo acids (<C16) or acids provided by feed or body mobilization (>C16).

Discussion

Milk fat concentration was in average reduced by 4%, and daily milk fat yield by 6% indoors and 9% on pasture (NS) in CLA-treated cows. Due to the strong documentation of the effect of C18:2trans10,cis12 CLA on milk fat synthesis (Baumgard et al 2001, 2002), there is no reason to doubt that this was an effect of the CLA supplementation. The present study was performed with NRF cows that have a higher average milk fat concentration than Holstein/Friesians, the cows were fed a more fibrous diet, 373 g NDF/kg DM, than in most other studies, they were supplemented with a CLA dose in the lower range of where effects are obtained (3.5 to 14 g/d) (Baumgard et al 2001, Castaneda-Gutierrez et al 2005), and the study was performed in early lactation, when higher doses are necessary than after peak lactation (Wheelock et al 2007). On this background, only a small effect on milk fat synthesis could be expected.

Table 3 Milk yield and composition

	CLA	Control	SEM	*P*
Indoor period (DIM 6 to 64)				
N	13	12		
Milk, kg	33.1	33.6	0.86	NS
ECM, kg	33.9	35.4	0.85	NS
Fat, g/kg	42.0	43.9	0.85	NS
Protein, g/kg	34.1	34.6	0.38	NS
Lactose, g/kg	47.2	47.0	0.37	NS
Urea, mM	4.66	4.91	0.148	NS
FFA, mEq/L	0.47	0.34	0.051	0.08
Fat, g/d	1371	1460	38.2	NS
Protein, g/d	1117	1146	30.9	NS
Lactose, g/d	1561	1580	41.3	NS
Pasture period (DIM 66 to 79)				
N	13	11		
Milk, kg	32.2	34.1	1.16	NS
ECM, kg	31.5	34.3	0.96	0.06
Fat, g/kg	39.0	40.7	0.79	NS
Protein, g/kg	32.4	33.5	0.39	0.09
Lactose, g/kg	47.0	47.3	0.39	NS
Urea, mM	5.16	5.53	0.155	NS
FFA, mEq/L	0.58	0.36	0.073	0.05
Fat, g/d	1243	1368	37.9	0.04
Protein, g/d	1042	1132	33.1	0.08
Lactose, g/d	1511	1608	59.1	NS

Increased feed intake before, but not after, calving, is also observed previously (Castaneda-Gutierrez et al 2005). Energy saved due to reduced milk fat synthesis may be directed to adipose tissue, which is the basis for higher EB and possibly improved fertility. Dønnem et al. (2011) found positive correlation between milk FFA and EB, in line with this study. Increased BW gain when feeding C18:2trans10,cis12 CLA was also obtained by Shingfield et al (2004).

The increased proportion of C18:0, C18:2trans10,cis12 CLA, and C20:0 in milk fat from cows fed the CLA supplement was a direct effect of increased supply of these acids in the diet. The absence of any increase in C18:2cis9,trans11 CLA in milk fat, in spite of an equal increase in supply, might be due to inhibition of Δ9 desaturase by C18:2trans10,cis12 CLA. While CLA-supplementation had a minor effect on fatty acid composition of milk, profound effects were found of stage of lactation (DIM 22 vs. 64) and of indoor feeding vs. grazing. Grazing nearly doubled C18:1trans11, C18:2cis9,trans11 CLA and C18:3cis9,cis12,cis15 proportions, but left C18:1trans10 and C18:2trans10,cis12 CLA proportions unchanged.

Conclusions

A recommended dose (5 g/d) of C18:2trans10,cis12 CLA that reduce milk fat concentration and daily milk fat yield of Holstein/Friesian cows on a high-concentrate diet in mid-lactation, did not significantly reduce milk fat concentration of NRF cows fed a typical Nordic, forage-based ration in early lactation. CLA-treatment did not influence milk fatty acid composition to any extent, whereas turn out on pasture significantly altered the concentration of almost all fatty acids, in a direction expected to give more healthy milk.

Table 4 Milk fatty acid composition, g/100 g fatty acids, average for DIM 22, 64 and 77

	CLA	Control	SEM	*P*
C4:0	3.98	4.07	0.057	NS
C6:0	2.26	2.33	0.035	NS
C8:0	1.37	1.38	0.031	NS
C10:0	2.98	2.90	0.084	NS
C12:0	3.27	3.14	0.092	NS
C14:0	9.77	9.51	0.148	NS
C14:1cis9	0.70	0.73	0.022	NS
C15:0	0.87	0.89	0.018	NS
C16:0	27.4	27.7	0.21	NS
C16:1cis9	1.30	1.38	0.041	NS
C17:0	0.46	0.46	0.008	NS
C18:0	10.6	10.0	0.15	0.01
C18:1trans9	0.25	0.25	0.004	NS
C18:1trans10	0.38	0.36	0.009	NS
C18:1trans11	1.88	1.88	0.052	NS
C18:1cis9	22.0	22.3	0.38	NS
C18:1cis11	0.79	0.81	0.019	NS
C18:2cis9,cis12 n6	1.72	1.68	0.030	NS
C18:2cis9,trans11 CLA	0.78	0.80	0.021	NS
C18:2trans10,cis12 CLA	0.021	0.012	0.0005	<0.001
C18:3 n3	0.50	0.48	0.011	NS
C20:0	0.15	0.14	0.002	0.03
C20:4 n6	0.077	0.072	0.0021	NS
C20:5 n3	0.060	0.059	0.0015	NS
C22:6 n3	0.021	0.021	0.0006	NS
Total	93.5	93.4	0.08	NS
Saturated (SFA)	63.1	62.5	0.40	NS
Monounsaturated (MUFA)	27.3	27.7	0.42	NS
Polyunsaturated (PUFA)	3.17	3.13	0.043	NS
n6/n3	3.29	3.31	0.066	NS

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Comparison of feed intake models for dairy cows

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Introduction

Feed intake in dairy cattle. Feed intake in dairy cows has a large effect on performance, in terms of milk production and body condition. The complex interaction between feed and feed intake in ruminants has therefore been investigated for decades (Ingvartsen, 1994). The production of large quantities of milk in high yielding dairy cows requires vast amounts of energy (Allen, 2000) and is sensitive to the profile of absorbed nutrients, resulting in an interest for modeling feed intake.

Why model feed intake? Feed intake, especially forage intake, accounts for most of the variation in productivity of dairy cows (Mertens, 2007). Dry matter intake (DMI) systems are necessary to optimize the dietary proportion of concentrate feeds to achieve a well-balanced and cost efficient diet (Ingvartsen, 1994; Zom et al., 2012). Over the years, feed intake models have become more advanced, moving from the simple model supposed by National Research Council (NRC; NRC, 2001), which only includes animal factors, towards more advanced models like the Nordic feed evaluation system (NorFor; Volden, 2011) and the total dry matter intake index (TDMI; Huhtanen et al., 2011), which include both dietary and animal factors. The latest feed intake model by Zom and co-workers ('Zom model'; Zom et al. 2012) focuses on additive feed characteristics of individual feeds and does not include milk yield as an animal factors.

Comparing feed intake models. Evaluation of feed intake models on independent data sets reveals not only the accuracy and robustness of the existing models but can lead to an extended understanding of model parameters effect on prediction performance, which can be used in development of new and improved models.

Short description of the feed intake models compared. The feed intake model proposed by NRC (2001) is the simplest model of the four evaluated here. This model includes only fat corrected milk yield (FCM), body weight (BW), and week of lactation (WL), but not dietary characteristics. In NorFor (Volden et al., 2011) the intake capacity (IC) expresses how much a cow can eat and is estimated from similar inputs as in NRC, i.e. days in milk (DIM), energy corrected milk yield (ECM), and BW, however the model depends also on parity and breed. Each feedstuff is given a fill value (FV), and both IC and FV are expressed in arbitrary units. Concentrates are given a constant FV, whereas FV's for forages are calculated from organic matter digestibility (OMD) and neutral detergent fibre (NDF) content. Adjustments of FV's are made depending on content of sugar and starch in the total ration and the quality of forage. The TDMI index (Huhtanen et al., 2011) is a sum of silage dry matter index (SDMI) and concentrate dry matter index (CDMI). SDMI is calculated based on digestible organic matter concentration (D-value) found by NIR-analysis, the concentration of total acid (TA), dry matter (DM), and

NDF, together with the proportion of regrowth, legume, and whole-crop silage (a, b, and c, respectively). CDMI is calculated based on allocated concentrate DM, supplementary concentrate crude protein (CCPI) (CP > 170 g/kg DM), and content of NDF (CNDF) and fat (Cfat) in concentrate. The prediction of DMI is calculated based on standardized ECM (sECM), BW, DIM, and the TDMI index of the ration. The prediction of DMI by the 'Zom model' is similar in approach to both NorFor and TDMI but does not include daily milk yield and BW as input in the estimation of feed intake capacity (FIC). FIC is calculated based on parity, DIM, and days in gestation. All feeds are assigned a satiety value (SV) resulting in a weighted SV for the ration. Depending on the type of forage SVs are calculated from contents of DM, CP, crude fiber (CF), and OMD.

Objective. The objective of this study was to compare the ability of different feed intake models to predict DMI in dairy cows fed total mixed rations (TMR).

Material and methods

Data used for evaluation. The evaluation of the four models was conducted on Danish intake data from eight different references described by Volden et al. (2011b), consisting of ten experiments with a total of 85 treatment means from lactating dairy cows of varying breed, parity (primi- and multiparous), and lactation stage (Table 1). Housing was registered as either tied up or loose housing.

Table 1 Experiments and breeds together with DMI, DIM and ECM expressed as mean ± SD

Data		Breeds			Feed intake (kg DM/day)		DIM		ECM (kg/day)	
Ref.	Exp.	Danish Holstein	Jersey	Danish Red	Mean	SD	Min	Max	Mean	SD
8	10	52	16	17	20.2	±2.6	25	275	31.1	±6.0

Feed and feeding practice. The ten experiments were conducted between 1999 and 2009 and included 11 different grass/clover-grass silages, eight maize silages, two whole-crop silages, and five alfalfa silages. All rations were fed as TMRs. Table 2 describes the variation in forage share, OMD, NDF, CP, sugar plus starch, and chewing time index (CI) as mean ± SD of the data.

Table 2 Nutritional value of the TMRs used to evaluate intake models.

	Forage share (% of DM)		OMD (%)		NDF (g/kg DM)		CP (g/kg DM)		Sugar + starch (g/kg DM)		CI (min/kg DM)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TMR	59	7.8	74.5	2.3	341	18	159	17	206	36	35	4

Modifications of equations used for prediction of DMI.

NorFor: The NorFor equations used in this evaluation has minor modifications compared to the equations published by Volden et al. (2011a). In the equation: FV_SubR (eq. 10.7; Volden et al., 2011a) the constants 0.1 and 0.05 are used to control the output for optimization purpose and were removed for the purpose of prediction. In equation: FV_MR (eq. 10.8; Volden et al., 2011a) the IC/8 variable was excluded in a newer unpublished revision of this equation (Nielsen, 2013; pers. com.).

TDMI: In the TDMI-model by Huhtanen et al. (2011) several of the variables were estimated or substituted by similar variables given by NorFor feed analysis. In the SDMI the D-value was substituted with OMD, calculated from in vitro organic matter solubility analysed by the Tilly and Terry method (Tilley and Terry, 1963). The parameters a, b, and c (Huhtanen et al., 2011) were estimated based on the information given in the data. In the CDMI-index, CCPI was calculated as the amount of CP (kg/d) given above the level of 170 g CP/kg DM. CEPD was set to 0.7 for all TMR's (Huhtanen, 2013, pers. com.).

'Zom model': In the 'Zom model' most of the input variables were available in the Danish data material. However, CF values were estimated based on a regression performed on feedstuff data supplied by Zom et at. (2012). CF (g/kg DM) in grass-, legume-, and whole crop silage was predicted from: -14.769 + 5.549*NDF (g/kg DM), and for concentrate: -9.972 + 0.454* NDF (g/kg DM). Maximum and minimum values of forage SV (Zom et al., 2012) were used as limitations for the calculation of SV for the given feedstuffs. FIC values for Jersey were corrected to 80% of ordinary FIC (Zom, 2013; pers. com).

Statistical criteria of testing accuracy. The accuracy of prediction by the feed intake models is evaluated by Mean Square Prediction Error (MSPE) as statistical criteria: The MSPE is calculated as follows:

$$MSPE = \sum (A - P)^2/n$$

where: A is the actual DMI, P is the predicted DMI and *n* is the number of pairs of A and P being compared. According to Bibby and Toutenberg (1977) the MSPE can be considered as the sum of three components: mean bias $(\bar{A} - \bar{P})^2$, which indicates the difference between the actual and predicted mean of DMI and is seen as the deviation of the intercept from 0, line bias $(S_P^2(1-b)^2)$, which indicates how much of the error is due to the fitted line and is seen as the deviation of the slope from zero, and random variation around the regression line (error of disturbance) of A on P$(S_A^2(1-r^2))$. Accordingly, MSPE is calculated as follows:

$$MSPE = (\bar{A} - \bar{P})^2 + S_P^2(1-b)^2 + S_A^2(1-r^2)$$

where: $\bar{A}$ is mean of the actual DMI, $\bar{P}$ is mean of the predicted DMI, S_A^2 is the variation of actual DMI, S_P^2 is the variation of predicted DMI, b is the slope of the regression of A on P with intercept zero, and r is the correlation coefficient of A and P (Bibby and Toutenberg, 1977).

Results and discussion

Observed and predicted DMI from the four intake models are presented as means in Table 3, together with the statistical criteria.

Table 3 Accuracy of four feed intake models predicting DMI in dairy cows fed TMR's.

Model[1]	DMI kg/day		Evaluation criteria					
	Mean pred.	Mean obs.	RMSPE (kg DM/day)	RMSPE (%)	MSPE	Mean bias	Line bias	Error of variation
NorFor	20.0	20.2	1.5	7.3	2.2	0.03 (1%)	0.91 (42%)	1.24 (57%)
NRC	21.7	20.2	1.8	9.1	3.4	2.08 (62%)	0.16 (5%)	1.12 (33%)
TDMI	20.7	20.2	1.4	6.7	1.9	0.21 (12%)	0.10 (5%)	1.54 (83%)
Zom	22.1	20.2	3.3	16.2	10.7	3.73 (35%)	2.49 (23%)	4.47 (42%)

[1]NorFor (Volden et al., 2011), NRC (NRC, 2001), TDMI (Huhtanen et al., 2011) and Zom (Zom et al., 2012)

As seen in Table 3, the TDMI model has the lowest RMSPE with a mean error of 1.4 kg DM/day. Decomposing MSPE into the three components shows a slight mean bias (12%), a minor line bias (5%), and a considerably part of the error explained by random variation in the data (83%). The NorFor model did nearly as well as the TDMI model, with a RMSPE of 1.5 kg DM/day. Decomposing MSPE of the NorFor model showed a very small mean bias (1%) but a noteworthy line bias (42%). The NRC model being the simplest model did surprisingly well with the third lowest RMSPE of 1.8 kg DM/day. The evaluation of the 'Zom model' showed the highest RMSPE of 3.3 kg DM/day resulting in the most inaccurate prediction result.

Figure 1 presents the centralised residual plot with residuals (observed-predicted) on the y-axis and the centralised predicted DMI on the x-axis. Mean bias is seen as the regression line's deviation from intercept at zero. An intercept at zero is a result of all predictions close to observed or equal amounts of positive and negative diversity from observed. Line bias is the deviation of the slope from zero. The higher the slope for the regression line across the centered residuals, the more line bias is associated with the model. The closer the regression line is to zero the smaller the line bias, resulting in models predicting equally well at low and high DMI. Prediction with the NorFor model resulted in residuals located in a downwards cloud. Here, the regression line almost intercepts at zero but shows a substantial slope explaining 42% of the error. The regression of the residuals from the NRC model is far from intercepting at zero and mean bias explain 62% of prediction error. The regression line indicates a downwards slope but line bias only explains 5% of prediction error. The regressed residual line from the TDMI model did not intercept zero. Mean bias explained 12% of prediction error and line bias deviation accounted for only 5% of the residual variation. The regressed residual line from the 'Zom model' shows an intercept different from zero, resulting in a mean bias explaining 35% and line bias accounting for 23% of the prediction error.

It is noteworthy that the NRC model, despite a general over-prediction of DMI and without inputs of dietary characteristics, predicted feed intake with a lower RMSPE compared with the 'Zom model'. This could be due to the lack of production traits when estimating FIC. The intention of the model by Zom and co-workers (2012) was to disconnect production traits, i.e. BW and ECM, from intake predictions because BW and ECM known at the time of prediction

are a result of the previous diet. The model evaluation conducted in this study hence indicates that animal production characteristics can be necessary for accurate prediction of DMI.

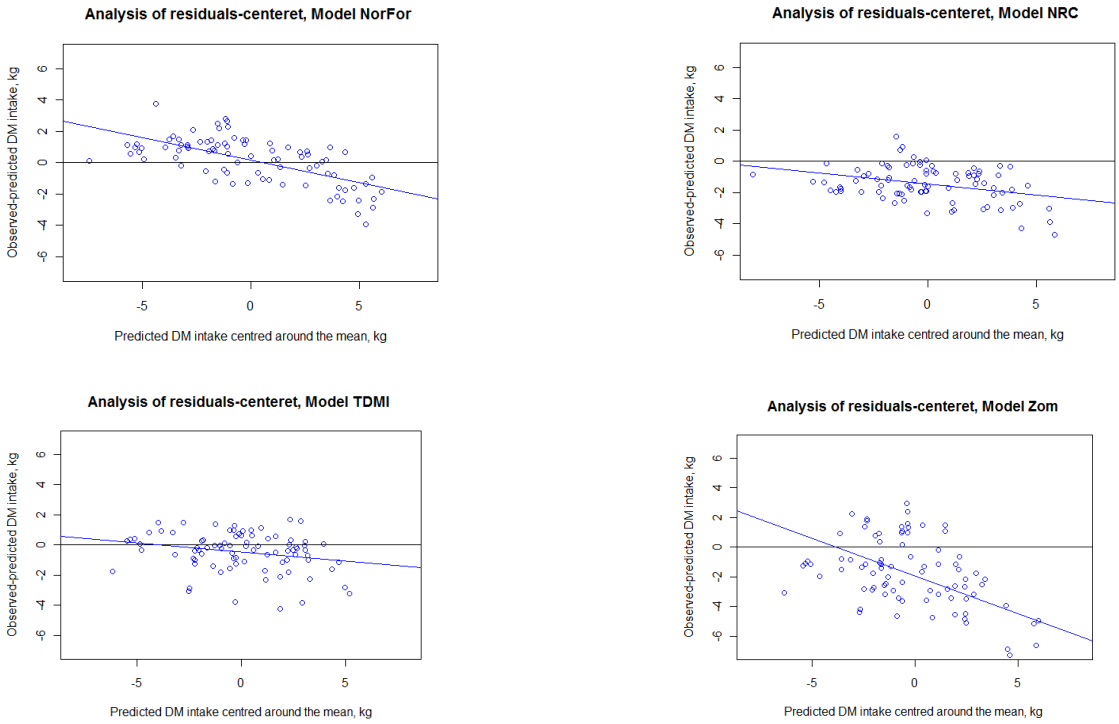

Figure 1 Centralised residual plots of the four models tested on 85 treatment means form Danish dairy cows fed TMR's.

The use of estimated CF in the prediction of DMI by the 'Zom model' may induce a minor error on CF compared to the analysed CF. The NorFor model included parameter settings for IC depending on breed (large breed, Jersey, and Icelandic breed), which was not the case for the three other models. However, the FIC values in the 'Zom model' were reduced by 20%, which corresponds to a commonly used breed correction for Jersey cows (Zom, 2013, pers. com.).

Conclusions

The TDMI and NorFor models produced the most accurate prediction of DMI in dairy cows fed typical Danish TMR. Furthermore, this study showed that feed intake models, which include production characteristic, in general are more robust than models lacking these characteristic.

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Using NorFor to Balance Diets for Amino Acids in Lactating Dairy Cows

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Introduction

The genetic code has codons for 20 amino acids (AA) that are used in protein synthesis in higher animals. Nutritionists know that nine of these AA cannot be formed in tissue metabolism but must be absorbed from the gastro-intestinal tract. The nine are classified as the essential AA (EAA) and are abbreviated as His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val. Another AA, Arg, is synthesized in the urea cycle and, hence, is not strictly an essential nutrient; however, dietary Arg supplementation may improve protein efficiency in some non-ruminant species when tissue synthesis is inadequate (Ball et al., 2007). Two additional AA, Cys and Tyr, are said to spare requirement of Met and Phe because these EAA are used partly to synthesize Cys (Met) and Tyr (Phe) when intestinal absorption of Cys and Tyr is insufficient. Therefore, Arg, Cys and Tyr are often referred to as semi-essential AA, while the remaining eight AA are non-essential. In this context, protein quality refers to EAA pattern, i.e., relative proportions of each EAA in a feed protein and how well these correspond to animal requirements. Rumen microbial protein is of better quality than many dietary ingredients commonly fed to domestic ruminants (Schwab, 1996). In addition, microbial ammonia utilization allows the feeding of some nonprotein N, such as urea, as well as capture of some recycled urea N that would otherwise be excreted in the urine.

It has been known for more than 60 years that, in productive ruminants such as lactating dairy cows, microbial protein synthesis is inadequate in itself and the animal depends partly on dietary rumen-undegraded protein (RUP) for additional metabolizable protein (MP), *i.e.* protein absorbed as AA from the small intestine. Thus, it is necessary to quantify both microbial protein formation and dietary protein escape in the ration formulation models used to estimate MP supply. Early models were simplistic, applying static constants in predicting microbial protein and RUP. However, it soon became obvious that protein digestion and metabolism are complex and dynamic, and rationing models have evolved to keep pace with new understanding. At last year's Nordic Feed Science conference, we compared predictions of milk and protein yield, made using the NRC (2001) and NorFor (2011) models, to yields observed in five feeding studies in which 21 different diets were fed (Broderick and Åkerlind, 2012). We found that the NorFor model gave more reliable estimates of observed milk protein production; recently we have attempted to extend the NorFor model to predicting EAA requirements and responses.

Optimizing dairy diets for essential amino acids

When first released, the NRC (2001) dairy nutrition model was hailed as innovative and reliable, and was widely accepted as the standard of comparison. Hanigan (2005) compared NRC-2001 to four other prominent models then in use and concluded that the new NRC model yielded more accurate predictions of MP supply. At that time, it was difficult to publish nutrition papers

in the Journal of Dairy Science without first subjecting experimental diets to evaluation in NRC-2001. However, cracks began to appear. A comparison of NRC-2001 predictions of rumen protein outflows to flows measured using omasal sampling indicated that the model overestimated RUP, underestimated microbial protein, while giving relatively close estimates of total MP flow (Broderick et al., 2010). Although total MP might be approximately correct, because MP origins differ in quantity from measured sources, it is suggested that the predicted EAA flows also might differ substantially from what is in fact available to the cow. Moreover, the NRC vs. NorFor comparison showed that the NRC model underestimated milk protein yield at low MP supply (Broderick and Åkerlind, 2012). We speculated that NorFor proved more reliable partly because it applies variable efficiency of MP utilization for milk protein production that declines as MP supply increases. The philosophy of variable, declining efficiency was extended to applying NorFor to balancing diets for EAA in dairy cow nutrition. NorFor (2011) used a quadratic regression of MP efficiency on MP availability/unit NEL to express the decline in MP utilization for milk protein synthesis as MP increases relative to energy supply. [Within NorFor and several other systems, MP is called AAT (total AA absorption in intestine); we will use MP and AAT interchangeably.] An AAT efficiency regression in this form, using data obtained under 108 different nutritional regimes (different diets and abomasal infusions) in 26 trials conducted in North America and the U.K. is in Figure 1. The regression coefficients are similar to those obtained in NorFor (equation 9.24; p. 95; Norfor, 2011) using regression of data from 16 Norwegian studies, which suggests that this approach is reliable for assessing efficiency of AAT (MP) utilization.

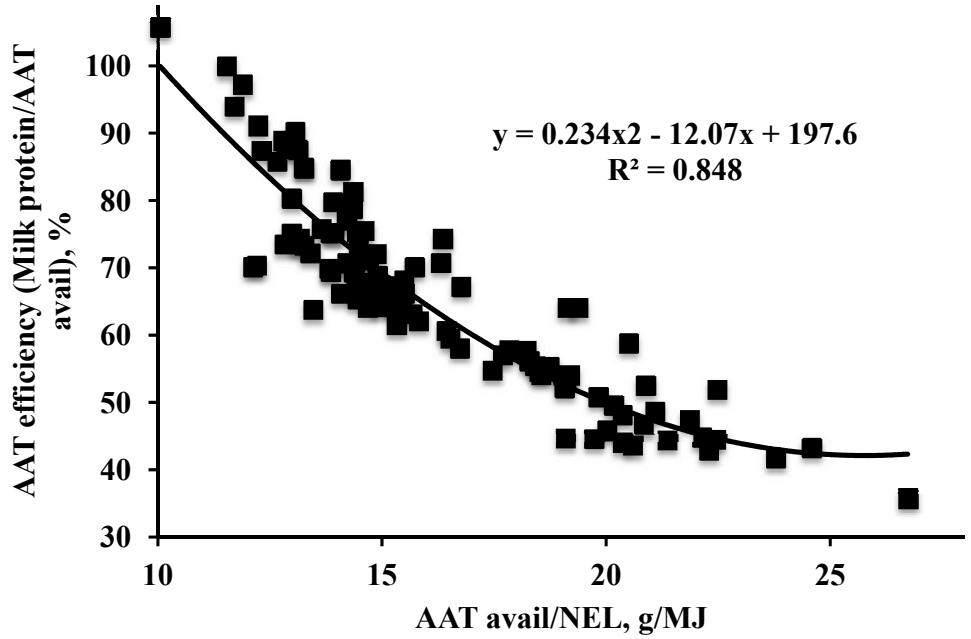

Figure 1 Regression of AAT (MP) efficiency (milk protein yield/AAT availabile; y-axis) on AAT available for milk production/NEL secreted in milk (x-axis) as estimated in the NorFor (2011) system.

Based on a large body of research from abomasal EAA infusions in lactating cows, Met and Lys (Schwab et al., 1976; Schwab, 1996) and His (Vanhatalo et al., 1999; Korhonen et al., 2000) are most likely to be limiting for milk protein synthesis. The approach applied in NorFor for AAT, and shown in Figure 1, was used to develop three quadratic equations that regressed Milk EAA/EAA available on EAA available/NEL, where EAA stands for Met, Lys or His, Milk EAA is the amount of each secreted in milk, EAA available is amount of each EAA available for milk synthesis, after subtracting amounts used for maintenance, growth (of 1st and 2nd parity cows), deposition (replacing EAA mobilized earlier in lactation) and gestation, and NEL is the energy secreted in milk (computed using Equation 9.2; NorFor, 2011). Calculations estimating EAA supply are described on p. 83 of NorFor (2011); EAA supply also includes EAA mobilized during lactation. These computations require knowledge of EAA composition of the proteins synthesized for maintenance functions plus whole body proteins (for mobilization or deposition) and the products of conception. We used protein compositions from Lapierre et al. (2007), who developed means from original data sources; milk composition is from NRC (2001). Except for milk, the values for which could be used directly (assuming 6.38% N in milk true protein), all AA data are recomputed as g AA/16 g N, using only AA-N plus amide N, assuming Glu was present in proteins as Gln. This is how all EAA concentrations, expressed in g EAA/16 g N, were computed. The quadratic expression developed for Met from the AAT dataset is in Figure 2. Please note that three results were deleted for the Met dataset (105 remaining) because they derived from abomasal infusions >20 g/d of Met. Satter et al. (1975) found that abomasal infusion of >40 g/d of Met depressed DM intake; thus, >20 g/d was deemed beyond normal nutritional requirements. The three quadratic equations developed (X = EAA available/NEL):

Y (Met efficiency) = -847.8 X + 803.0 X^2 + 274.4; inflection = 0.528 (R^2 = 0.848; 105 diets)
Y (Lys efficiency) = -291.0 X + 95.3 X^2 + 274.2; inflection = 1.527 (R^2 = 0.879; 108 diets)
Y (His efficiency) = -592.3 X + 509.6 X^2 + 221.5 (R^2 = 0.844; 108 diets)
[Inflection points for Met and Lys identify the minimal efficiencies for these two EAA.]

We propose that these efficiency equations be applied in the following way for evaluating results from milk production trials. Net AAT requirement for maintenance functions, deposition and gestation (computed in NorFor), and milk protein yield, are multiplied by their content of EAA, giving estimates of the total requirement for EAA for each function. The sum of all of these is the Net EAA requirement. The value, total EAA supply (computed in NorFor) minus EAA required for maintenance, growth, deposition and gestation, is divided by NEL required for milk secretion (energy-corrected milk yield x 3.14 MJ/kg), gives the estimated EAA available/NEL. This is the value of 'X' used in the appropriate quadratic regression to compute EAA efficiency. Dividing Net EAA requirement by computed efficiency gives the estimate of total EAA requirement for all functions. This value can be compared to the NorFor estimate of total EAA supply to assess whether this approach indicates milk protein secretion in a specific situation was limited by inadequate supply of Met, Lys or His.

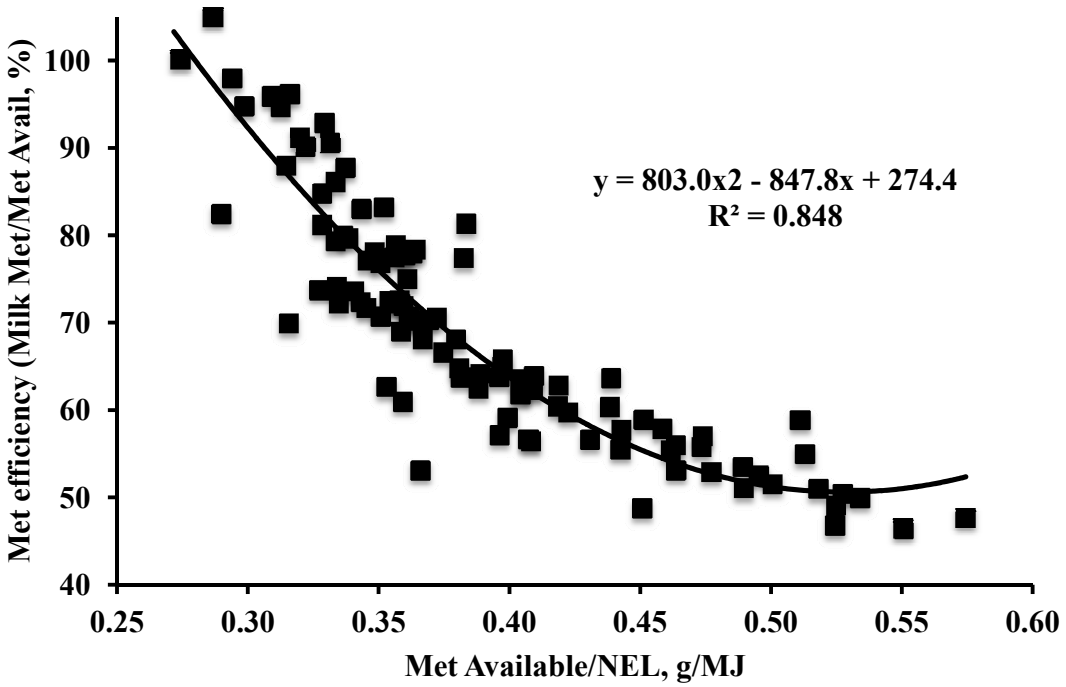

Figure 2 Regression of Met efficiency (milk Met secretion/Met availabile; y-axis) on Met available for milk production /NEL (x-axis) as estimated in the NorFor (2011) system.

Of course, in an advisory setting, a more likely application of this approach might be to predict milk protein yield on a specific diet. NorFor (2011), and most rationing systems, estimate AAT (MP) requirements for maintenance from body weight and DM intake, so these data would also be required. The individual feed ingredients of the diet in question are entered into NorFor along with other required inputs; NorFor generates an estimate of energy corrected milk yield as well as EAA required for various functions and total EAA supply. Dividing the value EAA available (total EAA minus EAA requirement for functions other than milk protein secretion) by predicted NEL yield gives the 'X' to use for computing EAA efficiency in the quadratic equations. Multiplying the EAA available by this efficiency yields an estimate of the amount that would be secreted in milk protein as EAA. The milk protein content of EAA (2.6, 7.6 and 2.6% for, respectively, Met, Lys and His) gives the equivalent yield of milk true protein. We suggest that this series of computations be conducted for all three EAA; the computations giving rise to the lowest milk protein estimate would, within this approach, be the predicted milk protein yield. This result could then be compared with yield predicted from AAT supply; it is expected that milk protein yield estimated from the most limiting EAA would be somewhat lower. This result would also indicate how the diet might be altered (such as by supplementation of rumen protected EAA) to increase supply of the limiting EAA so milk protein secretion matches that which could be supported by AAT supply.

Conclusions

The reliability of NorFor for predicting milk protein yields in our earlier work suggested that NorFor produces reliable estimates of AAT (MP) supply and gives us confidence that the system will yield reliable estimates when extended to individual EAA. This paper describes our early efforts at predicting and applying EAA efficiency computations within the NorFor system and should be considered a work in progress. We are exploring other ideas and approaches to define optimum levels of EAA in NorFor. Stay tuned for more developments in the near future.

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Effects on dairy cow urine volume and N metabolism at different K intake levels

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Introduction

Dairy cow rations may vary considerably in their concentration of several macrominerals. This is due both to ration composition and to variation within a certain feed type. Within the grass-legume forages, there is a large variation in especially potassium content. For the 20000 Swedish farm samples of grass-clover silage (Feed code 165) contained in the current Norfor Feedstuff table, K concentrations range from about 10 to 33 g/kg DM, when expressed as mean ± 2 standard deviations. These variations have implications for the metabolism of dairy cows, as they affect the liquid turnover and could be expected to give rise to secondary effects because of increased water intake and urine volume. Increased urine volume is associated with decreased milk urea concentration (De Campenere et al., 2006; Spek et al., 2012).

The objective of the experiment presented here was to assess the effects of incremental amounts of $KHCO_3$, with respect to urine volume, water intake and N metabolism measures such as rumen ammonia, milk urea, urinary urea and total urinary N.

Materials and Methods

Six ruminally cannulated lactating dairy cows of the Swedish Red breed (BW 618 ± 38 kg, parity 2.0 ± 0.0, DIM 86 ± 15 at experimental onset) were used in a 3 x 3 Latin square experiment. Period length was 14 days, with sampling days 10-14. The cows were kept in individual tie stalls and milked there at 06:00 and 17:00 h. The same basal ration was fed to all cows at individually fixed levels throughout the experiment to result in no refusals. The ration consisted of silage, a commercial concentrate (SOLID 620, Lantmännen, Stockholm, Sweden) and urea (AB Johan Hansson, Uppsala, Sweden) in the proportions 39.3:60.0:0.7 on a dry matter basis. The silage was a second cut from a timothy dominated grass-clover ley grown on an unfertilized organic soil and round baled at 56% DM with 375 g sodium bensoate/tonne fresh weight. Each bale was thoroughly cut and mixed in a TMR wagon, before feeding. Silage was fed in the manger with two equal meals at 05:45 and 16:45 h, respectively, and concentrates were fed in a separate through with four equal meals at 06:00, 09:00, 13:00 and 17:00 h, respectively. The two first concentrate meals were fortified with urea, manually mixed into the concentrates at feeding. Separate small amounts of silage and concentrate orts were collected immediately before silage feeding, weighed and sub-sampled once daily.

Experimental treatments were either basal ration only (Treatment L) or basal ration with food grade potassium bicarbonate (Univar Europe, Rotterdam, Netherlands) added to provide double (M) or threefold (H) daily potassium intake compared to the basal ration. Bicarbonate was manually mixed into each individual silage portion prior to feeding.

The cows were test milked for three consecutive days, and strip milking samples were obtained each second hour day 12, from 06:00 to 24:00 h. Samples from test milking were analyzed by

standard infrared methods for fat, protein, lactose and DM. Test milking samples as well as strip milking samples were also analyzed colorimetrically for urea by a standard diacetyl-monoxime procedure (Technicon, 1974). All further analyzes on the different sample categories collected were performed by the standard wet chemistry methods described by Eriksson et al. (2012) if not stated otherwise. Fecal spot samples were obtained twice daily during four days for analysis of acid insoluble ash (AIA). Quantitative urine collection was performed for three days and samples were analysed for urea, creatinine and Kjeldahl N. Ruminal liquid samples obtained at 19 different hours during four days were analyzed for pH, NH_3-N, α–amino-N and volatile fatty acids (VFA). Further, feeds and fecal samples were analyzed for DM, ash, NDF, Kjeldahl N and soluble N (feeds only). Feeds, refusals, feces and urine samples were analyzed for mineral elements by inductively coupled plasma-atomic emission spectroscopy (Spectro flame, SPECTRO Analytical Instruments, Kleve, Germany) after digestion with nitric acid.

Data were analyzed with Procedure Mixed of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with treatment as fixed factor and cow and period as random factors after initial tests for effects from previous treatment and interactions. Results are presented as least square means with single degree of freedom linear and quadratic contrasts. Probabilities for different least square means when reported are Tukey adjusted. Time series data were analyzed by a similar Procedure Mixed model that included time and the interaction time × treatment.

Results and Discussion

Intake of basal ration and of all constituents except for K was the same for all treatments in spite of some refusals (Table 1). Digestibility of DM, OM and NDF increased with K intake, which most likely could be attributed to the ruminal buffering from $KHCO_3$. Apparent digestibility of K increased, consistent with previous findings of a negative intecept for K digestibility (Fisher et al., 1994; Bannink et al, 1999). Ruminal pH increased linearly with $KHCO_3$ addition, accompanied by a shift from propionate to acetate in rumen VFA pattern. All shifts in VFA pattern occurred between treatments L and M, whereas the numerical pH increase continued also in treatment H. Ruminal NH_3-N concentration decreased linearly but α-amino-N levels were unaffected by K level.

Milk production and concentrations of milk components were not affected by treatment except for a linear decrease in milk urea concentration (Table 2). The magnitude of the decrease in milk urea concentration was 0.7 m*M*, similar to what Spek et al. (2012) found for increasing dietary Na content. Milk urea concentration was almost identical for the two experiments when plotted against the sum of daily K and Na intake on a molar basis (Figure 1). This was probably to a large extent due to very similar N intakes and production levels. However, the reduction in milk urea concentration was considerably less than the 1.4 m*M* that De Campenere et al. (2006) reported. Their experiments compared maize and ryegrass silages, respectively, and the outcome may have been influenced by factors other than ration mineral content. Morning diurnal milk urea pattern relative to feeding (Figure 2) was similar to Gustafsson and Palmquist (1993), with urea peaks about four hours post feeding. The same peak levels were not reached in the afternoon. This may, at least partially, be explained by silage, urea and concentrates being fed in the first morning meal, whereas the first afternoon meal was without silage and, hence, provided less total N.

There was a large linear effect on drinking water intake and urinary output with increased K intake (Table 2), similar to the experiment with KCl addition by Fisher et al. (1994). The slope for urinary output (kg/d) against K intake (g/d) was 0.058, the same coefficient that Bannink et al. (1999) reported and close to the 0.053 previously obtained for Swedish growing cattle and dairy cows (Eriksson, 2011). The correlation between K intake and urinary output has been ascribed to the maintenance of plasma or urinary osmolality (Bannink et al., 1999; Kume et al., 2008). Kume et al. (2008) showed that urinary K concentration increased with K intake to an asymptote of about 13 g/L. Above this level, urinary K excretion would occur entirely by increased urine volume. Treatment L in the current experiment had lowest urinary K concentration (P = 0.01), while the other treatments appeared to have reached the asymptotic level for urinary K concentration. The range of urinary K concentration within a dietary K level narrowed down with increasing K intake, so that the between cow standard deviation was 1.6, 1.0 and 0.5 g/kg for treatments L, M and H, respectively.

Table 1 Intake, digestibility and rumen liquid characteristics for mid lactating cows fed incremental amounts of $KHCO_3$ on top of a common basal ration

	K level				P for contrast	
	Low	Medium	High	SED	Linear	Quadratic
Intake						
Basal ration, kg DM/d	20.2	20.3	20.2	0.13	0.84	0.45
$KHCO_3$, g/d	0	616	1142	63	-	-
Total DMI, kg/d	20.2	20.9	21.3	0.18	<0.001	0.17
N, g/d	533	535	532	2.9	0.85	0.25
Soluble N, g/d	165	167	165	1.75	0.80	0.22
NDF, g/d	7103	7121	7060	73.5	0.59	0.59
K, g/d	240	483	686	27.5	-	-
Na, g/d	29.9	30.1	30.1	0.1	0.06	0.39
Digestibility						
DM	0.71	0.73	0.74	0.003	<0.001	0.10
OM	0.72	0.73	0.74	0.003	<0.001	0.25
NDF	0.59	0.62	0.64	0.010	<0.001	0.16
N	0.71	0.71	0.71	0.001	0.85	0.41
K	0.89	0.93	0.95	0.009	<0.001	0.14
Rumen liquid values						
pH	5.93	6.04	6.11	0.06	0.01	0.41
VFA, m*M*	121.5	120.3	119.9	2.73	0.55	0.83
Acetate proportion	0.649	0.672	0.672	0.009	0.04	0.13
Propionate proportion	0.204	0.176	0.178	0.012	0.06	0.14
Butyrate proportion	0.112	0.114	0.114	0.005	0.63	0.76
NH_3-N, mg/dL	6.65	6.51	5.84	0.35	0.05	0.55
α-amino-N, mg/dL	5.87	6.10	6.22	0.30	0.26	0.74

Table 2 Milk production, milk and urinary N compounds and liquid turnover for mid lactating cows fed incremental amounts of $KHCO_3$ on top of a common basal ration

	K level				P for contrast	
	Low	Medium	High	SED	Linear	Quadratic
Milk, kg/d	27.4	28.0	27.9	0.59	0.35	0.50
ECM, kg/d	28.6	29.3	29.3	0.70	0.36	0.58
Milk protein, g/d	944	958	950	22.1	0.75	0.57
Milk urea, m*M*	4.48	4.18	3.77	0.13	0.001	0.92
Urinary urea N, g/d	108	105	101	2.48	0.02	0.90
Total urinary N, g/d	132	143	134	3.26	0.55	0.01
Urine, kg/d	14.0	27.4	39.9	2.18	<0.001	0.18
Creatinine, g/d	15.5	15.5	14.9	0.32	0.12	0.49
Urinary K, g/kg	10.5	12.4	12.4	0.43	0.002	0.02
Drinking water int. kg/d	82.3	98.7	113.1	2.58	<0.001	0.14
Water balance, kg/d	20.7	21.9	25.7	1.31	0.006	0.47

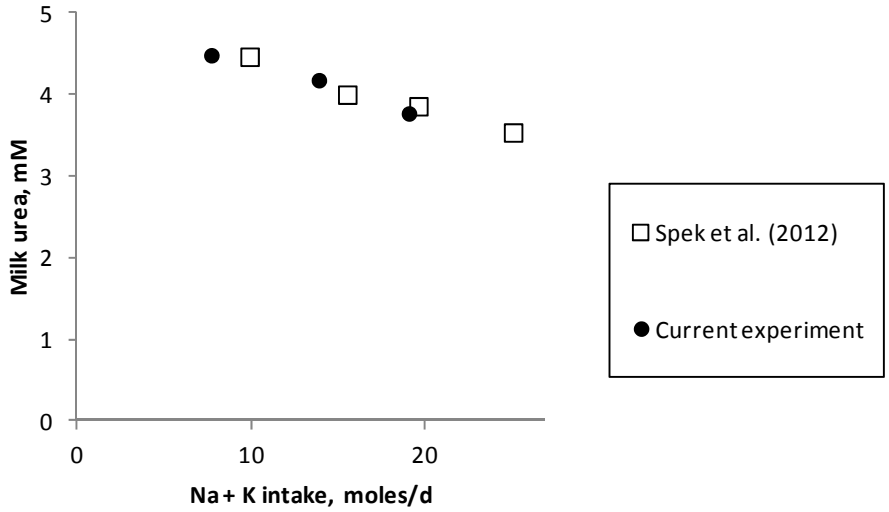

Figure 1 Milk urea concentration as a function of daily Na + K intake for mid-lactating cows consuming 519-533 g N/d and producing 25-28 kg milk/d. Data from the current experiment (incremental K amounts) and from Spek et al. (2012) (incremental dietary Na proportion).

Water balance (Table 2; Drinking water + feed water – milk water – urinary water – fecal water) was highest with the H diet ($P < 0.05$). Increasing water balance with increasing K intake was also reported by Fisher et al. (1994), but was unexplained also in their study. Creatinine excretion (Table 2) was similar to previous results at our laboratory (24.2 – 25.1 g/kg LW) and did not differ between treatments, indicating successful urinary collection.

Spek et al. (2012) found a linear increase in total urinary N excretion but no effect for urinary urea N excretion with incremental dietary Na proportion for dairy cows. In the experiment reported here, it was a curvilinear effect on total urinary N, with highest excretion for treatment M and a small, linear decrease in urinary urea N excretion for increased K intake (Table 2).

Conclusions

Dietary sodium and potassium reduce milk urea to a similar extent on a molar basis through increased drinking water intake and urinary output. This should be taken into account when interpreting milk urea results.

Acknowledgements

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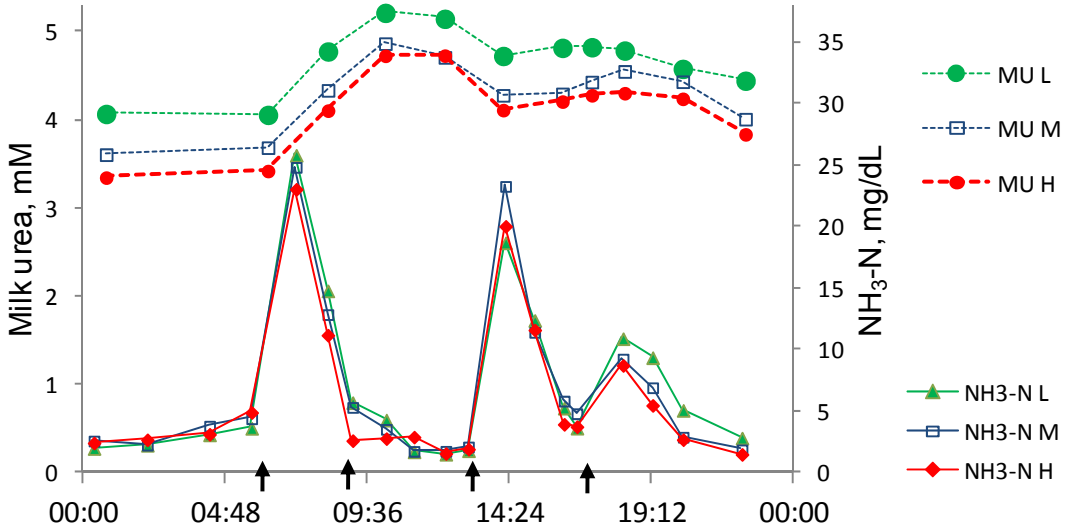

Figure 2 Diurnal concentrations of milk urea (MU, m*M*) and rumen NH_3-N in mid-lactating cows consuming low (L, 240 g/d), intermediate (M, 483 g/d) or high (H, 686 g/d) amounts of K. Arrows indicate meals (silage: 05:45 and 16:45 h; urea fortified concentrates 06:00 and 13:00 h; concentrates 09:00 and 17:00 h). Largest standard error of difference is 0.19 m*M* of milk urea and 2.0 mg NH_3-N/dL, respectively.

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Rapeseed or soybean meal to lactating dairy cows fed grass silage-based diets

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Introduction

Today, the topic of only domestic feeds within Swedish dairy production is of immediate interest. In order to have a fully homegrown diet for lactating cows, imported soybean meal has to be excluded and replaced with other protein rich feeds. Several studies have shown that it is possible to replace soybean meal with rapeseed meal in diets to dairy cows without limiting the production; it often even increases (Shingfield *et al*., 2003; Huhtanen *et a*l., 2011; Martineau *et al*., 2013). The positive production results have been explained in part by the fact that the concentration of histidine in rapeseed meal is higher than in soybean meal (Shingfield *et al*., 2003). Histidine is suggested the first limiting amino acid in cows fed grass silage-based diets (Vanhatalo *et a*l., 1999). In Sweden, the feeding of dairy cows is to a large extent based on grass silage. Because of this, the aim of this study was to investigate if rapeseed meal can increase milk production when replacing soybean meal under Swedish conditions.

Materials and Methods

Twenty-eight lactating Swedish Red cows were divided into four blocks in a cyclic change-over design according to Davis and Hall (1969) with 7 treatments in 2 × 4 factorial arrangement of treatments. One block was made up of primiparous cows and the rest of the animals were blocked according to their level of milk yield. Seven treatments were made up by either rapeseed meal (RSM) or soybean meal (SBM) to reach four incremental crude protein levels. A control treatment without supplementary protein feed was not replicated. The diets were fed *ad libitum* as total mixed rations during four experimental periods that lasted 21 days. Grass silage was supplemented with crimped barley and RSM or SBM. Four incremental crude protein (CP) levels were achieved by increasing RSM or SBM on the expense of barley in the diets (Table 1).

All seven diets included 8.8 % of a premix containing sugar beet pulp and molasses, oats, calcium-fat, NaCl, mineral and vitamin mix for provision of mineral requirements in the total mixed rations. Feed intake was recorded daily via a roughage intake control system (Insentec B. V., Marknesse, the Netherlands). The animals were milked at 06.00 and 15.00 h and milk yield was recorded daily using gravimetric milk recorders. Milk sampling was done at four subsequent milkings from the afternoon on day 19 until the morning of day 21 in each period. Morning and afternoon samples were pooled separately and analyzed for fat, protein, lactose and urea. Live weights were recorded on day 19-21 after morning milking. Blood from the tail vein were taken on day 19 after morning milking using 10 ml vacutainer tubes, spray-coated with K_2EDTA. The tubes were kept on ice until centrifugation and separation of plasma. The plasma was stored in -80°C. The concentrations of amino acids in plasma were determined according to Haque *et al*. (2012) using an ultra-performance liquid chromatography-mass spectrometry system equipped with an UV detector and a mass detector for the co-eluting peaks for arginine

and 3-methyl histidine. Data was collected during the last week of each period and processed using the General linear model of SAS (SAS Institute Inc., Cary, NC). Contrasts were added in the process and included comparison of rapeseed meal and soybean meal (R v. S), linear (Lin) and quadratic (Quad) response to protein level and the interactions between protein feed and protein level.

Table 1 Diet formulations (g/kg dry matter).

	Diets[a]						
	B	RL	SL	RM	SM	RH	SH
Silage	600	600	600	604	604	600	604
Premix	88	88	88	88	88	88	88
Barley, crimped	313	238	263	170	214	100	164
Rapeseed meal	0	75	0	138	0	213	0
Soybean meal	0	0	50	0	94	0	145

[a] B: only barley and no protein feed, RL; low level of rapeseed meal, SL: low level of soybean meal, RM: medium level of rapeseed meal, SM: medium level of soybean meal, RH: high level of rapeseed meal, SH: high level of soybean meal.

Results and Discussion

The feed intake tended to increase when the cows were fed RSM diets (Table 2). Increased crude protein level in the diet increased milk yield ($P < 0.05$) as well as energy corrected milk yield (ECM) ($P < 0.05$). Feeding RSM diets tended to increased milk yield ($P = 0.06$) and ECM yield ($P = 0.09$) compared to SBM diets (Table 3).

The concentrations of milk fat and milk protein did not differ between the diets (Table 3). On the other hand the cows produced more total milk protein per day when they were fed with RSM instead of SBM ($P < 0.02$).

Cows fed RSM diets had lower milk urea concentration ($P < 0.01$) (Table 3) and higher nitrogen efficiency ($P < 0.01$) than cows fed SBM diets. No differences in blood plasma histidine, branched-chain, essential and total amino acids concentration (Table 4) could be seen in the comparison between the two protein feeds, in contrast to Shingfield *et al.* (2003), who found significantly higher plasma histidine with rapeseed expeller compared with SBM. Overall, production responses to incremental CP intake were small (<10%) with both protein feeds suggesting that economically optimal level of supplementary protein is probably lower than current recommendations.

Table 2 Daily nutrient intake in kg/day if not otherwise mentioned. Shows mean treatment effects.

	Diets [a]								Significance		
											Int.[c]
	B[a]	RL[a]	SL[a]	RM[a]	SM[a]	RH[a]	SH[a]	SEM[b]	R v. S	P-Lin	R v. S * Lin
DMI	20.4	20.7	20.4	21.3	20.7	20.7	20.5	0.25	0.08		
Silage DMI	12.2	12.4	12.2	12.9	12.5	12.4	12.3	0.15			
OMI	18.9	19.1	18.9	19.6	19.1	19.0	18.9	0.23			
CP	3.11	3.51	3.52	3.92	3.93	4.15	4.28	0.051		< 0.01	
NDF	8.92	9.08	8.81	9.41	8.86	9.15	8.65	0.108	< 0.01		< 0.01
ME (MJ/day)	241	243	244	249	248	241	246	3.0			

[a] B: only barley and no protein feed, RL; low level of rapeseed meal, SL: low level of soybean meal, RM: medium level of rapeseed meal, SM: medium level of soybean meal, RH: high level of rapeseed meal, SH: high level of soybean meal [b] Standard error of mean; [c] Interactions.

Conclusions

The replacement of SBM with RSM resulted in greater milk protein yield. Further, RSM diets resulted in lower milk urea concentrations and improved milk nitrogen efficiency. This would indicate that RSM provides better conditions for transforming the total nitrogen in feed into milk. In the present trial similar plasma histidine concentration between RSM and SBM diets suggest that increased milk protein yield with RSM was not associated to increased histidine supply. Overall, this indication could not be explained by a greater histidine concentration in blood plasma.

Table 3 Mean treatment effects of milk production and nitrogen efficiency.

	Diets [a]							Significance			
											Int.[c]
	B	RL	SL	RM	SM	RH	SH	SEM[b]	R v. S	P-Lin	R v. S * Lin
Milk (kg/day)	26.7	28.0	27.5	29.0	28.0	28.8	28.0	0.47	0.06	< 0.01	
ECM (kg/day)	28.8	29.4	29.0	30.6	30.0	30.7	29.4	0.52	0.09	0.02	
Fat (g/day)	1197	1205	1184	1254	1252	1269	1206	25.5		0.07	
Protein (g/day)	943	969	959	1009	969	1010	965	15.5	0.02	0.01	0.03
Lactose (g/day)	1278	1336	1321	1389	1344	1370	1348	24.3		< 0.01	
Urea (mmol/l)	3.0	3.3	3.6	3.9	4.5	4.3	5.2	0.082	< 0.01	< 0.01	< 0.01
N-efficiency	295	272	268	252	241	239	220	3.50	< 0.01	< 0.01	< 0.01

[a] B: only barley and no protein feed, RL; low level of rapeseed meal, SL: low level of soybean meal, RM: medium level of rapeseed meal, SM: medium level of soybean meal, RH: high level of rapeseed meal, SH: high level of soybean meal. [b] Standard error of mean; [c] Interactions.

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Table 4 Mean treatment effects of blood plasma amino acid concentrations (µMol/L)

	Diets [a]								Significance				
	B	RL	SL	RM	SM	RH	SH	SME[b]	R v. S	P-Lin	P-Quad	R v. S *Lin	R v. S *Quad
Arginine	82	93	97	115	118	108	115	6.88		< 0.01			
Histidine	32	43	44	58	56	58	61	2.89		< 0.01	0.05		
Isoleucine	132	140	143	162	169	163	165	8.67		< 0.01			
Leucine	115	132	143	162	169	170	178	8.91		< 0.01			
Lysine	77	84	87	100	103	99	102	7.52		< 0.01			
Methionine	21	22	26	27	24	24	24	2.2					
Phenylalanine	51	51	61	58	63	57	61	3.04	0.02	0.02			
Threonine	118	123	124	138	127	123	125	7.17					
Tryptophan	45	44	50	48	48	47	45	2.46					
Valine	236	266	262	310	309	326	332	11.2		< 0.01			
Alanine	239	229	259	244	229	227	239	10.7					
Asparagine	89	88	101	102	105	93	107	6.37	0.06	0.08			
Aspartic acid	21	20	20	20	19	20	20	0.77					
Cysteine	11	13	12	14	13	12	13	0.84					
Glutamine	298	257	277	265	277	270	274	12					
Glutamic acid	36	35	31	35	31	32	34	1.77					0.03
Glycine	367	358	378	348	346	397	327	23.1				0.04	
3-MH [c]	5.7	5.5	5.6	5.5	5.1	4.7	4.5	0.17		< 0.01	0.06		
Ornithine	42	45	45	57	54	54	57	2.23		< 0.01			
Proline	99	96	108	107	111	98	111	3.43	< 0.01			0.06	
Serine	100	101	110	100	103	96	107	5.65					
Tyrosine	45	46	57	59	60	52	61	4.07	0.04	0.01			
BCAA[d]	484	539	548	635	646	659	675	27.1		< 0.01			
EAA[e]	911	1001	1043	1190	1198	1178	1224	52.2		< 0.01			
NEAA[f]	1305	1241	1347	1283	1282	1293	1275	45.7					
TAA[g]	2215	2242	2391	2474	2480	2471	2499	90.3		< 0.01			

[a] B: only barley and no protein feed, RL; low level of rapeseed meal, SL: low level of soybean meal, RM: medium level of rapeseed meal, SM: medium level of soybean meal, RH: high level of rapeseed meal, SH: high level of soybean meal; [b] Standard means of error; [c] 3- metyl histidine; [d] Branched-chain amino acids; [e] Essential amino acids; [f] Non-essential amino acids; [g] Total amino acids.

In situ disappearance of the minerals calcium, phosphorus and magnesium from grass and maize silages in the rumen of dairy cows

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Introduction

Loss of minerals to the environment can have a negative impact on the environment and thus should be prevented as much as possible. Therefore, the urge for efficient use of minerals will become higher in the near future. To feed dairy cows as efficient as possible regarding minerals, it is necessary to understand the degradability and usage of minerals in the rumen of dairy cows. Therefore, we set up an experiment in which maize, as well as grass silages, were incubated in the rumen using the *in sacco* technique with nylon bags. Disappearance of minerals was measured in order to estimate the availability of the minerals in the rumen of dairy cows.

Materials and Methods

An *in situ* study was set up in which 20 grass silages (no clover) and 20 maize silages were incubated in the rumen of dairy cows. The grass and maize silages were selected based on their nutritional composition including their phosphorous (P) content. The average nutritional composition of the silages is shown in Table 1 (starch was not analysed in grass silages). Nutritional composition was analysed with near infrared spectroscopy (ISO 12099: 2010). The average content of P, Mg and Ca in the grass and maize silages is shown in Table 2. Mineral composition was analysed by ICP-AES (derived from the standards: NEN-EN-ISO 11885:2009; NPR 6425: 1995).

Several different fractions of the minerals in the grass and maize silages were measured or calculated. The washable fraction (W) was analysed using a specialized washing machine. Subsequently nylon bags (pore size 37 µm, Nybolt, Zurich, Switzerland) containing the grass or maize silages were incubated in 3 lactating dairy cows for 2, 4, 8, 16, 32, 72 and 336 hours (at least 3 bags per incubation time, more bags for longer incubations). After removal from the rumen, the nylon bags were stored in ice water to stop microbial activity. Subsequently, bags were washed in a washing machine (no centrifuging), freeze dried, weighed, pooled and analysed for nutrient composition. The remaining residue after 336 h of incubation was considered the undegradable fraction (U). The degradable fraction (D) was calculated as 100-W-U. Rate of degradation of the D fraction was calculated according to the first order model of Robinson *et al*. (1986), including D, U and kd, e.g. residue at time t = $D*e^{(-kd)(t)}+U$.

Results and Discussion

As expected, the nutrient composition was more variable in grass silages than in maize silages (Table 1) and mineral content was lower in maize silages than in grass silages (Table 2). The washable fraction (W) of the minerals was high for P and Mg, but lower for Ca in both grass and maize silage (Table 3). This was probably due to the fact that in forages, Ca is required for the cell walls, and Ca will thus become available comparable to NDF, whereas especially Mg can be found in the cell contents and will thus become available much sooner.

Table 1 Nutrient composition (g/kg DM, unless stated otherwise) of the 20 grass and 20 maize silages (DM = dry matter, OMD = organic matter digestibility (%), NDF = neutral detergent fibre, ADF = acid detergent fibre and ADL = acid detergent lignin).

	Grass silage (N=20)				Maize silage (N=20)			
	Average	Standard deviation	Min.	Max.	Average	Standard deviation	Min.	Max.
DM (g/kg)	476	119	237	699	333	39	267	403
pH	5.0	0.71	3.9	6.7	3.9	0.14	3.6	4.1
Ash	101	16	72	131	36	6.9	26	51
OMD (%)	77.4	3.6	68.3	84.1	74.1	2.5	68.5	78.7
Crude protein	166	20	115	209	72	5.9	58	83
Sugar	76	43	10	175	10	2.4	7	15
Starch	-	-	-	-	341	26	303	401
NDF	500	53	381	599	395	32	330	447
ADF	284	29	228	354	223	21	190	270
ADL	21	5.6	14	35	20	5.4	12	34

Table 2 Mineral composition of grass and maize silages

	Grass silage (N=20)				Maize silage (N=20)			
	Average	Standard deviation	Min.	Max.	Average	Standard deviation	Min.	Max.
P	4.0	0.7	3.2	5.5	2.0	0.4	1.4	2.9
Mg	2.5	0.5	1.6	3.3	1.3	0.2	0.9	1.7
Ca	5.9	1.2	4.5	9.0	1.9	0.6	1.2	4.0

Table 3 The washable (W) fraction (%) of phosphorus (P), calcium (Ca) and magnesium (Mg) in the grass and maize silages.

	Grass silage (N=20)			Maize silage (N=20)		
	P	Ca	Mg	P	Ca	Mg
Average	75.7	18.1	63.1	85.1	21.1	80
Standard deviation	8.2	26.8	8.4	2.6	19.3	4.0
Minimum	56.6	0.0	50.3	79.5	0.0	70.9
Maximum	87.7	59.2	78.6	88.4	57.3	90.0

As for P, this mineral is available in cell walls (phospholipids) as well as cell content (ATP), and because of its role at the energy metabolism, it will also be highly available.
After incubation, the Ca concentration in the silage seemed to rise (Figure 1, left), probably due to microbial contamination. It was only at t=32h that residual Ca (expressed in % of the original, incubated silage) was lower than washable Ca. For P, the disappearance was variable, probably due to the high P concentration in saliva and rumen fluid. The P

concentration in the rumen of sheep was 16-25 mmol/litre rumen fluid (Beardworth et al., 1989; Dias et al., 2013; Johnson and Jones, 1989). The concentrations of Ca and Mg were clearly lower: 0.2-2.5 mmol Ca and 0.2-4.5 mmol Mg/litre rumen fluid (Johnson and Jones, 1989).

The U fraction was higher for Ca than for P and Mg in grass silages as well as in maize silages (Table 4). The U fraction for calcium was much higher for maize silages than for grass silages. This was probably due to contamination by microbes and/or rumen fluid, since the effect of contamination by microbes and/or rumen fluid on calcium disappearance is most likely higher when calcium content of the maize silage is low. For phosphorous in grass silages, the W fraction and U fraction were quite high, resulting in a low D fraction (Figure 2). The high W fraction for P in maize silages resulted in unreliable numbers: the D and U fraction could not be estimated. This is due to the low P content in combination with the high presence of P in the rumen.

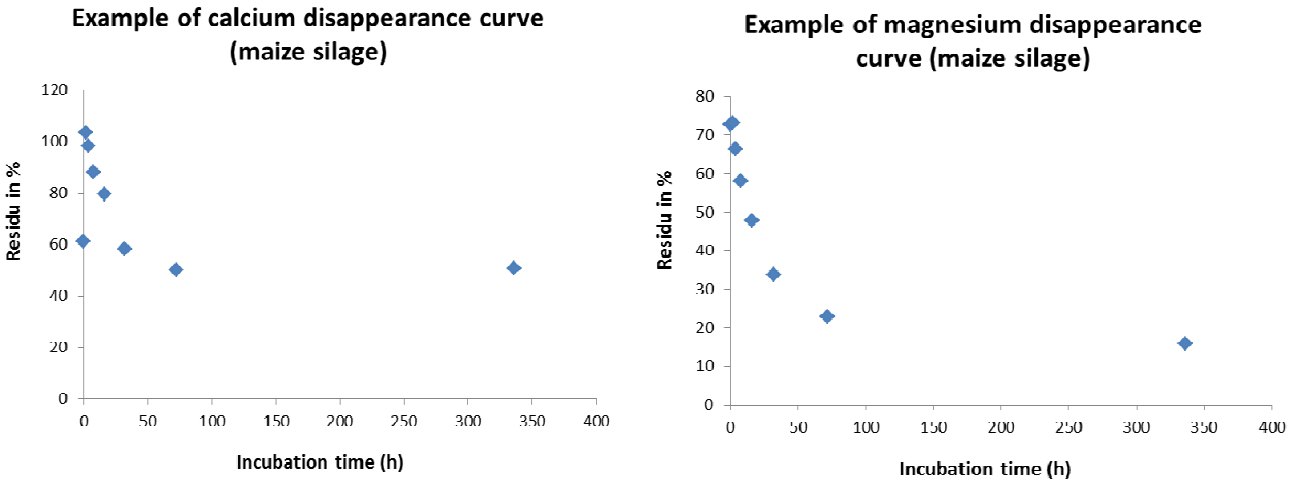

Figure 1. Disappearance curves of calcium and magnesium in maize silage.

Table 4. The U fraction of P, Ca and Mg in grass and maize silages (%).

	Grass silage			Maize silage		
	P	Ca	Mg	P	Ca	Mg
Average	13.9	38.5	6.1	-	55.9	9.9
Standard deviation	3.8	10.2	2.5	-	8.0	3.0
Minimum	6.8	21.5	3.1	-	43.5	6.8
Maximum	20.6	57.9	12.6	-	73.7	16.0

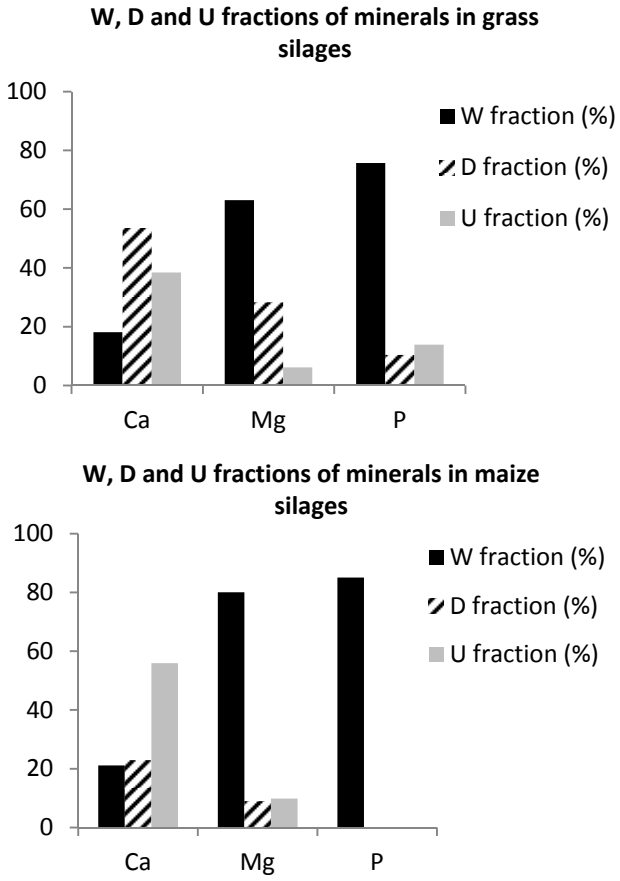

Figure 2. W, D and U fractions of Ca, Mg and P in grass and maize silages.

Based on the degradation curve (as in Figure 1), the rate of degradation (kd) was estimated (Table 5). Large differences between samples can be observed from the standard deviations. However, this was also partly due to the fact that all errors accumulate in the kd. Magnesium was quickly released in the rumen, with an average rate of 15%/h for grass silages and 11%/h for maize silages.

Table 5. The rate of degradation (kd) of P, Ca and Mg in grass and maize silages (%/h).

	Grass silage			Maize silage		
	P	Ca	Mg	P	Ca	Mg
Average	4.0	6.5	15.3	-	5.45	10.9
Standard deviation	7.5	1.8	4.54	-	3.2	9.9
Minimum	0.13	4.3	9.3	-	0.04	2.02
Maximum	11.9	10.5	24.6	-	13.2	33.7

Rumen available and bypass fraction

Based on the rate of degradation, the W, D, U fractions and the passage rate, the fermentable and bypass fraction has been calculated, according to the following formulae:

Bypass fraction = U+D*(kp/(kp+kd))

Rumen available fraction = W+D*(kd/(kd+kp))

It is clear that the available fractions of P and Mg were high in both maize and grass silages, as W for P was 76% for grass silages and 85% for maize silages and the W for Mg was 63% for grass and 80% for maize silages. It is assumed that the W fraction will be directly available in the rumen. Furthermore, the D fraction will be degraded at a high rate (kd of 15%/h for grass silage and 11% for maize silage). As a result, the estimated bypass fraction was low: 13% for both grass and maize silages.

For Ca, the average W fraction was much lower (18 and 21% for grass and maize silages, respectively) and thus the available fraction was much lower, about 50% of maize silages and 55% of grass silages. The low availability was probably linked with the relatively low degradability of NDF; the average fermentable fraction of NDF in this dataset was 55% for grass silages and only 36.5% for maize silages (pers.comm. Bruinenberg).

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Relationships between ewe body condition score, production traits and nutrition, on organic sheep farms

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Introduction

The nutritional requirements of ewes differ during the reproductive cycle. The requirements are high during the second half of the gestation period, especially for ewes bearing twins or triplets, as well as during the suckling period. During these periods the ewes cannot cover their nutritional requirements from consumed feed alone, but have to use their body reserves to support their own and the lamb nutritional demand (Mendizabal et al., 2011). Losses in ewe body condition may impair fertility and productive traits, decrease the amount of milk available for lambs, lower lamb birth weights and thus increase lamb mortality (Petrovic et al., 2012). There is therefore a need to plan a feeding strategy that enables the maximisation of ewes' productive potential and also ensures unimpaired growth of lambs. Several authors (Russel, 1984; Fthenakis et al., 2012.) have recommended using ewe body condition scoring (BCS) as a tool to evaluate the nutritional status of ewes on farm. It should be borne in mind that covering the nutritional requirements on organic sheep farms has become more complicated from 1st January 2008 since when, on all EU organic farms, animals must be fed 100% with organically produced feed. In Estonia, despite the rapidly increasing number of organic sheep farms, sheep nutrition on organic farms has not previously been investigated. Therefore, the aim of this project was to study the ewe body condition scores at important time points in the reproductive cycle in relation to production traits.

Materials and Methods

The work was carried out on three organic sheep farms; Farms A and B, (both Estonian Whiteface sheep) and farm C (Estonian Blackface sheep) over a three year period (2010 to 2012). During the period between weaning and mating, while at pasture, it was estimated that the ewes consumed from 6 to 8 kg of forage per day. During the mating period the ewes were fed either lucerne silage (Farm A), pasture forage (Farm B) or forage in the first month and then hay (Farm C). Pasture forage contained grass and legumes with <25 % legumes on farm B and 50-75% legumes on farms A and C. During the gestation period, on all farms, the ewes were fed in groups; hay for the first three months, from the fourth month a mix of hay and silage (farms A and B) or hay and whole oats (farm C). Feed samples were analysed regularly and rations were calculated to study if they met nutritional requirements. Ewe BCS on a scale of 1 (emaciated) to 5 (obese), with 0.5 point graduations, was assessed before mating, at lambing and at weaning by two observers at the same time during the whole study, and the score was given consensually. Lambing dates, lamb birth weights and 100-day body weights were recorded. Data was analysed statistically to evaluate the association of the ewe body condition scores with litter size, lamb birth weights and lamb 100-day bodyweights. To manage the dataset and calculate the group frequencies, mean values and test the statistical significance of body condition score groups on lamb birth weights and 100-day body weights with analysis of variance, MS Excel and the statistical package SAS 9.2 (SAS 9.2 Online Doc., 2013) were used.

Results and Discussion

Throughout the reproductive cycle the ewes gained or lost body condition (Fig.1). Ewes consumed only pasture feed, containing metabolizable energy of 10.5 to 11.0 MJ/kg DM per day, during the period between weaning and mating, while at pasture, which sufficiently covered their needs for energy and protein.

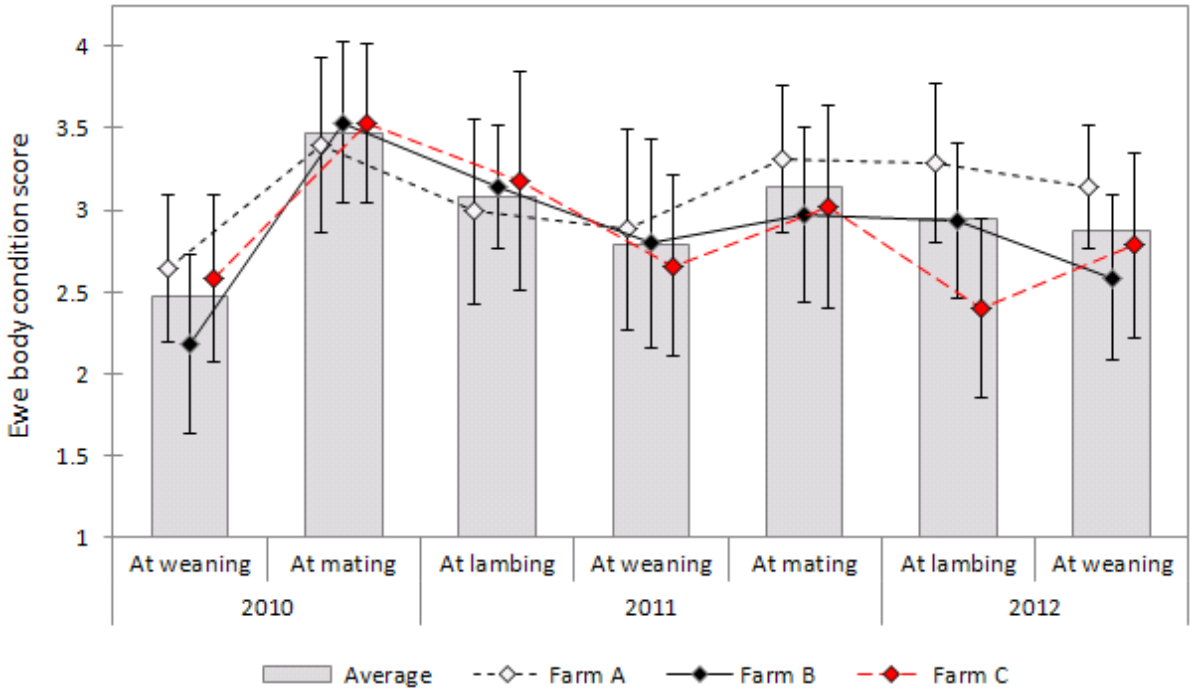

Figure 1 The dynamics of ewe body condition scores (average ± standard deviation) on three farms.

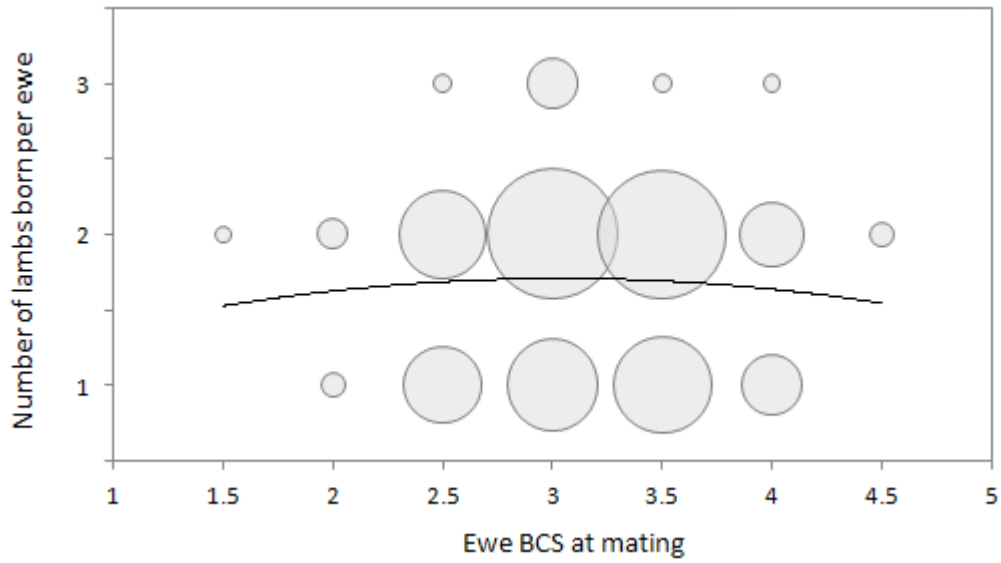

Figure 2 The relationships between ewe BCS at the time of mating and number of lambs born per ewe. The area of the circles represents the number of ewes with the indicated number of lambs born. The continuous line shows the predictive curve for lambs born from ewe BCS at mating (NS; $F_{2,261}$=0.32, p=0.72).

In both study years, the ewes improved their body condition between weaning and mating and reached, by the time of mating, mean body condition scores of 3.47 and 3.14 during the first and second study years, respectively, which were within the optimal range for this period (Kenyon et al., 2004). In the current study, the number of lambs born per ewe was highest (1.78; NS) in ewes whose body condition at mating was 3.0 (Fig. 2).

The most critical time, from the aspect of ewe nutrition, was the gestation period, when mean ewe BCS change on all three farms was biggest -0.39 ±0.57 and -0.20 ±0.65 points, in the first and second study years, respectively. In the second study year, the ewes were fed hay and silage during the last two month of the gestation period on farms A and B, which enabled them to better cover their energy demands, and the BCS change was minimal (farm A -0.04 ±0.52; farm B -0.04 ±0.62 points). On farm C in the second study year, the ewes were fed only hay in the gestation period and the BCS loss was -0.61 ±0.7 points, in contrast to the first year when the ewes were fed hay with 0.35 kg oats per day and the BCS loss was 0.35±0.62 points. Maintaining the BCS in the gestation period is important, as lamb birth weight and 100-day weight were related to the ewe body condition change during the gestation period (Table 1); the birth weight of single lambs was statistically significantly different depending on ewe BCS change during the gestation ($p=0.006$). The 100-day body weights differed in multiple lambs with different BCS change of ewes during the gestation ($p<0.001$). The ewe BCS at lambing was also related to 100-day body weight of both single ($p=0.004$) and multiple lambs ($p<0.004$; Table 2); the 100-day body weights of lambs were greater in those ewes whose body condition was higher at lambing.

Table 1 Lamb birth weight and 100-day body weight in relation to the ewe BCS change over the gestation period (↓ declined; – no change; ↑ increased)

Indicator	Lambs born	BCS change	Farm A	Farm B	Farm C	Total	p-value
Number of ewes	Single	↓	18	5	16	39	
		–	16	7	4	27	
		↑	15	12	1	28	
	Multiple	↓	27	28	17	72	
		–	36	14	5	55	
		↑	27	14	1	42	
Lamb birth weight (kg)	Single	↓	3.81	5.30	3.76	4.01	$F_{2.80}=5.40$
		–	4.57	5.57	3.98	4.76	$p=0.006$
		↑	4.19	5.32	4.00	4.65	
	Multiple	↓	3.43	4.39	3.63	3.84	$F_{2.163}=1.13$ $p=0.33$
		–	3.57	4.49	3.00	3.75	
		↑	3.71	4.45	4.00	3.97	
Lamb 100-day weight (kg)	Single	↓	29.47	27.25	21.67	26.83	$F_{2.72}=1.35$ $p=0.27$
		–	29.50	26.33	24.67	28.04	
		↑	32.38	25.88	27.00	29.77	
	Multiple	↓	24.69	20.19	20.08	21.92	$F_{2.155}=10.30$ $p<0.001$
		–	23.67	21.33	19.08	22.85	
		↑	29.49	20.71	25.00	26.60	

Table 2. Mean 100-day body weights of lambs in relation to the BCS of ewes at the time of lambing.

Lambs born	Ewe BCS at lambing	Farm A	Farm B	Farm C	Mean	p-value
Single	≤2	25.00 (n=1)	-	21.00 (n=9)	21.57 (n=10)	$F_{4.77}$=4.29
	2.5	26.43(n=7)	25.00 (n=1)	23.67 (n=6)	25.55 (n=14)	p=0.004
	3	29.58 (n=13)	26.00 (n=18)	24.75 (n=6)	27.27 (n=37)	
	3.5	31.45 (n=24)	26.50 (n=14)	-	30.04 (n=38)	
	≥4	33.40 (n=5)	23.00 (n=2)	-	31.67 (n=7)	
Multiple	≤2	23.75 (n=2)	19.38 (n=8)	16.25 (n=4)	19.33 (n=14)	$F_{4.174}$=7.66
	2.5	25.83 (n=9)	20.22 (n=29)	19.95 (n=12)	21.19 (n=50)	p<0.001
	3	23.87 (n=26)	20.11 (n=23)	21.38 (n=7)	22.22 (n=56)	
	3.5	26.50 (n=42)	21.50 (n=14)	22.50 (n=1)	25.31 (n=57)	
	≥4	26.90 (n=11)	-	-	26.90 (n=11)	

Conclusions

This analysis has shown that lamb birth weight and lamb 100-day weight were affected by ewe BCS and BCS change at certain periods of the reproductive cycle on organic sheep farms. As the number of lambs born per ewe was highest in ewes whose BCS at mating was 3.0., improvement of body condition during the period after weaning is very important. Maintaining the BCS during the gestation period is also important as lamb birth weight and 100-day weight was related to ewe body condition change during the gestation period. The ewe BCS at lambing was related to single and multiple lambs' 100-day body weight. However, it is also important to avoid large BCS loss during lactation; otherwise ewes can't restore BCS during the free period before the next mating period. Body condition scoring appears to be a useful tool for monitoring herd nutritional status of organically farmed sheep.

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A comparison of herbivore digestion efficiency in vitro using moose spring and summer foods

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Introduction

Domesticated ruminants (e.g. *Bos taurus*) are adapted to utilize fibrous plant material efficiently because of their digestive system based on microbial fermentation in the fore-stomachs and selective retention of feed particles in the rumen. Browsers have a different morphological and physiological adaptation than grazers, which allow them to selectively forage on highly digestible plant material (Hofmann, 1989). Many laboratory techniques for ruminant feed evaluation have been developed based on in vitro methodology with cattle rumen fluid as inoculum. The moose (*Alces alces*) is a ruminant herbivore and a browser, which feeds on nutritious twigs and shrubs. During winter, the moose forage foremost twigs of a variety of tree species such as pine and juniper shoots. In summer, they mainly consume seedlings, leaves and herbs. It is claimed that the moose seeks food high in protein and low in fiber during summer. Poor summer-autumn nutrition has resulted in lower calf growth, reduced pregnancy rates and lower winter survival in wild ruminants (Cook et al., 2004). Herfindal et al. (2006) concluded that body mass of Norwegian moose was mostly affected by access to fresh vegetation in early spring and to variables influencing slow plant maturation. Recent research has explored strategies to alleviate grazing damage on agricultural and forest lands caused by the moose. However, little attention has been given to food preference of moose with regard to feed nutritive value. This project was aiming at using chemical characterization and more advanced in vitro measurements to evaluate the nutritive value of both natural and offered spring and summer food resources to moose. In addition, we examined the effect of rumen fluid source from moose or dairy cow on digestion parameters measured in vitro.

Materials and methods

A set of 12 botanical samples were collected in Umeå (63°45´N, 20°17´E) between May 1 and August 14 in 2012. Two samples consisted of goat willow (*Salix caprea*) and European white birch (*Betula pubescens*) twigs. The herb fireweed (*Chamerion angustifolium*), the root of the white water lily (*Nymphaea alba*), and leaves from aspen (*Populus tremula*), white birch and rowan (*Sorbus aucuparia*) trees were collected in mid-June and in early or mid-August with an average of seven weeks between the collections. The samples were acquired from clear-cut areas and the white water lily roots in a nearby lake. Additionally, samples of red clover (*Trifolium pratense*), rapeseed plant (*Brassica napus*), common vetch (*Vicia sativa*) and alsike clover (*Trifolium hybridum*) were collected from a cultivated game field in Södermanland county (58°53´N, 15°58´E) in August 21, 2012. All feed samples were dried at 60°C for 48 h and ground through a 1.0-mm screen before chemical analysis and in vitro gas incubations using a stationary cutting mill (Retsch SM 2000; Retsch GmbH, Haan, Germany).

Rumen fluid was collected from the same two dry cows for all three in vitro incubations. The moose rumen fluid was taken from animals shot during the hunting season between

September 22 and October 20 in 2012 in Vindeln (64°12´N, 19°43´E) and Robertsfors (64°12´N, 20°51´E) municipalities in Västerbotten county. The moose shot in Vindeln municipality was an approximately 6 months old female calf with empty carcass weight of 65 kg. In Robertsfors municipality, rumen fluid was collected from a 3-year old bull and an 8-year old cow with empty carcass weights of 215 and 219 kg, respectively. The rumen fluid was filtered through two layers of cheesecloth into heated thermos flasks flushed with CO_2. Gas production was measured with an automated system (Cone et al., 1996). All samples were incubated for 96 h in three consecutive runs including duplicate samples of blanks. Mean blank gas production within run was subtracted from the sample gas production. In vitro true organic matter (OM) digestibility (TOMD) was determined for all samples in all runs by analysing the neutral detergent fiber (NDF) concentrations in the residues after the 96 h incubations. Mean blank true in vitro digestibility within run was subtracted from the sample in vitro TOMD. Measurements, analysis and production of CH_4 in vitro were made according to Ramin and Huhtanen (2012). One mL of rumen fluid was collected at 50 h of incubation from the bottles and immediately stored at -20°C until processed for volatile fatty acid (VFA) determination. Sampled amount of CO_2 gas was flushed through the T-tube to each bottle in order to empty the tube to the content of the bottles. The individual and total VFA productions were calculated by subtracting mean blank VFA concentration from the sample concentration.

Residual moisture of all feed samples was determined by oven drying for 16 h at 105°C. Ash concentration was determined by ignition of the dried sample at 500°C for 4 h. The samples were analyzed for NDF using heat stable α-amylase and sodium sulfite (aNDF) by autoclaving at 105°C for 1 h. The insoluble residue was retained by vacuum filtration in 100-mL filter crucibles holding a porosity of 40–100 μm (Saveen & Werner AB, Limhamn, Sweden) and fitted with a glass microfiber filtering aid to trap small particles (934-AH; Whatman Inc., Piscataway, NJ, USA), washed sequentially with hot water and acetone, and oven-dried at 105°C for 16 h. The NDF was expressed free of residual ash (aNDFom). The aNDFom concentration in in vitro residues was determined following the same procedure except that the vacuum filtration was replaced by centrifugation according to Udén (2006). Lignin (lignin (sa)) concentration of the feeds was determined by solubilization of cellulose in 12 *M* sulfuric acid after extraction with acid detergent. The same glass microfiber filters as described above was used in the recovery. Concentrations of N were determined by Kjeldahl digestion of 1.0 g sample in 12 *M* sulfuric acid using Foss Tecator Kjeltabs Cu (Höganäs, Sweden) in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). The individual VFA concentrations were determined by high performance liquid chromatography (Ericson and Andre, 2010).

The data were analyzed using the General linear model of SAS (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC) by applying a model correcting for the effect of run, feed, species, and the interaction between feed and species. Least square means are reported and mean separation was done by least significant difference to test differences between treatments.

Results and discussion

The twig samples displayed the highest concentrations in aNDFom and lignin (sa), and lowest CP concentrations among all samples (Table 1). The aNDFom concentration increased

in botanical samples collected later in the season, but the decrease in CP concentration between the early and late collection time was more pronounced. Both clover samples and the rapeseed plant were low in aNDFom, which suggests that the samples were regrowth material as a consequence of the late collection time and that the game has had access to the fields the whole season.

Table 1 Chemical composition of experimental samples

	DM	OM	CP	NDF	Lignin (sa)
Feed sample[a]	g/kg	g/kg of DM			
White birch twigs	519	972	63	513	329
Goat willow twigs	486	961	74	452	221
Fireweed 1	172	929	177	142	36
Fireweed 2	271	963	95	193	61
White water lily root 1	87	882	86	178	58
White water lily root 2	106	912	41	170	51
Aspen leaves 1	250	943	202	210	90
Aspen leaves 2	401	941	120	260	101
Rowan leaves 1	305	935	158	175	61
Rowan leaves 2	394	945	66	189	79
White birch leaves 1	322	963	161	176	79
White birch leaves 2	398	958	105	268	119
Alsike clover	168	895	203	228	61
Red clover	210	907	166	266	75
Rapeseed plant	111	869	152	252	43
Common vetch	256	931	210	316	90

[a]The number 1 indicate the collection time in mid-June and number 2 the collection in early to mid-August.

All measurements derived from the gas in vitro incubations are presented in Table 2. All traits showed feed × species interactions ($P < 0.01$; Table 2) except the TVFA and the molar proportion of butyric acid production. Both these traits displayed effects of feed and species ($P \leq 0.01$; Table 2). Total gas production was generally greater for samples incubated in moose rumen fluid, in agreement with generally more TVFA produced, except for one sample of aspen leaves and the common vetch incubated in moose rumen fluid. This indicated a more extensive fermentation when samples were incubated in moose rumen fluid compared with cow rumen fluid. Further, it could be speculated from these results that less feed energy is used for microbial growth in the moose compared to the dairy cow (Clauss et al., 2006), and that they therefore need to selectively forage on plants high in digestible carbohydrates and protein during summer. The game field plants were slightly higher in aNDFom than the botanical samples, which were reflected in numerically higher proportion of acetic acid compared to what was generated from the botanical samples, and induced a less favorable fermentation pattern with regard to methane produced in vitro. Else, the methane produced in vitro was comparable across species except for the white water lily root that generated more than the double amount when incubated in rumen fluid from the moose compared to the cow rumen fluid. These feed× species interactions were also reflected in the molar proportions of acetic and propionic acid. The significant feed × species interaction of

the TOMD values ($P < 0.01$; Table 2) indicates that differences were not consistent between the two species and suggest a potential for different microbial populations in moose and dairy cows.

A global estimation of CH_4 production from wild animals such as moose is difficult due to lack of sufficient data on animal populations, intake and food digestion. The proportion of CH_4 to the gross energy intake is typically between 6 and 7% in dairy cows, but can vary from 2 to 12% depending mainly on type of diet and physiological state of the animal (Johnson and Johnson, 1995). In the current study, the average proportion of CH_4 to the gross energy intake was estimated to be 5.0 and 4.8% for moose and cow, respectively.

Conclusions

This study implies that fermentation of moose spring and summer food differs depending on the rumen fluid inoculum applied to the samples. The strong interactions between feed and animal species further indicate that rumen fluid from dairy cows cannot even be used to rank the nutritive value of moose food resources. A larger population of game field plants and plants collected at different time points throughout the whole season need to be evaluated in order to appropriately compare game field plant nutritive value to the summer food preferred by the moose.

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Table 2 Measurements derived from the automated gas in vitro system of all experimental feeds incubated in moose or dairy cow rumen fluid[a]

																	P - value[b]		
Parameter	BT	GW	FW 1	FW 2	WR 1	WR 2	AL 1	AL 2	RL 1	RL 2	BL 1	BL 2	AC	RC	R	CV	SEM	F	S	F x S
TOMD, g/kg																				
Moose	482	648	882	807	884	861	864	833	848	762	944	823	823	728	787	764	20.7	<0.01	0.06	<0.01
Dairy cow	449	602	860	795	811	784	805	808	886	859	872	775	827	794	835	754				
CH_4, mL/g OM																				
Moose	11.0	16.6	10.7	13.2	29.7	37.6	22.7	20.0	25.7	20.4	20.9	17.0	28.6	27.7	29.7	30.8	1.91	<0.01	0.28	<0.01
Dairy cow	11.6	16.3	10.3	15.7	11.2	17.9	31.3	21.9	28.8	21.6	18.9	13.8	31.0	32.8	32.2	35.2				
TVFA, mmol																				
Moose	0.9	1.7	1.8	2.0	2.6	3.2	2.3	2.4	2.6	2.5	2.4	2.2	2.6	2.1	2.5	2.1	0.19	<0.01	<0.01	0.07
Dairy cow	0.7	1.4	1.5	1.7	1.5	2.2	2.3	2.1	2.5	2.26	2.1	1.8	2.2	2.2	2.5	2.2				
AcA, mol/mol																				
Moose	689	725	757	686	617	602	623	725	662	711	718	736	674	665	663	661	9.7	<0.01	<0.01	<0.01
Dairy cow	717	763	798	741	566	548	725	797	735	745	760	773	730	742	732	728				
PA, mmol/mol																				
Moose	247	200	180	242	284	299	284	192	245	222	210	196	229	249	231	248	12.0	<0.01	<0.01	<0.01
Dairy cow	199	178	147	175	355	383	180	146	155	178	190	182	184	179	177	191				
BA, mmol/mol																				
Moose	65	75	63	72	99	99	93	84	93	67	73	69	97	86	106	91	8.7	<0.01	0.01	0.10
Dairy cow	84	59	55	84	79	69	95	57	110	77	50	46	86	79	92	82				
GV, mL/g OM																				
Moose	116	190	235	232	386	380	261	261	294	292	263	226	274	272	332	259	14.1	<0.01	<0.01	<0.01
Dairy cow	64	151	141	178	240	280	271	231	269	266	213	192	268	260	316	269				

[a]The number 1 in feed sample name indicate the collection time in mid-June and number 2 the collection in early to mid-August.

[b]Probability of a significant effect of feed (F), species (S) and interaction between F and S (F×S). (*) indicates p<0.05; (**) p<0.01

BT, white birch twigs; GW, goat willow; FW, fireweed; WR, white water lily root; AL, aspen leaves; RL, rowan leaves; BL, white birch leaves; AC, alsike clover; RC, red clover; R, rapeseed plant; CV, common vetch; SEM, standard error of mean; TOMD, true OM digestibility; TVFA, total volatile fatty acids; AcA, acetic acid; PA, propionic acid; BA, butyric acid; GV, gas volume at 96 h of incubation.

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