Proceedings of the 9th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
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Rapport 298
Report

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Foreword

The aim of the 9th Nordic Feed Science Conference is still to create an arena for Nordic feed scientists to meet and discuss ruminant feeds and feeding.

Coming together in Uppsala is something that, at least, we at SLU in Uppsala always eagerly look forward to. We believe that a small conference, participants from northern Europe and other parts of the world in combination with invited and distinguished speakers create an atmosphere conducive to exchange of ideas and a possibility for future collaborations and building friendship.

This year, we have tried to pull together presentations dealing with feed processing, which is a subject of interest, perhaps even more so, in feeding of monogastric animals and in biogas production.

We estimate a total of 32 papers to be presented at the conference covering topics related to feed processing and facilities for this, forage conservation, laboratory and feed evaluations, animal responses to variation in feed composition, etc.

More than 60 participants have registered for the conference this year and we particularly thank our two sponsors – Bioprocess Control Sweden AB and Ramcon from RAMC AB, Sweden, who will demonstrate and/or display their products at the conference.

You are all most welcome to the conference! For downloading proceedings of earlier conferences, please go to our homepage (https://www.slu.se/en/departments/animal-nutrition-management/news/nordic-feed-science-conference-2018/contributions-nfsc-2018/) where you also find a list of all previous titles.

Uppsala 2018-06-01

Peter Udén

2016 Survey answers (% of 25 to 26 answers)

When?		How often?		Keynote speakers?		Where?		Proficiency testing & anal. methods?	
Feb.	4	Every year	60	2 - 3	76	Uppsala	54	In NFSC or satellite	84
April	4	Every other year	40	More	16	Nordic rotation	46	Separate conference	16
May	4			Fewer	8				
June	81								
Aug.	4								
Nov	4								

Program suggestions (work in progress, reviews, etc.)

- It was very interesting as it was!
- Error propagation is ultra-low gas flow measures
- New research. Ruminant nutrition. More ruminant behavior and grazing related research.
- Questions related to analyses and near-market topics
- Maybe to expand the organizing committee to the Nordic countries, including the Baltic states.
- Work in progress, status and reviews regarding forage, concentrate feed, feed processing, analysis and feeding practice, feeding strategies, NorFor
- Focusing on effects of different raw materials (and rations) on effect for milk and meat production.
- Exciting results from young scientists. Good to see what is going on at the Nordic universities.
- In each conference version it will be nice to organize one satellite event.
- Focus on feed, feeding and results in research. Less presentations on tests of Commercial products.
- Work in progress

Your comments to individual questions above

- First time to join the conference, and a very interesting and pleasant experience! Thank you for organizing!
- It is important to get more people outside Sweden and more people in total.
- none
- For my schedule this years' conference was held at perfect time. Also I think that Uppsala is most convenient place for conference; easy to get to and everything is at nice reachable distance. So the best place is Uppsala and the best time is in the middle of June.
- I think it is a good and nice conference. Good work!
- The NFSC is extremely cozy event with very good keynote speakers and it should be continue in future too. I guess the Uppsala is best place as it is in the center of Nordic countries. Perhaps there should best poster award for PhD student to attract them to participate.
- place for conference does not matter, but it is important with a short every year conference and it seems to me, that only you Swedish people so far have given it the higher priority.
- "It would be nice to include some organized social event with the purpose of getting people to get to know some more (new) people. Maybe as a workshop session, or some kind of group activity/game during the dinner.
- It would also be nice to offer coffee during registration hours just before the start of the conference. That would make people more relaxed, alert and focused during the first session, so it would be an even better start of a good conference. "
- Will the key note speakers attract participants? It was very ambitious with the SARA speakers. Did that give what you wanted? The laboratory presentations were a disappointment. Were north European laboratories invited?
- Regarding satellite event: such as workshops in statistics (i.e. mixed models), analytical methods (i.e. fiber determinations), feed processing (i.e. pelleting) etc.
- The proposal for also include topics related with monogastric animals is also interesting.
- Patrik N: I could see a point where NorFor could be interested to take a more active organization part in the Conference. However that might harm the original intentions and objectives. I would like to suggest that speaker should be somehow quality tested before entering.

Contents

Ruminant nutrition

Posters

Density of pelleted feeds to dairy cows and passage to the small intestine

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Introduction

Concentrate feedstuffs are often industrially processed to compound mixtures and subsequently pelletized to ease on-farm allocation. For cattle, conventional pellet presses are typically used to pelletize compound concentrates where rollers press the compound meal through a steel dye. In the fish feed industry, however, cooking extrusion is used to obtain compound feed pellets with high nutrient availability combined with specific physical functional properties of pellets suitable for allocation in water, e.g. high water stability and specific sinking velocities (Kraugerud et al., 2011; Sørensen, 2012). In comparison, pellets produced by conventional pelleting typically have low water stability and high sinking velocity.

Separate allocation of pelletized concentrate as compared with allocation in TMR has been observed to decrease rumen pH (Maekawa et al., 2002) and to reduce feed intake and milk production (Ingvartsen et al., 2001). This effect has often been attributed to acidification of the rumen and decreased fibre digestion as starch rich concentrates are known to decrease rumen pH (Owens et al., 1998; Khafipour et al., 2009b); however, feeding pellets low in starch e.g. pelletized alfalfa has also been observed to induce acidic conditions in the ventral rumen (Khafipour et al., 2009a). Hence, it could be speculated that the local acidic conditions in the ventral rumen induced by separate allocation of pelletized feeds is caused by a limited intra-ruminal mixing of feed pellets with the forage part of the diet. Therefore, feeding compound concentrates with physical functional properties that potentially allow better intra-ruminal mixing of feed pellets with the forage part may beneficiate rumen environment and cow performance.

The study aimed to investigate rumen nutrient degradability kinetics, intra-ruminal mixing and ruminal outflow of pelletized compound concentrates with different physical functional properties as assessed by postprandial starch concentrations in medial rumen content, rumen environmental measures, and duodenal starch and protein flow in dairy cows fed a basal diet low in starch. The current paper is based on publications of *in situ* investigations (Razzaghi et al., 2016) and of *in vivo* investigations (Larsen et al., 2018).

Materials and methods

The present experiments complied with Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and care of experimental animals.

Experimental concentrates

Six compound concentrate meals were produced containing either 100% wheat (as is basis, Table 1), 100% maize, 50% wheat + 50% soy bean meal (SBM), 50% maize + 50% SBM, 50% wheat + 50% sugar beet pulp (SBP), or 50% maize + 50% SBP. Meals were produced using a hammer mill (P50SP, President, Herning, Denmark) with a 2-mm screen. Each concentrate meal was subdivided into three portions for pelleting (3 mm) by either conventional pelleting with steam to 81°C (M-6K with 3 × 50 mm dye, Jesma-Matador, Esbjerg, Denmark) or by cooking extrusion (BC-45 twin screw with 2.4- or 2.6-mm circular

dye, Clextral, Firmini, France) using two distinct settings giving pellets of either high density (HD) or low density (LD). Low-density pellets were obtained by steam addition to 115°C resulting in concentrate meal expansion when extruded through the dye. High-density pellets were obtained by limiting the extent of concentrate meal expansion by water cooling of the extruder barrel to keep temperature on maximum 90°C.

Bulk density of the cereals and the cereal mixtures was determined in triplicate by measuring weight of each concentrate in a hard plastic container with defined volume. Water stability index (WSI) of experimental concentrate pellets was determined by measuring DM retained in 3 mm mesh net baskets after soaking for 120 min in 25°C deionised water (Baeverfjord et al., 2006).

In situ procedures

A detailed description of the *in situ* experiment has been published by Razzaghi et al. (2016). In brief, the *in situ* procedure for measuring rumen degradation was performed according to the NorFor system (Åkerlind et al., 2011). Approximately 2 g of each ground concentrate sample was weighed into 38 µm Dacron bags with 11 × 8.5 cm dimensions (Saatifil PES 38/31, Saatitech S.p.A., 22070 Veniano, Como, Italy) giving approximately 15 mg sample per cm^2 effective bag of surface area. Bags were incubated for 0, 2, 8, 24, and 48 h in the rumen of three dry Holstein cows. In the first week, a total of 24 bags (4 processing techniques × 6 concentrates) were incubated in each animal for each incubation time. This procedure was repeated the following week in order to obtain within animal replicates.

In vivo procedures

A detailed description of the *in vivo* experiment has been published by Larsen et al. (2018). In brief, a 3 × 3 Latin square digestibility trial was conducted with 2-day periods and the three pelletizing methods as treatments for each compound concentrate. One cow had to be replaced after two squares as intake was affected by sampling. Thus, four lactating Danish Holstein cows (weighing 655 ± 57 kg, 209 ± 98 days in milk, and yielding 22 ± 10 kg milk/d at shift between 2^{nd} and 3^{rd} square) fitted with ruminal, duodenal, and ileal cannulas were used.

Experimental concentrates were allocated separately with 3 kg as is at the 8.00 h morning milking. To ease palatability of experimental concentrates, 2 kg as is of a low starch pelletized concentrate was manually mixed with experimental concentrates at feeding. Residual concentrate was removed and weighed 45 min after allocation. The afternoon milking was at 15.30 h where 3 kg as is of the low starch concentrate was allocated. Half an hour after each milking, at 9.00 and 16.00 h, a partial mixed ration (PMR) including an external marker (TiO_2) was allocated at 90-95% of pre-experimental ad libitum DMI.

Samples were obtained at the last day of each period. Eleven sample sets of rumen ventral fluid, rumen medial digesta and fluid, and duodenal digesta was obtained at -0.5, 0.5, 1.5, 2.5, 4.0, 5.5, 7.0, 8.0, 9.0, 10.0, and 11.5 h relative to feeding the experimental concentrates at 8.00 h. Rumen ventral fluid was sampled by suction strainer. Rumen medial content was sampled by grab sampling two hand-full of the particulate matter 10 cm under the surface area in the mid-section. Fluid was obtained from one hand-full by squeezing through one layer of cheesecloth and the other hand-full was dried at 60°C for chemical analysis. Duodenal digesta samples (200 mL) were immediately transferred to plastic beakers and dried at 60°C for chemical analysis.

Calculations and statistical analysis

Rumen degradation parameters of starch, crude protein, and NDF were estimated using the NLIN procedure of SAS. The following model was used: PD(t) = a + b [1 – exp (- ct)] where, PD is degraded proportion at time t, a is the fraction that immediately disappeared from bags, b is the fraction that is potentially degradable over time, c is the fractional rate of degradation (h^{-1}), and t is incubation time (h). Effective degradability values (ED) of starch, protein, and NDF in the rumen were estimated as ED = a + [(b × c) / (c + k)], where a, b, and c are the degradation constants estimated as described and k is the fractional rate of passage assumed to be 0.05 h^{-1}. The same degradation model and fractional rate of passage was used for all nutrients to facilitate comparisons.

Duodenal DM flow was calculated using TiO_2 as indigestible marker assuming hourly rumen TiO_2 outflow as daily intake divided by 24 h. Daily duodenal starch flow was estimated as area under the curve and correcting for basal starch flow using the flow observed at -0.5 h relative to feeding. Rumen escape starch was calculated from daily duodenal starch flow estimated in the 12-h sampling window. Flow measurement were used as the midpoint flow between two sampling times, and in the 12-h post sampling window using the average between 11.5- and 23.5-h flow as the midpoint flow where the 23.5-h flow is assumed equal to the -0.5-h flow.

Data was subjected to ANOVA by concentrate using the MIXED procedure of SAS. Rumen degradation kinetic parameters from the *in situ* experiment were analysed using a model with processing (Meal, Pelleting, Extruding HD, and Extruding LD) and period (1, 2) as fixed effects, and animal as random factor. Serial variables from the *in vivo* experiment were analysed with a model having period, treatment (Trt), time relative to feeding (Time) and Trt × Time as fixed effects, and cow as random factor. Time within cow × period was considered a repeated measurement using the spatial power covariance structure. The following predefined contrasts were tested: Pelleting versus Extrusion, and Extrusion HD versus Extrusion LD. Significance was claimed when $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

Results and discussion

The chemical composition of experimental concentrates is in Table 1, which reflects ingredient composition. Obtained bulk densities for pure cereals and cereals+SBM varied between 756 and 797 g/L for conventional pellets, between 633 and 701 g/L for extrusion HD, and between 428 and 516 g/L for extrusion LD pellets. Bulk densities for cereals+SBP varied more due to difficulties in controlling the extrusion process, in particular for the extrusion HD pellets. The WSI (g DM in intact pellets/kg DM) of experimental concentrate pellets ranged from 15.6 to 980 for conventional pellets, from 223 to 884 for extrusion HD pellets, and from 664 to 855 g/L for extrusion LD pellets.

In situ experiment (Razzaghi et al., 2016)

The untreated wheat and maize meals used in the current study differed in their rate and extent of ruminal starch degradation with wheat being degraded to a greater extent than maize (Figure 1a and 1b). Thus, effect of processing on starch degradation was more pronounced in maize than in wheat. Pelleting increased effective starch degradability (ESD; $P < 0.01$) and extrusion further increased the ESD in maize ($P < 0.01$) with the most intensive extrusion i.e. steam addition (extrusion LD) giving the highest ESD. Overall, ESD in maize and maize

mixtures was increased with increasing intensity of processing (extruding LD > extruding HD > pelleting > meal). This finding was in line with the observation by Offner et al. (2003).

Table 1 Chemical composition, bulk density, and water stability of pelletized compound concentrates by either conventional pelleting or by cooking extrusion giving pellets with high density (HD) or low density (LD)

			Composition, g/kg DM			Density	Water stability[1]
Concentrate	Processing	DM, g/kg	Crude protein	Starch	NDF	g/L	g intact/kg DM
100% wheat							
	Meal	889	126	667	100	712	-
	Pelleting	883	125	673	93	760	818
	Extrusion HD	915	130	681	98	683	884
	Extrusion LD	954	129	677	97	458	842
50% wheat + 50% soybean meal							
	Meal	896	329	342	99	736	-
	Pelleting	884	318	365	85	756	980
	Extrusion HD	924	329	344	95	701	757
	Extrusion LD	949	332	337	97	516	771
100% maize							
	Meal	895	90	713	93	639	-
	Pelleting	901	90	726	75	797	38.5
	Extrusion HD	921	95	728	79	633	745
	Extrusion LD	947	92	715	78	428	855
50% maize + 50% soybean meal							
	Meal	892	307	362	86	716	-
	Pelleting	884	312	363	80	763	467
	Extrusion HD	929	308	354	88	691	584
	Extrusion LD	949	313	340	86	505	757
50% wheat + 50% sugar beet pulp							
	Meal	901	107	361	258	719	-
	Pelleting	878	108	349	262	800	103
	Extrusion HD	929	111	337	254	565	664
	Extrusion LD	938	113	327	262	467	753
50% maize + 50% sugar beet pulp							
	Meal	902	93	348	258	712	-
	Pelleting	886	99	362	256	761	15.6
	Extrusion HD	958	97	359	257	501	223
	Extrusion LD	971	97	364	257	452	455

[1]g DM in intact pellets after soaking in net baskets for 120 min in 25°C water bath (Baeverfjord et al., 2006).

Pelleting tended to decrease effective protein degradability (EPD) for maize+SBM ($P = 0.06$; Figure 1c and 1d), but did not affect EPD for wheat+SBM ($P = 0.43$). For wheat+SBM, extruding decreased EPD ($P = 0.02$) as compared to meal, and EPD tended to be further decreased by extruding LD ($P = 0.09$). Heat treatment by extrusion with steam addition reduced EPD considerably in both cereal–protein mixtures indicating that the high protein content of SBM masked the effect of extrusion on maize protein. The general decreasing effect on EPD of heat treatment was mainly caused by decreased rate of degradation as it decreased with increasing intensity of processing for both cereal-protein mixes.

In concentrates with high NDF content, wheat+SBP and maize+SBP, effective rumen degradability of NDF was not affected by conventional pelleting ($P = 0.77$; Figure 1e and 1f), but extrusion tended to increase effective rumen degradability of NDF ($P \leq 0.11$) as compared to pelleting. Level of effective rumen degradability of NDF was similar in both untreated cereal-fibre meals, likely reflecting that SBP is the dominating NDF source in these mixes. Processing did not appear to affect effective degradability of NDF in concentrates

with low NDF content (<10% of DM); however, extrusion without steam addition increased the effective NDF degradability in the maize-SBP mixture.

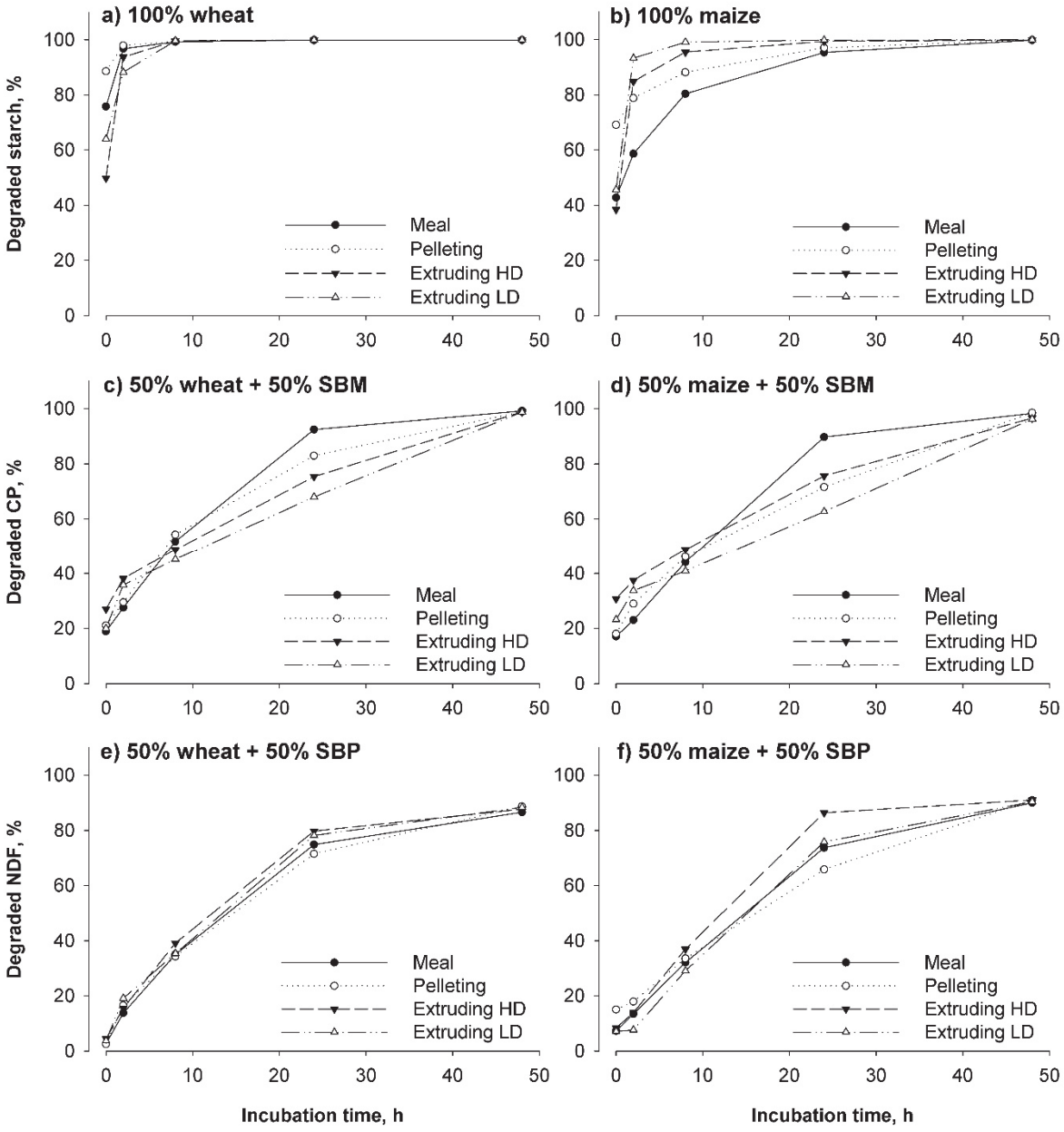

Figure 1 *In situ* degradation profiles of starch (a and b), protein (c and d), and NDF (e and f) in compound concentrates as meal (●), conventional pellets (○), and as pellets produced by extruding yielding either high bulk density (HD; ▼) or low bulk density (LD; Δ) pellets (Razzaghi et al., 2016).

In vivo experiment (Larsen et al., 2018)

Cereal+SBP mixtures were not investigated in the *in vivo* experiment due to problems of producing pellets by cooking extrusion for fibre rich mixtures as mentioned previously. Palatability of experimental pelleted concentrates produced by cooking extrusion was observed to be low during pre-trial testing; hence, an amount of low starch pelletized concentrate was mixed with all experimental concentrates. It was observed that extrusion LD

pellets of the 100% grains were particularly difficult to eat as the pellets became sticky in contact with saliva and water. Yet, cows consumed nearly all of the experimental concentrates within 20 min after allocation.

For intra-ruminal mixing of concentrate pellets, the underlying hypothesis was that concentrate pellets with high stability in liquid combined with low density, e.g. floating in water, would have a greater likelihood of being captured by the rumen dorsal particulate matter and thus allowing a better mixing avoiding local acidic conditions in the ventral rumen. Thus, when feeding a basal diet low in starch, it was expected that starch concentration in medial contents would be greater and that fermentation variables in medial and ventral contents would reflect a shift in starch fermentation from ventral to medial rumen to occur with cooking extrusion LD pellets. However, no clear effects on starch concentration or pH in rumen medial contents was observed for either of the two concentrate pellets produced by cooking extrusion (Table 2). Looking at the postprandial starch concentration in the rumen medial content, relative large variations in starch concentration was observed during the first ~4 hours after feeding. This indicates substantial animal-to-animal variation in how fast rumen motility moves ingested concentrate pellets caudally from the oesophageal region into the ruminal cavity.

For ruminal escape of concentrate pellets, the underlying hypothesis was that concentrate pellets with high stability in liquid combined with high density, e.g. sinking in water, would have a greater likelihood of passing out of the rumen and, hence, increase the proportion of rumen escape starch. Hence, with the basal diet low in starch, cooking extrusion HD pellets were expected to have the highest ruminal outflow of starch. However, with the exception of 100% maize compound, conventional pellets had the largest ruminal outflow of starch compared with the other compounds (Table 2). This was quite intriguing as conventional pellets normally disintegrate quickly in liquid (Larsen and Raun, 2018); hence, a low chance for rumen escape of feed particles would be expected. However, the current conventional pellets having a high proportion of rumen escape starch were very stable in water as evidenced by the WSI (Table 1). In comparison, WSI of 24 commercial pelletized concentrates ground to pass a 3.5-mm screen for dairy cows ranged from 21 to 198 g intact/kg DM (Larsen and Raun, 2018). It can be speculated that the current combination of fine grinding, e.g. using a 2-mm screen and steam heating during pelletizing would guarantee firm and stable pellets. A further indication of this was that the 100% maize compound, which had a low WSI and did not have higher rumen escape starch, was, in fact, not pelletized at more than ~55°C due to problems with expansion of the starch in the dye causing blockages.

Table 2 Effect of pelletizing compound concentrates by either conventional pelleting (Pe) or by cooking extrusion (Ce) giving pellets with high density (HD) or low density (LD) on 12 h postprandial patterns of ruminal concentration of starch, VFA and pH, and duodenal starch, and crude protein (CP) flow in dairy cows

		Cooking extrusion			*P* values				
Item	Pelleting	HD	LD	SEM[1]	Trt	Time	Trt × Time	Pe vs Ce	HD vs LD
100% wheat									
Rumen contents									
Medial starch, g/kg DM	33	40	45	7.2	0.29[T]	<0.01[T]	0.89[T]	0.20[T]	0.37[T]
Medial pH	5.84	5.79	5.68	0.11	0.16	<0.01	0.97	0.16	0.18
Ventral pH	6.23	6.29	6.20	0.095	0.66	<0.01	0.91	0.88	0.38
Duodenal starch, flow g/h	45	33	37	4.1	0.37[T]	<0.01[T]	0.09[T]	0.22[T]	0.54[T]
Rumen escape starch, g/g	0.36	0.27	0.28	0.025	0.19	-	-	0.10	0.89
50% wheat + 50% soybean meal									
Rumen contents									
Medial starch, g/kg DM	30	32	35	4.1	0.45	<0.01	0.04	0.34	0.41
Medial pH	5.79	5.78	5.94	0.14	0.02	<0.01	0.28	0.16	0.01
Ventral pH	6.26	6.30	6.39	0.15	0.36	<0.01	0.85	0.31	0.32
Duodenal starch, flow g/h	24.6	20.0	18.2	1.0	0.01	<0.01	0.05	<0.01	0.30
Rumen escape starch, g/g	0.35	0.29	0.26	0.013	0.07	-	-	0.04	0.22
100% maize									
Rumen contents									
Medial starch, g/kg DM	46	40	38	3.2	0.39[T]	<0.01[T]	>0.99[T]	0.18[T]	0.79[T]
Medial pH	5.71	5.60	5.76	0.060	0.19	<0.01	0.73	0.73	0.08
Ventral pH	6.29	6.14	6.24	0.050	0.13	<0.01	0.25	0.13	0.18
Duodenal starch, flow g/h	37	27	41	9.4	0.21[T]	<0.01[T]	0.44[T]	0.28[T]	0.11[T]
Rumen escape starch, g/g	0.29	0.21	0.29	0.020	0.15	-	-	0.21	0.11
50% maize + 50% soybean meal									
Rumen contents									
Medial starch, g/kg DM	34	29	33	2.5	0.30[T]	<0.01[T]	0.70[T]	0.50[T]	0.13[T]
Medial pH	5.91	5.92	5.84	0.098	0.16	<0.01	0.04	0.39	0.08
Ventral pH	6.50	6.32	6.33	0.075	<0.01	<0.01	0.62	<0.01	0.78
Duodenal starch, flow g/h	27	19	19	3.8	0.02[T]	<0.01[T]	0.69[T]	0.01[T]	0.51[T]
Rumen escape starch, g/g	0.42	0.26	0.29	0.014	0.02	-	-	0.01	0.22

[T]*P* values are for the $\log_{10}$-transformed variable.

Conclusions

The effect on starch and protein degradability were highly dependent on conditions applied during processing (temperature and moisture) where extruding at higher temperature increased starch degradation for most concentrates and efficiently reduced ruminal degradation of protein, especially in protein mixtures. The study indicated that concentrate pellets with very differing physical functional properties had limited effects on intra-ruminal mixing with the particulate mat. Yet, rumen escape of conventional pellets might be of a substantial magnitude if pellets with both high density and stability in liquid could be produced. Indeed, further investigations are needed.

Acknowledgements

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References

Åkerlind, M., Weisbjerg M.R., Eriksson, T., Thøgersen, R., Uden, P., Olafsson, B.L., Harstad, O.M. & Volden, H., 2011. Feed analysis and digestion methods. In: Norfor the Nordic feed evaluation system. EAAP publication (ed. Volden H.) no. 130, p. 41-54. WageningenAcademic Publishers, Wageningen, the Netherlands.

Baeverfjord, G., Refstie, S., Krogedal & P., Åsgård, T., 2006. Low feed pellet water stability and fluctuating water salinity cause separation and accumulation of dietary oil in the stomach of rainbow trout (Oncorhynchus mykiss). Aquacult. 261, 1335-1345.

Ingvartsen, K.L., Aaes, O. & Andersen, J.B., 2001. Effects of pattern of concentrate allocation in the dry period and early lactation on feed intake and lactational performance in dairy cows. Livest. Prod. Sci. 71, 207-221.

Khafipour, E., Krause, D.O. & Plaizier, J.C., 2009a. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. J. Dairy Sci. 92, 1712-1724.

Khafipour, E., Krause, D.O. & Plaizier, J.C., 2009b. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92, 1060-1070.

Kraugerud, O.F., Jørgensen, H.Y. & Svihus, B., 2011. Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. Anim. Feed Sci. Technol. 163, 244-254.

Larsen, M. & Raun, B.M.L., 2018. Effect of compound composition on water stability of pellets. In: Udén, P., Spörndly, R. (Eds.), Proc. 9th Nordic Feed Sci. Conf. Swedish University of Agricultual Sciences, Uppsala, Sweden (in press).

Larsen, M., Lund, P., Storm, A.C. & Weisbjerg, M.R., 2018. Effect of conventional and extrusion pelleting of compound concentrates on intra-ruminal mixing of pellets in dairy cows. Livest. Sci. (submitted).

Maekawa, M., Beauchemin, K.A. & Christensen, D.A., 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85, 1165-1175.

Offner, A., Bach, A. & Sauvant, D., 2003. Quantitative review of in situ starch degradation in the rumen. Anim. Feed Sci. Technol. 106, 81-93.

Razzaghi, A., Larsen, M., Lund, P. & Weisbjerg, M.R., 2016. Effect of conventional and extrusion pelleting on in situ ruminal degradability of starch, protein, and fibre in cattle. Livest. Sci. 185, 97-105.

Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquac. Nutr. 18, 233-248.

Can we increase digestibility of green forages by physical treatment before ensiling?

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Introduction

Milk production in Denmark is based on forages supplemented with concentrates. High digestible forages are required to combine healthy cows with high feed intake and high milk production (Alstrup et al., 2014; Alstrup et al., 2016; Weisbjerg et al., 2017). In ruminants, non-fibre components are close to 100% digestible (Weisbjerg et al., 2004), it is therefore quantity and quality of the fibre fraction which determines overall (organic matter) digestibility of forages (Huhtanen et al., 2006). For green forages, increased growth period (maturity) results in an increased fibre concentration and reduced fibre digestibility mainly due to increased lignification. High digestible forages can be obtained by short growth and regrowth periods. However, short growth periods might hamper dry matter production and an increased number of harvests can seriously increase harvest costs and damage soil fertility due to compaction. Forage productivity in Denmark is expected to be stimulated by the climate change due to a longer growing season, while a higher temperature might have a negative effect on fibre digestibility in green forages.

Physical treatment of forages (maceration, shredding, hereafter referred to as shredding) has been shown to increase fibre digestibility in some situations, but the effect is probably both dependent on efficiency of the macerator (Hintz et al., 1999) and on type and maturity of the forage. Until now, physical treatment has mainly been studied in the US, and these studies do not seem to have had been widely implemented, probably due to type of machine, power requirements and risk of leaf losses.

It is therefore expected, that physical treatment of green forages could increase forage fibre digestibility, and, if done before ensiling, silage compaction and quality might be enhanced (Lehmann et al., 2017).

This paper report some early results from an ongoing project with physical treatment of green forages before ensiling including a digestibility experiment with fistulated cows. The aim of the experiment was to examine the effect of physical treatment of grass-clover silage before ensiling on in vivo digestibility in dairy cows.

Materials and methods

Four rumen, duodenal and ileal fistulated Holstein cows in late lactation (300 – 640 days in milk) were used in a crossover experiment with two periods of four-week length each. Cows were fed one of two pure silage diets as treatments, with two cows per treatment per period resulting in a total of four observations per treatment. Milk yield varied from 13 to 26 kg, and live weight from 612 to 831 kg in the experiment.

The two treatments were silages made from a developed autumn grass-clover ley. The cutting date was 30 September, and botanical composition was 25% perennial ryegrass, 47% red clover, and 28% white clover on dry matter (DM) basis. The grass-clover was ensiled in round bales after wilting. Half of the bales were wrapped for ensiling, the other half were transported to a concrete area, de-baled and treated in an experimental shredder where the crop was shredded between a rotating drum and a curved shell around the drum, thus creating

a treatment channel. The drum and shell were fitted with steel ridges, creating a proper ratio between cutting and rubbing treatment. The drum rotated at a fixed speed to allow material to be fed through the treatment channel. This created a treatment, whereby the fibre was shredded without being chopped. The grass was fed into the treatment channel at a continuous flow. The shredded material was then baled and plastic wrapped as the original silage. Time span from de-baling until re-baled after shredding was less than one hour. In total 7120 kg (9 bales @ 791 kg) of untreated and 5780 kg (6 bales á 963 kg) of shredded silage were produced.

The cows were housed in a tie stall on rubber mats with sawdust as bedding and were milked twice daily at 05:30 and 16:30 h. Experimental silages were offered ad libitum twice daily, 06:00 and 17:00 h. Amount of silage offered and leftovers were recorded daily. No concentrates were offered, but 100 g of a granulated mineral-vitamin mixture was offered daily and 20 g of vitamins were offered at the morning feeding twice a week. The cows had free access to water from individual drinking bowls fitted with water gauges. Flow at duodenum, ileum and faecal output was measured using markers, where 10 g chromium(III) oxide (Cr_2O_3) and 13 g titanium(IV) oxide (TiO_2) were placed in the rumen via the cannula twice daily in connection with daily milkings. Markers were supplied throughout the experiment (Day 1-19) except for respiration chamber days.

Twelve subsamples of both duodenal and ileal chyme and faeces were taken over a 94-h period on day 15–19 in each period to cover the diurnal variation (6-8 h between samples, resulting in one sample every second h of the 24-h day). Faecal samples were collected when cows defecated or by grab sampling. Each sample type was pooled within cow and period and freeze dried before analysis. Rumen fluid from the ventral part was collected through the rumen cannula using a plastic syringe and a suction strainer. Rumen fluid pH was measured immediately with a pH meter and subsamples were frozen until analysis.

Total rumen evacuations were performed at 12.00 h on the last day (day 28) in each period. Rumen content was divided in mat and free fluid fractions using a sieve basket and weight of each fraction was recorded before subsampling and compositing of pooled sample by weight, for dry matter (DM) determination (60^{o}C). Rumen samples were freeze dried for further analysis. Faecal consistency was scored using a 5-point (1–5; 1 is loose and 5 is firm) visual observation score (Johansen et al., 2017a).

Chewing time was measured twice during 24 h in the second week of each period using the IGER system. Data were analysed in the PC program “Graze” (Ultra Sound Advice) by designating jaw movements as eating or ruminating chews (Rutter, 2000).

Methane production was measured using individual open-circuit respiration chambers on four continuative days in the fourth week (day 24-28).

For more detailed description of our experimental praxis in experiments with fistulated cows see Johansen et al. (2017b) and for description of respiration chambers see Helwing et al. (2012).

Samples for chemical analyses were ground to pass a 1-mm screen (ZM 200 mill, Retsch GmbH, Haan, Germany). Ash concentration was determined in all samples by combustion at 525°C for 6 h. Nitrogen was determined using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany) following the Dumas method (Hansen, 1989) and multiplied by 6.25 to determine CP. The neutral detergent fibre (aNDFom) concentration was analysed

according to Mertens (2002) in a Fibertec M6 System (Foss Analytical, Hillerød, Denmark) including sodium sulphite and heat stable amylase treatments and corrected for ash content. Titanium oxide was measured by digestion of samples with sulfuric acid and measuring of absorbance after addition of hydrogen peroxide (Myers et al., 2004). Chromium oxide was determined by spectrophotometry after oxidation with sodium peroxide to chromate (Schürch et al., 1950).

In vitro digestibility was according to the Tilley and Terry (1963) rumen fluid procedure. Indigestible neutral detergent fibre (NDF) (iNDF) was estimated as NDF residue in ANKOM F57 bags after a 12-d rumen incubation. Silages extracts were prepared by blending 100 g of silage with 1 L of water, followed by centrifugation (2300 × *g*, 20 min, 10°C).

In silage extracts and rumen fluid, volatile fatty acids were analysed by gas chromatography as described by Kristensen et al. (1996). Ammonia N (NH_3-N) was determined using a Cobas Mira auto-analyser (Triolab A/S, Brøndby, Denmark) and a kit based on glutamate dehydrogenase (AM 1015; Randox Laboratories Ltd, Crumlin, UK). Glucose and L-lactate were determined with membrane-immobilised substrate specific oxidases using an YSI 2900 Biochemistry Analyser (YSI Inc., Yellow Springs, OH, USA). Alcohols and alcohol esters in silage extracts were determined by headspace GC–MS (Kristensen et al., 2010). Free amino acids were analysed by gas chromatography–mass spectrometry following ethyl chloroformate derivatization according to Kristensen et al. (2010).

Energy corrected milk (3.14 MJ/kg) was calculated according to Sjaunja et al. (1991) using the formula ECM = 0.01 × milk yield (kg) + 12.2 × milk fat (kg) + 7.7 × milk protein (kg) + 5.3 × milk lactose (kg), where lactose was measured as lactose monohydrate.

Digesta flow was calculated from marker concentrations in duodenal and ileal digesta and in faeces, assuming quantitative passage and excretion of added marker. Flow of DM used was average of DM flow estimated for each of the two markers. Digestibility in rumen was calculated as feed-duodenal disappearance over intake, in small intestine as duodenal-ileal disappearance over duodenal flow, hind gut digestibility as ileal-faecal disappearance over ileal flow, and total digestibility as feed-faecal disappearance over intake. Rate of passage was estimated as faecal iNDF output ((kg/day)/(24hour/day)) over rumen iNDF pool (kg). Rate of digestion was estimated as digested aNDFom (feed – faeces) ((kg/day)/(24hour/day)) over rumen digestible aNDFom (dNDF) pool (kg).

Statistical calculations were performed using Proc GLM (Proc MIXED for chewing time data to account for repeated measures) in SAS with diet, period and cow as fixed effects. Statistical significance was declared for $P<0.05$, and tendency for $0.05<P<0.10$.

Results and discussion

Silages were similar in chemical composition as planned (Table 1). The rather low aNDFom and high CP concentrations reflected the high clover proportion. The crop was well developed at harvest, but digestibility (*in vitro* and *in vivo*) were still above average literature values for digestibility found by Johansen et al. (2018), although they were in the lower range of silages used in Denmark. Silage extracts contents of free amino acids, ammonium, short chain fatty acids, alcohols and esters were similar for the two silages (results not shown). Lactate concentration in % of DM was 6.4 in untreated and 5.4 in shredded silage, and NH3-N in % of total N was 8.5 in untreated and 8.3 in shredded silage. pH was 4.8 in untreated and 4.6 in shredded silage, reflecting the high DM concentration in the silages.

Milk yield (kg/d) and energy corrected milk yield, respectively, were 16.0 and 16.5 for untreated and 16.8 and 17.1 for shredded silage treatment, respectively, but did not differ statistically (P=0.6; P=0.7, respectively). Also milk fat and protein concentrations and yields were not affected by treatment (results not shown). However, cows were selected to be in late lactation to allow for feeding grass-clover silage as the sole feed, and therefore major responses in milk yield were not expected.

Feed intake (Table 2) was not (P=0.2) higher on shredded silage compared to untreated. Rumen digestibilities (in vivo) of DM, organic matter (OM), aNDFom and CP either were higher or tended to be higher (DM: P=0.07; OM: P=0.05; aNDFom: P=0.09; CP: P=0.06) for shredded compared to untreated silage.

Table 1 Composition of experimental silages

	Untreated	Shredded
Dry matter (%)	45.1	46.3
Ash (% DM)	12.1	11.6
NDF (% DM)	29.9	30.7
Crude protein (% DM)	23.2	23.5
Crude fat (% DM)	3.23	3.21
In vitro organic matter digestibility (% OM)	73.2	73.5

Total tract digestibilities (*in vivo*) were not higher for shredded than for untreated, but there was a tendency for a higher digestibility of aNDFom for shredded silage. Small intestinal and hind gut digestibilities were generally not affected by physical treatment before ensiling. Faecal score was also not affected by treatment, and was 3.0 for untreated and 3.25 for shredded silage treatment.

Rumen evacuation data showed a high total rumen load of nearly 100 kg (Table 3), but was not affected by silage treatment. The highest individual value recorded was 134 kg (data not shown). Rumen aNDFom pool was approximately 5.8 kg, and consisted of about half dNDF and half iNDF. Rate of passage of iNDF was less than 1% per h, and rate of dNDF digestion was 5.1 % per h for shredded silage, 0.5%/h higher than for untreated, but did not differ between treatments.

Rumen fluid pH and rumen short chain fatty acid concentrations were not affected by treatment (Table 4), but a tendency for higher L-lactate concentration was found for shredded silage. Rumen ammonia concentration was lower for shredded silage.

Chewing time measures showed lower eating time daily and per kg of DM intake (DMI) for shredded silage (Table 5), whereas rumination time only tended to decrease per kg DMI per day.

Table 2 Feed intake and *in vivo* digestibility of silages

	Untreated	Shredded	SEM	P
Dry matter intake kg/day	14.3	15.2	0.6	0.2
Digestibilities (%)				
Dry matter				
Rumen	31.1	35.1	1.6	0.07
Small intestine	48.3	46.0	1.7	0.2
Hind gut	14.6	12.2	2.8	0.4
Total	70.5	71.3	1.3	0.5
Organic matter				
Rumen	43.1	46.6	1.1	0.05
Small intestine	48.7	48.1	1.0	0.5
Hind gut	8.3	6.4	2.0	0.3
Total	73.3	74.1	1.0	0.4
aNDFom				
Rumen	73.8	76.0	1.0	0.09
Small intestine	-3.6	-3.0	3.0	0.8
Hind gut	11.0	7.2	5.1	0.4
Total	76.1	77.2	0.6	0.1
Crude protein				
Rumen	11.1	14.1	1.1	0.06
Small intestine	62.9	63.2	1.9	0.8
Hind gut	11.7	9.6	5.4	0.6
Total	71.0	71.7	1.3	0.5

Table 3 Composition of rumen content, pool sizes and calculated digestion and passage rates

	Untreated	Shredded	SEM	P
Total content (kg)	99.7	98.0	5.9	0.7
Free fluid (% of total)	24.2	20.5	5.2	0.4
Dry matter (%)	13.1	13.9	0.7	0.2
Dry matter (kg)	13.1	13.7	0.4	0.2
Composition (% of DM)				
Ash	13.3	13.6	1.0	0.7
Organic matter	86.7	86.4	1.0	0.7
NDF	43.7	42.4	1.0	0.2
Crude protein	24.3	25.4	1.4	0.4
Rumen pools (kg)				
Ash	1.71	1.85	0.13	0.3
Organic matter	11.4	11.9	0.42	0.2
Crude protein	3.18	3.48	0.28	0.3
NDF	5.76	5.84	0.13	0.5
iNDF[1]	2.96	2.99	0.42	0.9
dNDF[2]	2.80	2.85	0.42	0.9
Rates (%/h)				
Digestion, dNDF	4.60	5.11	0.7	0.4
Passage, iNDF	0.798	0.878	0.2	0.6

[1]Indigestible NDF found as residue after 12 d rumen *in situ* incubation; [2]Digestible NDF found as total aNDFom – iNDF.

Table 4 Concentration of short chain fatty acids (SCFA), ammonia and pH in rumen fluid

	Untreated	Shredded	SEM	P
pH	6.70	6.71	0.23	0.8
Ammonia (mmol/l)	13.8	12.1	0.5	0.04
Total SCFA[1] (mmol/l)	104.4	98.6	8.7	0.4
Mole/100 mole				
L-lactate	0.118	0.296	0.09	0.1
Acetate	65.0	64.4	0.4	0.2
Propionate	19.8	20.5	0.8	0.4
C2/C3[2]	3.28	3.14	0.13	0.3

[1]Total SCFA is the sum of L-lactate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, caproate; [2]Ratio between acetate and propionate

Table 5 Time used for eating, rumination, and total chewing time (min/d)

	Untreated	Shredded	SEM	P
Total chewing time	870	829	18	0.12
Rumination	423	430	9	0.6
Eating	445	398	17	0.06
Minutes/kg DM intake				
Total chewing time	59.4	54.5	0.2	0.02
Rumination	28.8	27.9	0.1	0.09
Eating	30.6	26.6	0.1	0.02

Gas measures showed no effects of treatments on any gas exchange (Table 6), except a lower H_2S production for the shredded silage. Methane production per kg feed DM was 0.5 l higher for shredded than for untreated, but far from significant.

Table 6 Use of oxygen and production of methane, CO_2, H_2 and H_2S measured in respiration chambers

	Untreated	Shredded	SEM	P
O_2 (l/d)	5332	5374	185	0.8
CO_2 (l/d)	5415	5509	201	0.6
Methane (l/d)	393	393	6	1
H_2 (l/d)	3.80	4.53	1.83	0.6
H_2S (l/d)	0.128	0.102	0.0038	0.01
CH_4 (l/kg DM)	25.1	25.6	0.7	0.4
CH_4/CO_2	0.0723	0.0712	0.0016	0.4

Physical treatment of the grass-clover in the present experiment increased rumen digestibility and reduced eating time. The higher digestibility was obtained despite a somewhat higher DM intake for shredded silage. Higher feed intake would normally result in reduced digestibility, therefore, the higher digestibility obtained for shredded silage is even more convincing. Rumen pool sizes were not affected by treatment, but rates of iNDF passage and dNDF digestion indicated, although treatment effects were not significant, that the increased digestibility was due to increased rate of digestion and not decreased rate of passage. Improved fiber digestibility due to maceration of lucerne has been found in several in vivo, in situ and in vitro studies (Hong et al., 1988a,b; Broderick et al., 1999). For ryegrass, the only study found (Broderick et al., 2002) revealed decreased fibre digestibility due to maceration, possible because maceration increased field losses of leaves as indicated by increased NDF and decreased protein concentration in the macerated silage compared to untreated.

Generally, in the literature, largest effects have been found for lucerne stems and more mature lucerne, whereas for immature lucerne and ryegrass less effect have been seen. This could be caused by machinery built to fit lucerne stems, which do not efficiently treat

immature lucerne and ryegrass or losses of leaves (Broderick et al., 2002), or it could indicate that effects can only be expected on more mature forages. The present study shows that some positive effects also can be found for a normal to a relatively mature clover-rich grass-clover mixture.

Conclusions

Shredding of grass-clover before ensiling decreased eating time and increased rumen digestibility.

References

Alstrup, L., Weisbjerg, M.R., Hymøller, L., Larsen, M., Lund, P. & Nielsen, M., 2014. Milk production response to varying protein supply is independent of forage digestibility in dairy cows. J. Dairy Sci. 97, 4412-4422.

Alstrup, L., Søegaard, K. & Weisbjerg, M.R., 2016. Effect of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. J. Dairy Sci. 99, 328-340.

Broderick, G.A., Koegel, R.G., Mauries, M.J.C., Schneeberger, E. & Kraus, J., 1999. Effect of feeding macerated alfalfa silage on nutrient digestibility and milk yield in lactating dairy cows. J. Dairy Sci. 82, 2472-2485.

Broderick, G.A., Koegel, R.G., Walgenbach, R.P. & Kraus, T.J., 2002. Ryegrass or alfalfa silage as the dietary forage for lactating cows. J. Dairy Sci. 85, 1894-1901.

Hansen, B., 1989. Determination of nitrogen as elementary-N, an alternative to Kjeldahl. Acta Agric. Scand. 39, 113-118.

Hellwing A.L.F., Lund, P., Weisbjerg M.R., Brask, M. & Hvelplund, T., 2012. Test of a low-cost and animal-friendly system for measuring methane emissions from dairy cows. J. Dairy Sci. 95, 6077-6085.

Hintz, R.W., Koegel, R.G., Kraus, T.J. & Mertens, D.R., 1999. mechanical maceration of alfalfa. J. Anim. Sci. 77, 187-193.

Hong, B.J., Broderick, G.A., Koegel, R.G., Shinners, K.J. & Straub, R.J., 1988a. Effect of shredding alfalfa on cellulolytic activity, digestibility, rate of passage, and milk production. J. Dairy Sci. 71, 1546-1555.

Hong, B.J., Broderick, G.A., Panciera, M.P., Koegel, R.G. & Shinners, K.J., 1988b. Effect of shredding alfalfa stems on fiber digestion determined by in vitro procedures and scanning electron microscopy. J. Dairy Sci. 71, 1536-1545.

Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R. & Nørgaard, P. 2006. Digestion and passage of fibre in ruminants. In: Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, 2006, p. 87-135.

Johansen, M., Hellwing, A.L.F., Lund, P. & Weisbjerg M.R., 2017b. Metabolisable protein concentration increased with increasing dry matter concentration in grass-clover silage measured in fistulated dairy cows. Anim. Feed Sci. Technol. 227, 95–106.

Johansen, M., Lund, P. & Weisbjerg M.R., 2018. Feed intake and milk production in dairy cows fed different grass and legume species: a meta-analysis. Animal 12, 66-78.

Johansen, M., Søegaard, K. Lund, P. & Weisbjerg, M.R., 2017a. Digestibility and clover proportion determine milk production when silages of different grass and clover species are fed to dairy cows. J. Dairy Sci. 100, 8861-8880.

Kristensen, N.B., Danfær, A., Tetens, V. & Agergaard, N., 1996. Portal recovery of intraruminally infused short-chain fatty acids in sheep. Acta Agric. Scand. Sect. A. Anim. Sci. 46, 26–38.

Kristensen, N.B., Sloth, K.H., Højberg, O., Spliid, N.H. & Jensen, C., Thøgersen, R., 2010. Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. J. Dairy Sci. 93, 3764–3774.

Lehmann, J.O., Kristensen, T. & Weisbjerg, M.R., 2017. Review: Mechanical treatment of grasses and clovers to produce high-quality silage for dairy cows. http://pure.au.dk/portal/files/117877185/BEGROME_litteratur_review_final.pdf. Intern rapport, 20 pp.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217-1240.

Myers, W.D., Ludden, P.A., Nayigihugu, V. & Hess, B.W., 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82, 179-183.

Rutter, S.M. 2000. Graze: A program to analyze recordings of the jaw movements of ruminants. Behav. Res. Methods Instrum. Comput. 32, 86–92.

Schürch, A.F., Lloyd, L.E. & Crampton, E.W., 1950. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41, 629-636.

Sjaunja, L. O., Baevre, L. Junkkarinen, L. Pedersen, J. & Setälä. J., 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Performance Recording of Animals—State of the Art 1990. EAAP Publ. 50, 156–157.

Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18, 104–111.

Weisbjerg, M.R. Hvelplund T. & Søegaard, K., 2004. Prediction of digestibility of Neutral Detergent Solubles using the Lucas principle. J. Anim. Feed Sci., 13, suppl. 1, 239-242.

Weisbjerg, M.R. & Johansen, M., 2017. A note on the response in feed intake and milk yield on increased forage organic matter digestibility. NFSC, Nordic feed Science Conference, Proc. 8th Nordic Feed Sci. Conf., Uppsala, Sweden, 13-14 June 2017. Report 296, SLU, p. 77-80.

Contribution of forages for the nutrient use efficiency in ruminant nutrition

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Introduction

Proper feeding of ruminants is a challenging issue. Despite a high nutrient and energy availability in concentrate feeds, most ruminant diets of high yielding animals are based on forages. Forage nutrient and energy content varies also among forage plant species, between cutting frequencies, among cutting dates (season), and forage conservation practices. Next to obtaining highest forage nutritive value and resulting optimal animal performance, increasing issues in ruminant feeding are efficiency of nutrient and energy utilization, environmental friendly production of foods of animal origin, and expectation of consumers to obtain animal products from systems taking into consideration animal welfare issues.

The main objective herein was to integrate knowledge about evaluation of nutritive value (mainly protein) with that of grassland science in order to optimize feeding of high yielding dairy cows. In this way, it should be possible to obtain a better understanding of processes influencing variation in nutritive value of forage crops and related them to possible relevant aspects in ruminant nutrition physiology.

Home grown protein production – still a challenge

An increasing demand for so called “home grown proteins” is being discussed for animal feeding. Although the European Union (EU) covers its own demand for vegetable oil, the EU is protein deficient. This deficit makes EU the world's largest importer of soybean meal and the second-largest importer of soybeans, just behind China (USDA, 2018). Import of protein sources is questioned as it is linked to additional transport and environmental costs, as well as problems with traceability and nutritive value. Farmers in Europe are facing the challenge to meet requirements of high yielding animals, which in turn becomes difficult considering high feed costs at the farm. The objective is to optimize formulation of diets for high yielding dairy cows with local feed resources, for instance, by increasing utilization of forage legumes or share of high productive grasses in temporary grassland production systems (Søegaard et al., 2007). Temporary grassland is used as an alternative to optimize nutrient fluxes in the farming system, allowing nutrient exchange between crop and livestock production. Such measures may reduce the amount of concentrate necessary for similar milk yields, reducing impact of nutrient losses to the environment. A forage production system towards cultivation of temporary grassland combines the increasing genetic potential for high yielding dairy cows with utilization of newly released cultivars of forage species or utilization of those with high nutritive value, especially with high energy contents (Gierus et al., 2005). This is important, as the main task for the majority of specialized dairy farms is to increase N use efficiency.

Forages are important sources of protein to high yielding dairy cows. Determination of protein quality should necessarily represent estimation of escape protein content of a specific feed or feed mixture to reduce excessive losses of intra-ruminally degraded protein. Increasing amounts of escape protein available in the diet has resulted in positive influences on non-ammonia N (NAN) flow to the small intestine, improving efficient of dietary N utilization (Santos et al., 1998; Volden, 1999). However in most studies, concentrates with

high amounts of escape protein are fed to correct for undegradable protein requirements of high yielding cows, increasing N load in the dairy farm. Higher levels of escape protein from forages are seldom used as a strategy. Due to their biological N fixation capacity, forage legumes generally have a higher protein content compared to grasses. In contrast, available energy is lower in proportion to protein content. Consequently, in forage legume based production systems, protein is poorly utilized due to energy deficiency and an extensive degradation of protein in the rumen (Beever et al., 1986a; 1986b). In addition, characterizing protein quality by protein fractionation varies considerably depending on defoliation system and available forage legume species (Kleen et al., 2011). Furthermore, forage legume species determine feed quality and yield of binary mixtures with perennial ryegrass (Gierus et al., 2012) in the first production year.

Protein quality of forages as breeding strategy

Plant breeding has contributed with 4-5% per decade to an increase in dry matter production, in addition to an increase of 10 g/kg digestible dry matter per decade in North Western Europe since the 1950's (Wilkins and Humphreys, 2003). However, one of the main problems in grassland based systems is the inefficiency of ruminants to convert plant biomass into animal products, as mentioned above. Taking into account that only 20-30% of ingested N can be converted to meat or milk (Dewhurst et al., 1996), ruminant feeding contributes to environmental N pollution. Considering improvements in forage quality, high crude protein (CP) contents and rapid protein degradation rates of forages in the fore stomach of the ruminant contribute to inefficiency. Assuming forages as important sources of proteins for high yielding dairy cows, determination of protein quality should necessarily include the estimation of escape protein content of a specific forage species or mixture to reduce excessive losses of intraruminal degraded protein and N losses.

The possibility to improve protein quality of perennial ryegrass by breeding to save concentrate imports is not often considered. For feeds such as soybean meal and rape seed meal, the amount of escape protein is increased artificially through physical (heat) or chemical (formaldehyde) treatments. Also in silages, there are possibilities to positively influence protein quality, or at least avoid extensive losses on protein quality. Wilting, applications of lactic acid producing bacteria, or chemicals (e.g. organic acids) are examples of this. Although the proportion of true protein in fresh forages is higher compared to forage conserved as silage (Messman et al., 1994; Krawutschke et al., 2013b), in grazed forages possibilities to manipulate protein quality are limited. Advances have been done by plant breeding strategies (Coulman et al., 1999; Miller et al., 2001; Gierus et al., 2016). Therefore, breeding for protein quality in perennial ryegrass needs to consider also grazing systems to increase substantially progress in efficient nutrient utilization by ruminants.

Another option to the selection of cultivars for balanced nutrient and energy utilization is to increase availability of readily fermentable carbohydrates by e.g. breeding for high sugar cultivars of *Lolium perenne* (e.g. Miller et al., 2001). A limitation of this approach is that sugar levels are dependent on climatic conditions of the growing site. Another possibility to achieve synchrony of protein and energy release in forage based diets is to slow down the rate of protein degradation of the forage protein. An attempt to reduce initial degradation rate obtained was tested for alfalfa (*Medicago sativa* L.), resulting in LIRD-cultivars (low initial rate of degradation), which is a breeding target using measurements simulating protein degradation in the rumen. Coulman et al. (1999) used 4-hour incubation periods with the *in*

situ technique to determine initial rate of dry matter disappearance in fresh leaf alfalfa material. Cultivars which were characterized as LIRD were selected for subsequent breeding programs. Initial rate of degradation during the vegetative phase of selected cultivars could be reduced by approximately 15%. However, this difference was only manifested in the early vegetation stage and disappeared with increasing plant maturity.

Based on such observations, it is suggested that breeding of new genotypes for improved feed quality should be based on animal responses (Casler and Vogel, 1999; Wilkins and Humpreys, 2003). In this regard, the Cornell Net Carbohydrate and Protein System (CNCPS) represents one of several mechanistic models developed to predict nutritional value of diets by taking into consideration degradation processes of carbohydrates and proteins in the rumen. Hence, CNCPS could be used as a tool to gain information whether and to which extent differences in carbohydrate and protein composition might enhance animal performance (Salama et al., 2010).

Benefits of forage legume based systems

Due to their biological N fixation capacity, forage legumes generally have higher protein contents compared to grasses. Therefore, legume proportion and persistence under prevailing defoliation systems (cutting or grazing, or a combination of both) determine crude protein content of a grass-legume mixture in the short-term (Kleen et al., 2011; Wahyuni et al., 2018). In contrast, available energy is lower in proportion to protein content compared to grasses. Consequently, in forage legume based production systems, protein is poorly utilized due to energy deficiency and extensive degradation of protein in the rumen.

In forage legume based production systems, nutritive value of forages can be improved by increasing the amount of escape protein. In this case, forage legumes with secondary plant compound like tannins or polyphenol oxidase may be advantageous in contributing to an increased amount of escape protein. Different studies have demonstrated that with fast degradation rate and high losses of N in urine, absorption of amino acids in the small intestine may limit performance of lambs, lactating ewes and dairy cattle (Rogers et al., 1980; Barry, 1981). In contrast, lambs grazing forage legumes with condensed tannins showed significant higher average daily gains compared to grazing of ryegrass (Speijers et al., 2004). More feed protein would pass rumen undegraded as condensed tannins complex with proteins, protecting them from extensive ruminal degradation (Kardel et al., 2013). In addition, these complexes are expected to dissociate largely at the low pH conditions of the abomasum (Jones and Mangan, 1977), making protein available for absorption in the small intestine. However, tannin containing forage legumes such as birdsfoot trefoil (*Lotus corniculatus* L.), greater birdsfoot trefoil (*Lotus pedunculatus* Cav.) or sainfoin (*Onobrychis viciifolia* L.) have poorer agronomic performance and have, therefore, not yet become competitive with traditionally grown forage legumes like white clover, red clover or lucerne.

Determination of protein fractions as part of the CNCPS definition of protein quality indicated both higher quality for red clover and birdsfoot trefoil as compared to white clover or lucerne (Kleen et al., 2011). Higher proportions of fraction A (non-protein N) are linked to low escape protein contents and therefore suggests poor utilization of protein, which will be excreted mainly in urine. Although productivity and increase in nutritive value has received attention in several studies, protein quality of white clover and lucerne is rather poor. Compared to birdsfoot trefoil and red clover, white clover and lucerne showed higher protein degradation rates (Broderick und Albrecht, 1997) and higher contents of fraction A under

cutting systems (Krawutschke et al., 2013a). *In vivo* trials comparing white clover with ryegrass showed higher N losses with white clover in the diet of grazing cattle (Beever et al., 1986a) and cattle fed fresh forage indoors (Beever et al., 1986b). Differences between digestion processes, either biologically (enzymes, microbial activity) or physically driven (particle breakdown, passage rate) influence N losses (Dewhurst et al., 2003). *In vivo* work need to formulate isonitrogenous and isoenergetic diets for comparison purposes between forage species (Broderick et al., 2001).

Legumes have a high impact on forage quality in grass-legume based systems (Gierus et al., 2012). The choice of legume species is based on its adaptation to management system, climate and soil conditions, its biological N_2 fixation capacity or a combination of these factors. Tannin-containing legumes may be favourable to increase N use efficiency of the ruminant, but up to now, poor yields have made species like birdsfoot trefoil less attractive. More knowledge about protein quality of different forage crops, i.e. degradation rate, protein content and protein fractions as influenced by forage species and grassland management, are important to develop feeding strategies to enhance nutrient utilization and N use efficiency of the grazing animal, and to contribute to modelling of N losses in soil-plant-animal systems.

Conclusions

- Focus on home grown proteins should also consider the contribution of forage production systems for the energy and protein supply of ruminants. Information on protein and carbohydrate fractionations is helpful for forage characterization and comparisons of nutritive value
- Breeding of new genotypes for improved feed quality should be based on animal responses
- Forage legume species determine feed quality and yield of binary mixtures with perennial ryegrass in the first production year

References

Barry, T.N., 1981. Protein metabolism in growing lambs fed fresh ryegrass/white clover pasture. 1. Protein and energy deposition in response to abomasal infusion of casein and methionine. Br. J. Nutr. 46, 521-531.

Beever, D.E., Dhanoa, M.S., Losada, H.R., Evans, R.T., Cammell, S.B. & France, J., 1986b. The effect of forage species and stage of harvest on the processes of digestion occurring in the rumen of cattle. Br. J. Nutr. 56, 439-454.

Beever, D.E., Losada, H.R., Cammell, S.B., Evans, R.T. & Haines, M.J., 1986a. Effect of forage species and season on nutrient digestion and supply in grazing cattle. Br. J. Nutr. 56, 209-225.

Broderick, G.A. & Albrecht, K.A., 1997. Ruminal *in vitro* degradation of protein in tannin-free and tannin containing forage legume species. Crop Sci. 37, 1884-1891.

Broderick, G.A., Walgenbach, R.P. & Maignan, S., 2001. Production of lactating dairy cows fed alfalfa or red clover silage at equal dry matter or crude protein contents in the diets. J. Dairy Sci. 84, 1728-1737.

Casler, M.D. & Vogel, K.P., 1999. Accomplishments and impact from breeding for increased forage nutritional value. Crop Sci. 39, 12-20.

Coulman, B., Goplen, B., Majak, W., McAllister, T., Cheng, K.-J., Berg, B., Hall, J., McCartney, D. & Acharya, S., 1999. A review of development of a bloat-reduced alfalfa cultivar. Can. J. Plant Sci. 80, 487-491.

Dewhurst, R.J., Mitton, A.M., Offer, N.W. & Thomas, C., 1996. Effects of the composition of grass silages on milk production and nitrogen utilization by dairy cows. Anim. Sci. 62, 25-34.

Dewhurst, R. J., Evans, R.T., Scollan, N.D., Moorby, J.M., Merry, R.J. & Wilkins, R.J., 2003. Comparison of grass and legume silages for milk production. 2. *In vivo* and *in sacco* evaluations of rumen function. J. Dairy Sci. 86, 2612-2621.

Gierus M., Jahns U., Wulfes R., Wiermann C. & Taube F., 2005. Forage quality and yield increments of intensive managed grassland in response to combined sulphur-nitrogen fertilization. Acta Agric. Scand. Section B – Soil Plant Sci. 55, 264-274.

Gierus M., Kleen J., Loges, R., Taube F. & 2012. Forage legume species determine the feed quality of binary mixtures with perennial ryegrass in the first production year. Anim. Feed Sci. Technol. 172, 150-161.

Gierus M., Loesche M., Salama H., Herrmann A. & Taube F., 2016. Protein degradation rate as affected by plant proteases among fresh samples of perennial ryegrass cultivars (*Lolium perenne* L.) Die Bodenkultur: J. Land Manag. Food Environ. 67, 61-68.

Jones W.T. & Mangan J.L., 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with Fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28, 126-136.

Kardel M., Taube F., Schulz H., Schütze W. & Gierus M., 2013. Different approaches to evaluate tannin content and structure of selected plant extracts – review and new aspects. J. Appl. Bot. Food Qual. 86, 154-166.

Kleen, J., Taube, F. & Gierus, M., 2011. Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. J. Agric. Sci. Camb. 149, 73-84.

Krawutschke M., Kleen J., Weiher N., Loges R., Taube F. & Gierus M., 2013a. Changes in crude protein fractions of forage legumes during the spring growth and summer regrowth period. J. Agric. Sci. Camb. 151, 72-90.

Krawutschke, M., Thaysen, J., Weiher, N., Taube, F. & Gierus, M., 2013b. Effects of inoculants and wilting on silage fermentation and nutritive characteristics of red clover-grass mixtures. Grass Forage Sci. 68, 326–338.

Messman, M.A., Weiss, W.P. & Koch, M.E., 1994. Changes in total and individual proteins during drying, ensiling, and ruminal fermentation of forages. J. Dairy Sci. 77, 492-500.

Miller L.A, Moorby, J.M., Davis, D.R., Humphreys, M.O., Macrae, J.C. & Theodorou, M.K., 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): Milk production from late-lactation dairy cows. Grass Forage Sci. 56, 383-394.

Rogers, G.L., Porter, R.H.D., Clark, T. & Stewart, J.A., 1980. Effect of protected casein supplements on pasture intake, milk yield and composition of cows in early lactation. Austr. J. Agric. Res. 31, 1147-1152.

Salama, H., Lösche, M., Gierus, M., Herrmann, A. & Taube, F., 2010. Insights into carbohydrate and protein fractionation in perennial ryegrass (*Lolium perenne* L.) genotypes using the Cornell Net Carbohydrate and Protein System. 2. Ruminal kinetics. Grassl. Sci. Eur. 15, 551-553.

Santos F.A.P., Santos J.E.P., Theurer C.B. & Huber J.T., 1998. Effect of rumen-undegradable protein on dairy cow performance: a 12 year literature review. J. Dairy Sci. 81, 3182-3213.

Søegaard, K., Gierus, M., Hopkins, A. & Halling, M., 2007. Temporary grassland - challenges in the future. Grassl. Sci. Eur. 12, 27-38.
Speijers, M.H.D., Fraser, M.D., Theobald, V.J. & Haresign, W., 2004. The effects of grazing forage legumes on the performance of finishing lambs. J. Agric. Sci. 142, 483-493.
USDA. Economic Research Service. https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/trade.aspx (access: 9/4/2018).
Volden H., 1999. Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. J. Anim. Sci. 77, 1905-1918.
Wahyuni R.D., Pötsch E.M. & Gierus M., 2018. Impact of different N-fertilization level on metabolizable energy content of functional plant groups in permanent grassland. 17. BOKU Symp. Tierernähr., Vienna, Austria, Apr 12, 2018. In: Carbohydrates in Animal Nutrition, Slama, J; Gierus, M. (Eds.), ISBN: 978-3-900932-52-7
Wilkins P.W. & Humphreys M.O., 2003. Progress in breeding perennial forage grasses for temperate agriculture. J. Agric. Sci. Camb. 140, 129-150.

Feed intake, milk production and dry matter digestibility in cows fed CTMR

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Introduction

Total mixed ration (TMR) is an established feeding strategy for dairy cows in Sweden. One of the challenges with total mixed rations is to prevent sorting of the feed ration (Leonardi & Armentano, 2003). Sorting of TMR has been linked to health conditions such as sub-acute ruminal acidosis (Shaver, 2002). A development of the total mixed ration, called compact total mixed ration (CTMR), has recently been suggested as a way to reduce sorting of the ration (Kristensen, 2015). The CTMR is a wetter and more finely chopped version of the TMR. The dry matter (DM) target is 37 % and feed components should be close to indistinguishable from each other (Kristensen, 2015). Practical results from farms in Denmark using CTMR have shown that it can lead to increased milk production and improved feed efficiency (Kristensen, 2015). However, no controlled experiments have previously been done using this procedure.

The aim of this study was to evaluate the effects of reduced particle size and DM content in a TMR fed to lactating dairy cows on feed intake, milk production and DM digestibility.

Materials and methods

Forty dairy cows in early to mid-lactation, 48±19 (mean ± standard deviation; SD) days in milk at the start of the experiment were housed in a loose housing system and batch milked two times a day in an automatic milking rotary (DeLaval, Tumba, Sweden). Both primi- and multiparous cows were used and were allocated into seven blocks according to calving date and parity. Within the blocks, cows were randomly allocated to one of two groups. The experiment had a change-over design with two dietary treatments and two treatment periods, each 21 days. The first 14 days were used for adaption to the diet while the last 7 days within a treatment period were for sampling and measurements.

The dietary treatments TMR and CTMR were total mixed rations that differed in forage particle size and dry matter content. The diets were similar in chemical composition and based on the same forage and concentrate. The forage to concentrate ratio was 60:40, according to standards for organic milk production (KRAV, 2017). The forage was a second harvest grass silage chopped to 2 cm theoretical length of cut and preserved in a bunker silo. The concentrate was in the form of crushed pellets and produced from ingredients available as organically produced. Nutritional values for forage and concentrates are in Table 1. Both diets were mixed in a mixer wagon with a vertical auger without knives (DeLaval, Tumba, Sweden). The CTMR diet was altered in forage particle size and dry matter content. To decrease forage particle size, the forage in CTMR was mixed in a vertical auger with knives (SiloKing, Tittmoning, Germany) for 60 minutes before added to the mixer wagon. To alter DM content of CTMR, water was added into the mixer to achieve a 37% DM content, while TMR DM content averaged 58.1%. Dry matter content in forage was measured weekly for adjustment of the recipe. The diets were distributed in individual feeding mangers on scales three (TMR) or two (CTMR) times a day with the goal to provide *ad libitum* access.

Table 1 Nutritional composition of forage and concentrate used in the experiment

Forage			**Concentrate**[6]		
DM[1]	41	%	DM	88.1	%
Energy[2]	10.7	MJ ME kg DM^{-1}	Energy	13.4	MJ ME kg DM^{-1}
Crude protein[3]	165	g kg DM^{-1}	Crude protein	170	g kg DM^{-1}
NDF[4]	452	g kg DM^{-1}	Crude fat	55	g kg DM^{-1}
Ca[5]	8.2	g kg DM^{-1}	Crude fiber	66	g kg DM^{-1}
P[5]	2.2	g kg DM^{-1}	Ash	62	g kg DM^{-1}
Mg	2.3	g kg DM^{-1}	Ca	8	g kg DM^{-1}
K[5]	27.3	g kg DM^{-1}	P	6	g kg DM^{-1}
			Mg	3	g kg DM^{-1}
			K	9	g kg DM^{-1}
			Na	3.2	g kg DM^{-1}

[1]Dry matter, analysed by drying in 60°C over night; [2]Estimated by analysis of VOS (Lindgren, 1979) and calculated using the equation from Lindgren (1983); [3]Estimated by the Kjeldahl method; [4]Analysed according to Chai and Udén (1988); [5]Analysed by plasma emission spectroscopy; [6]Data on nutrient composition of concentrate were provided by the manufacturer (Lantmännen).

The Penn State Particle Separator with 19 and 8 mm sieves was used to assess particle size distribution of the diets. Feed samples were collected at feeding four times during each sampling period. Particle size proportions of the diets were calculated on fresh and DM basis. Dry matter intake was measured using data collected from the feed mangers and were recalculated using the DM content of each diet. Drinking water intake was measured using water cups fitted with water meters and transponder sensors (Biocontrol A/S, Rakkestad, Norway). Total water intake was calculated by using registered water intake from water cups, feed intake and DM content of diets. Milk yield data were collected from all milkings during the sampling week. Milk composition was determined by analysis of milk samples derived from morning and evening milking for two consecutive days during the sampling week within each period. Milk samples were analysed with a Delta Combiscope (Combiscope FTIR 300, Delta instruments, the Netherlands) for fat, protein and lactose content. Dry matter digestibility was measured by analysis of acid insoluble ash (AIA) in feed and faeces. Forage and concentrates were analysed separately in duplicates during both treatment periods. Faeces were spot sampled three times on three separate days for each cow during the sampling weeks and frozen. The three samples from the same animal and treatment period were then thawed and composited to one 180-g sample for further analysis. The 180-g samples were freeze dried, ground to pass a 1-mm sieve, combusted at 550°C, boiled in hydrochloric acid to remove acid soluble components and then filtered (Van Keulen & Young, 1977).

Data were analysed by SAS 9.4 with procedure Mixed and class variables: animal, block breed, period and treatment. Random variable was animal and fixed factors were breed, block, period and treatment. The covariance between samples within animal was modelled with a spatial power covariance structure. Significant results were considered when $P<0.05$.

Results and discussion

In this study, the effect of forage particle size and DM content was evaluated as one factor and therefore it is not possible to determine which of them affected the results, or if there was an interaction between them. The CTMR diet had a smaller forage particle size and a lower DM content. The fraction of DM in the diet that remained on the top sieve in the Penn State

particle separator was 32 % for the TMR diet and 6 % for the CTMR diet. Dry matter intake decreased, even though fresh matter intake increased, when cows were fed CTMR diet compared to TMR diet (Table 2). This was inconsistent with previous literature that studied the effect of forage particle size in forage based TMR's. Reducing forage particle size has been seen to increase DM intake in dairy cows in forage based TMR diets (>50 %, DM basis) (Kononoff & Heinrichs, 2003; Maulfair *et al*., 2010). However, decreasing DM content of a TMR to below 50 % has previously been shown to decrease DM intake (Miller-Cushon & DeVries, 2009; Felton & DeVries 2010). Total water intake was higher when CTMR was fed compared with when TMR was fed (Table 2). This was a result from the added water to the CTMR ration, since water intake from water troughs was lower when cows were fed CTMR (Table 2). It has previously been suggested that adding water to a TMR can decrease DM intake because of water's ruminal filling effect, as well as that the rumen's capacity to transport water is exceeded (Robinson *et al*., 1990; Miller-Cushon & DeVries, 2009). Therefore, it is suggested that the effect of reduced forage particle size was overridden by the effect of lowered DM content in the CTMR diet and that this caused the decrease in DM intake.

Table 2 Effects of feeding total mixed rations TMR[1] and CTMR[2], differing in forage particle size and dry matter content

	TMR	CTMR	SEM	P-value
Dry matter intake, kg day^{-1}	28.6	26.8	0.6	<0.001
Water intake, kg day^{-1}	109.6	98.9	2.6	<0.001
Total water intake, kg day^{-1}	136.3	144.3	3.0	<0.001
Dry matter digestibility, %	61.35	62.1	0.01	0.187
Milk yield, kg day^{-1}	35.2	35.1	0.4	0.495
Milk yield, kg ECM day^{-1}	34.7	33.8	0.8	0.151
Fat content, %	3.83	3.71	0.01	0.239
Fat yield, kg day^{-1}	1.36	1.28	0.04	0.062
Protein content, %	3.24	3.25	0.01	0.621
Protein yield, kg day^{-1}	1.15	1.13	0.03	0.189
Lactose content, %	4.77	4.78	0.01	0.748
Lactose yield, kg day^{-1}	1.71	1.68	0.04	0.237

[1] TMR was forage from the bunker silo and dry matter content 58.1%. [2]CTMR was re-chopped forage and water added to achieve dry matter content 37%.

Even if dietary treatments affected DM intake, there was no effect on milk production or milk composition (Table 2). This was consistent with TMR studies on the effect of forage particle size (Kononoff & Heinrichs, 2003; Maulfair *et al*., 2010) and effect of DM content (Miller-Cushon & DeVries, 2010; Felton and DeVries, 2012). It can be speculated that the higher energy intake on the TMR diet was used for body fat synthesis rather than milk synthesis and that with time, this should result in a weight gain and increase in body condition score. There was a tendency towards a lower milk fat yield when CTMR diet was fed (Table 2). Reduction of forage particle size has previously resulted in reduced milk fat percentages when the forage proportion of a TMR was 40 % (Krause & Combs, 2003).

Since CTMR resulted in lower DM intake at the same time as milk production was maintained, the reduced forage particle size of CTMR could have increased digestibility. There was, however, no effect of dietary treatment on DM digestibility (Table 2). This is

inconsistent with previous work that used forage based TMR (>50 %, DM basis) where DM digestibility increased when forage particle size was reduced (Kononoff & Heinrichs, 2003). There are, however, previous research where forage proportions were <50% of DM and no effect of forage particle size on DM digestibility could be found (Krause *et al*., 2002; Alamouti *et al*., 2009). The AIA concentration of the forage was higher in one of the treatment periods, for unknown reasons. The higher AIA concentration occurred in both duplicate samples and therefore not thought to be due to an analytical error. This led to that an effect of period on calculated DM digestibility was found. Also, there was a significant interaction between dietary treatment and period, showing that the CTMR increased DM digestibility with 2.2 percentage units in the second treatment period ($P<0.05$), although not in the first period. A dietary treatment's effect on DM digestibility could therefore not be fully established.

Conclusions

When CTMR, where particle size as well as DM content of the diet were decreased, was fed to dairy cows in early to mid-lactation, DM intake decreased and total water intake increased, compared to when a conventional TMR was fed. However, no effects on milk production or milk composition was shown. The effect of CTMR on DM digestibility could not be fully established.

References

Alamouti, A.A., Alikhani, M., Ghorbani, G.R. & Zebeli, Q., 2009. Effects of inclusion of neutral detergent soluble fibre sources in diets varying in forage particle size on feed intake, digestive processes, and performance of mid-lactation Holstein cows. Anim. Feed Sci. Technol. 154, 9-23.

Chai, W. & Udén, P., 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. Anim. Feed Sci. Technol. 74:281-288.

Felton, C.A. & DeVries, T.J., 2010. Effect of water addition to a total mixed ration on feed temperature, feed intake, sorting behavior, and milk production of dairy cows. J. Dairy Sci. 93(6), 2651-2660.

Kononoff, P.J. & Heinrichs, A.J., 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86(4), 1445-1457.

Krause, K.M. & Combs, D.K., 2003. Effects of forage particle size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows. J. Dairy Sci. 86(4), 1382-1397.

Krause, K.M., Combs, D.K. & Beauchemin, K.A., 2002. Effects of forage particle size and grain fermentability in midlactation cows. I. Milk production and diet digestibility. J. Dairy Sci. 85, 1936-1946.

KRAV, 2017. Standards for organic production. Uppsala, Sweden.

Kristensen, N. B., 2015. Implement Compact TMR to increase productivity, feed efficiency and health in dairy herds. In: Djurhälso- & utfodringskonferensen. Växa Sverige, Sollentuna, Aug 25-26th, (p. 68-79.

Lindgren E., 1979. Vallfodrets näringsvärde bestämt in vivo och med olika laboratoriemetoder. Report 45. Dept. of Animal Nutr. and Managm., Swedish Univ. Agric. Sci., Uppsala, Sweden (English summary).

Lindgren, E. 1983. Nykalibrering av VOS-metoden för bestämning av energivärde hos vallfoder. Dept. of Animal Nutr. and Management. Swedish Univ. Agric. Sci., Uppsala, Sweden.

Maulfair, D.D., Zanton, G.I., Fustini, M. & Heinrichs, A.J., 2010. Effect of feed sorting on chewing behavior, production, and rumen fermentation in lactating dairy cows. J. Dairy Sci. 93, 4791-4803.

Miller-Cushon, E.K. & DeVries, T.J., 2009. Effect of dietary dry matter concentration on the sorting behavior of lactating dairy cows fed a total mixed ration. J. Dairy Sci. 92, 3292-3298.

Robinson, P.H., Burgess, P.L. & McQueen, R.E., 1990. Influence of moisture-content of mixed rations on feed-intake and milk-production of dairy-cows. J. Dairy Sci. 73, 2916-2921.

Van Keulen, J. & Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44, 282-287.

What raw material processing technology is available at the BTC- Biomass Technology Centre, Umeå?

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Introduction

The Biomass Technology Centre (BTC) is a research pilot plant designed for refining of solid biomass and industrial biobased by-products. We work with a wide range of technologies along the upgrading chain where biomass or side-products are transformed into industrial biobased feedstock. We have 18 years of experience in pilot scale research, development, and innovation. Our infrastructure is designed for handling and storage of large quantities of materials. We can perform reliable and representative sampling and prepare specific assortments through fractioning, sieving, milling, briquetting, and pelletizing.

At BTC, we have advanced systems for performing experimental research designs for production of pellets and briquettes from a wide range of feedstock, feedstock mixes and additive blends. Research results from the pilot scale setup have been shown to correspond well to industrial scale pelletizing conditions and have been directly implemented. Hence, process optimization can be performed in designed experiments with well-defined materials and under controlled conditions at low cost.

Materials and methods

Pelletizing and briquetting

Bio-based, recycled and waste materials can be pelletized or briquetted at BTC in amounts ranging from 10 kg to 10 tons. We have 15 years of experience from pellet and briquette production from a large variety of forestry and agricultural biomasses, waste streams, thermally treated biomass materials, and various animal feed assortments. Thus, we dare to say that our expertise in pilot-scale biomass pelletizing technology is world-leading. We can offer our expertise and equipment in collaborative R&D projects, but also provide pellet or briquette production as a service.

Figure 1 Chips and pellets of torrefied wood.

Figure 2 Wood chips and briquettes.

Sampling

We can offer representative sampling of large and inhomogeneous bio-based, recycled and waste material batches, characterization of ash and moisture content and preparation and sending of samples to analytical laboratories. Our unique abilities lie in whole chain equipment for blending, drying, and comminution and in our expertise and long term experience in biomass and waste sample handling. Sampling and sample preparation is performed according to ISO 18135:2017 Solid biofuel sampling procedures.

Comminution

BTC has unique abilities for comminution of bio-based, recycled and waste materials. Pilot scale equipment consists of a slow-rotating shredder (screen size: 15 or 30 mm) and a hammer mill (screen size: 2-8 mm) which can be utilized to produce several tons of chips, chops, and/or powder. This process line tolerates feedstock moisture contents up to 20-30%. On-site drying of moister materials can sometimes be offered. Wood chipping can be performed with an electric chipper (5-12 mm chip length).

Our laboratory comminution equipment (kg/h) consists of two types of cutting mills and a hammer mill. They can be utilized for milling of smaller material amounts (100 g – 50 kg). Samples can, if needed be dried on-site before milling.

Figure 3 Representative sampling for further analysis.

Figure 4 Shredder.

Drying

Drying of bulk materials in amounts ranging from tens of kilos to a few tons can be performed at BTC. Several different drying technologies are available. Bench-scale equipment is available (5-50 kg) for convective drying at temperatures ranging from 40 to 105°C and for cyclone drying at 60 to 85°C. Pilot scale equipment (100 kg – 3 tons) is available for plane drying at 40 to 90°C or cyclone drying at 70 to 100°C.

Mechanical fractioning

Fractioning and sorting of bio-based bulk materials can be performed at BTC by combining different methods of comminution with fractioning according to particle sizes and particle density. Particle size based fractioning can be performed for amounts ranging from 5 kg to 2 ton. Fractioning according to particle density is performed on amounts of 100 kg to 2 ton.

Figure 5 Cyclone dryer.

Figure 6 a) and b) Mechanical size fractioning equipment.

Flow properties

Characterization of biomass arching tendencies and silo flow properties can be performed at BTC. Flow properties are evaluated by discharging bulk materials from a 0.5 or a 1 m^3 test silo with adjustable hopper angle and outlet opening size. This method can be used to compare flow properties of bulk materials and for measuring impact of different treatments on mechanical flow properties.

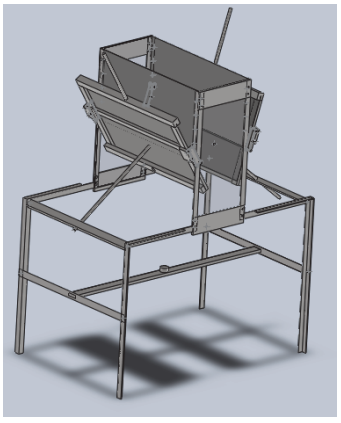

Figure 7 Silo with adjustable hopper angle and outlet opening size

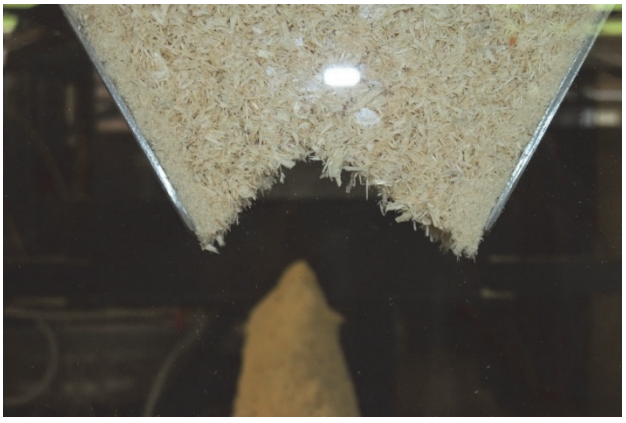

Figure 8 Arching of wood powder

Results and discussion

A large number of studies on pilot scale pelletizing of different biomass feedstock for production of fuel pellets have been performed at BTC. Focus of these studies has e.g. been to: i) determine optimum feedstock and process settings for maximum pellet quality and process performance (e.g. Larsson et al., 2008; Samuelsson et al., 2009; Larsson & Rudolfsson, 2012; Samuelsson et al., 2012; Larsson et al., 2013; Segerström & Larsson, 2014; Rudolfsson et al., 2017a) and ii) develop pelletizing hardware equipment for improved feedstock versatility (Larsson & Rudolfsson, 2012; Larsson et al., 2012; Rudolfsson et al., 2017b), and iii) determine storage off-gassing of permanent gases and VOCs from thermally treated biomass materials throughout the pellet feedstock and production chain (Borén et al., 2018a; Borén et al., 2018b).

Scientific studies are performed in experimental designs with triplicate measurements of all parameters to obtain statistically solid data, both for process parameters and pellet quality measures.

Some major findings from pellet oriented studies are: i) the strong moisture influence (on both process performance and pellet quality) and that optimum moisture content to maximize the two major pellet quality parameters; bulk density and durability, do not coincide (Figure 9 a) and b) and ii) that a better process stability and better pellet quality can be obtained when pelletizing straw materials by actively lowering die temperature (Figure 10) and that process stability and pellet quality can be maintained by adding water through a nozzle in the press nip when pelletizing torrefied materials (Figure 11).

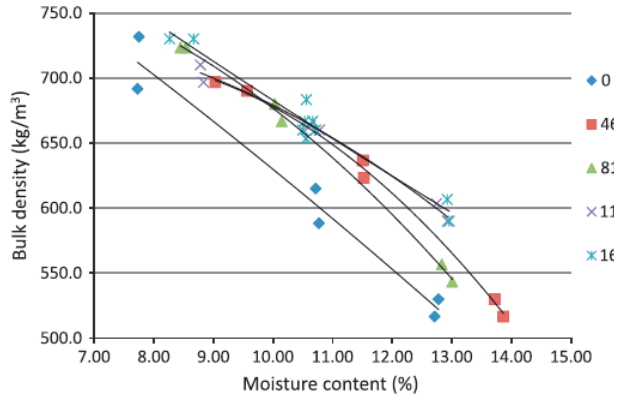

Figure 9 a) Bulk density of pellets made from pine sawdust which had been stored for 0-160 days (from Samuelsson et al., 2012)

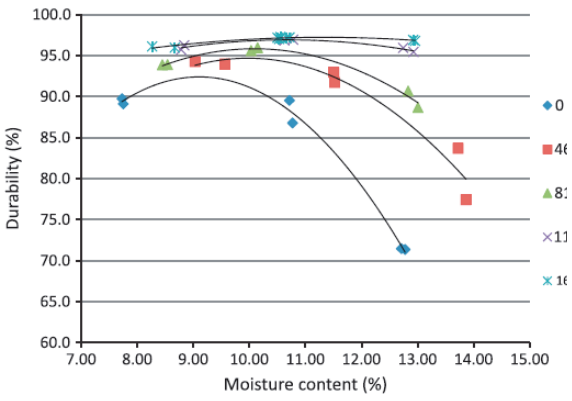

Figure 9 b) Durability of pellets made from pine sawdust which had been stored for 0-160 days (from Samuelsson et al., 2012)

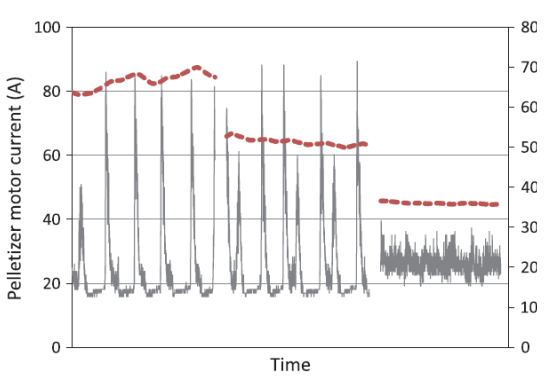

Figure 10 Pelletizer motor current (grey) during pelletizing of reed canary grass at three different die temperature levels (red dotted) (from Larsson et al., 2012).

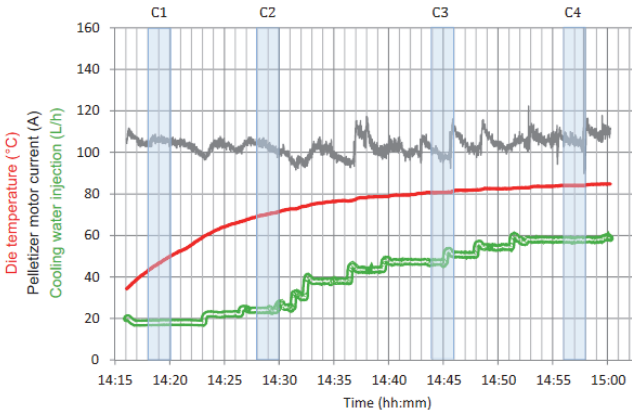

Figure 11 Pelletizer motor current (grey), die temperature (red), and water injection (green) during pelletizing of torrefied willow (from Rudolfsson et al., 2017b).

The studies on off-gassing patterns of thermally treated biomass were performed to determine possible hazardous occupational emissions along the supply chain for these types of pellets. An important finding from these studies was that emission levels, which decline during storage of treated material, are sparked anew to higher levels when the material is pelletized (Figure 12).

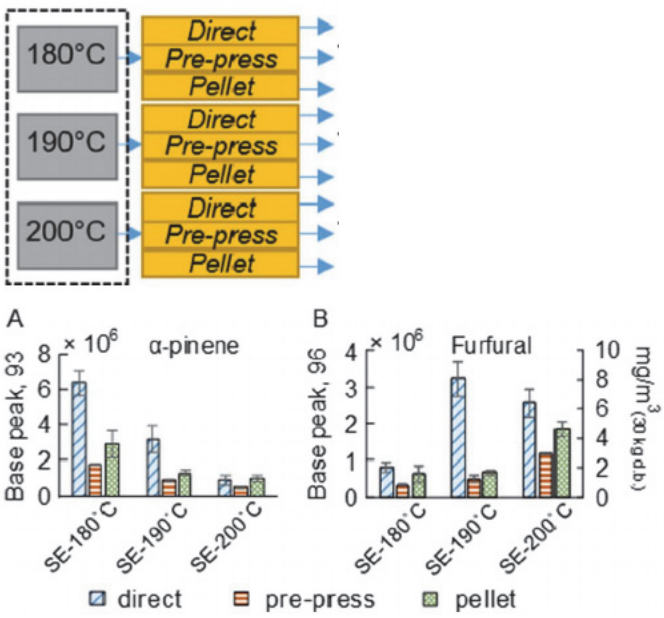

Figure 12 Emissions of α-pinene and furfural from bark steam-exploded at 180, 190 and 200°C at different steps in the pellet production chain: directly after steam-explosion, after 72 h of storage - sampled pre-press and directly after pelletizing (from Borén et al., 2018b).

Several feed pelletizing studies have also been performed at BTC, e.g. for production of reindeer fodder from different types of forage and to determine the effect of various types of additives in ruminant feed pellets. None of those studies are published.

Conclusions

The Biomass Technology Centre, BTC, at SLU in Umeå, Sweden provides a versatile whole-chain pilot scale biomass preparation and pelletizing infrastructure for research, development, and innovation with experienced technical personnel to facilitate tailored project setups. Contact us for further questions! Contact person: Sylvia Larsson sylvia.larsson@slu.se Web page: www.slu.se/sbt/btc

References

Borén, E., Larsson, S.H., Averheim, A., Thyrel, M. & Broström, M., 2018a. Reducing volatile organic compound off-gassing during the production of pelletized steam-exploded bark: Impact of storage time and controlled ventilation. Energy and Fuels, 32, 5181-5186.

Borén, E., Larsson, S.H., Thyrel, M., Averheim, A. & Broström, M., 2018b. VOC off-gassing from pelletized steam exploded softwood bark: Emissions at different industrial process steps. Fuel Process. Technol. 171, 70-77.

Larsson, S.H. & Rudolfsson, M., 2012. Temperature control in energy grass pellet production - effects on process stability and pellet quality. Appl. Energy 97, 24-29.

Larsson, S.H., Rudolfsson, M., Nordwaeger, M., Olofsson, I. & Samuelsson, R., 2013. Effects of moisture content, torrefaction temperature, and die temperature in pilot scale pelletizing of torrefied Norway spruce. Appl. Energy 102, 827-832.

Larsson, S.H., Rudolfsson, M., Thyrel, M., Örberg, H., Kalén, G., Wallin, M. & Lestander, T.A., 2012. Temperature controlled feed layer formation in biofuel pellet production. Fuel, 94 81-85.

Larsson, S.H., Thyrel, M., Geladi, P. & Lestander, T.A., 2008. High quality biofuel pellet production from pre-compacted low density raw materials. Biores. Technol. 99, 7176-7182.

Rudolfsson, M., Boren, E., Pommer, L., Nordin, A. & Lestander, T.A., 2017a. Combined effects of torrefaction and pelletization parameters on the quality of pellets produced from torrefied biomass. Appl. Energy 191 414-424.

Rudolfsson, M., Larsson, S.H. & Lestander, T.A., 2017b. New tool for improved control of sub-process interactions in rotating ring die pelletizing of torrefied biomass. Appl. Energy 190, 835-840.

Samuelsson, R., Larsson, S.H., Thyrel, M. & Lestander, T.A. 2012. Moisture content and storage time influence the binding mechanisms in biofuel pellets. Applied Energy, 99, 109-115.

Samuelsson, R., Thyrel, M., Sjöström, M., Lestander, T.A., 2009. Effect of biomaterial characteristics on pelletizing properties and biofuel pellet quality. Fuel Process. Technol. 90, 1129-1134.

Segerström, M. & Larsson, S.H., 2014. Clarifying sub-processes in continuous ring die pelletizing through die temperature control. Fuel Process. Technol. 123, 122-126.

The Centre for feed technology – FôrTek

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Introduction

Feed compounding and manufacturing is far more than milling and mixing ingredients. Globally, feed manufacturing has reached the point where MSc and PhD degrees are necessary in order to understand processes developed decades ago, based on practical experience of trial and error.

A decade ago, the Centre for Feed Technology – FôrTek was established with an overall goal to serve the feed industry by carrying out research, educational and developmental assignments in the fields of feed manufacturing processes and animal and fish feed nutrition. The initial idea of an experimental feed production plant came from the feed industry and scientists from the university. Such idea later received support from other institutions and companies. Since 2005, FôrTek is owned 100% by the Norwegian University of Life Sciences (NMBU), situated in the village of Ås, 35 km south of the Norwegian capital Oslo. Ever since, FôrTek has had a mission to be a full value chain supplier, from developing technology to running animal trials together with the Animal Production Experimental Centre.

Some of the services provided by FôrTek

- Production of research feeds and process technology development
- Training university students on MSc and PhD levels and industry personnel. Conduct various seminars related to feed technology, extrusion, drying, aqua feeds and pet food with support from the Norwegian University of Life Sciences, its centres of excellence and industrial partners
- Equipment evaluation

Equipment and capabilities

A unique and flexible collection of feed production equipment for animals and fish makes FôrTek a state-of-the-art feed manufacturing complex.

FôrTek is designed to make and test new ingredients in feed formulations and their physical handling characteristics as well as to evaluate variations of formulas to improve nutritional value and product quality. FôrTek's production lines are available for production of research feeds for fish and other monogastric and ruminant animals. Receiving, grinding, proportioning and mixing as well as conventional and non-conventional conditioning, expanding, extruding and pelleting processes are possible with precise liquid and micro-ingredient addition.

FôrTek is co-located with the faculty of Life Sciences, Department of Animal and Aquacultural Sciences as a research and production section within the Animal Production Experimental Centre. The interaction between those two divisions enable researchers to monitor the complete feed chain, from feed ingredients processing and its technical characteristics to the effects on nutrition, health, welfare of various farm animals. Thus, the

main strategy of FôrTek is to serve as base for research institutions on know-how principles by combining national and international knowledge in the area of feed technology and animal nutrition. This all makes FôrTek a universal host that can offer a complete research package for feed research and development.

Scientific work and quality-based system

FôrTek produces feeds in agreement with the Norwegian regulation policy for feed production, "Regulation of feedstuffs" dated 7th November 2002, and "Regulation of feed additives" dated 12th April 2005, which is in correspondence with EU regulation policy for feed production. FôrTek operates under the ISO9001 quality system.

Numerous experiments have been performed at FôrTek, resulting in a large number of scientific papers published in peer-reviewed journals and applied journals (Miladinovic, 2009; Miladinovic and Zimonja, 2010; Miladinovic *et al.*, 2013; Miladinovic, 2014a; Miladinovic, 2014b).

Education

Since year 2002 the Norwegian University of Life Sciences with help from FôrTek have educated generations of Masters of Science within the feed manufacturing area. The Feed Manufacturing Master of Science program taught in English language provided national and international feed companies with highly qualified individuals. These skilled individuals are facilitating feed manufacturing improvements and breakthroughs across the world.

References

Miladinovic, D. 2009. Knife condition and its influence on the pellet quality. Afma Matrix 3, 24-26

Miladinovic, D., Zimonja, O., 2010. Influence of the die design, screw speed and filling grade on physical properties, processing parameters and output rate of the extruded fish feed.

Proc. Extrusion Technol. Feed and Food Process, pg. 53-61, Feed-to-Food FP7-REGPOT-3 project. 14th Int. Symp. Anim. Feed Technol., Quality and Safety. Funded under: FP7-REGPOT, Novi Sad, Serbia.

Miladinovic, D., Sørensen, M., Svihus, B., 2013. Strength and durability of feed pellets influenced by different particle size distribution, pellet volume and dehydration techniques. Ann. Transact. Nordic Rheol. Soc 21, p. 107-115.

Miladinovic, D., 2014a. Enzymes make the pelleting process less power consuming. AllAboutFeed 22, 10-12.

Miladinovic, D. 2014b. Vacuum dehydration of feed studied. AllAboutFeed 22, 22-25.

What will the Feed Technology Laboratory in Uppsala be able to do in the near future?

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Introduction

Feed technology laboratory (FTL) is the newest addition to the Department of Animal Nutrition and Management (HUV) at SLU. HUV has a strong competence in animal nutrition and animal feeds for various animal groups. While feed science with focus on ruminants has been strongly represented at HUV, research in technology of feed processing for monogastric animals has been scarce, especially for areas of aquaculture feeds and pet food. This has mainly been due to lack of relevant competence but also suitable infrastructure. With FTL, HUV aims to develop competence in this area, enabling strengthening of both internal and external collaboration through existing and future projects.
FTL will work on optimization of production conditions for various new materials on a small scale, utilizing advanced and common processing steps applied by the feed industry, including, but not limited to controlled extrusion technology, vacuum coating and physical pellet quality. FTL's role is thus two-fold - producing feed batches in support of research at HUV and developing solutions for challenging new raw materials using laboratory grade equipment.

Materials and methods

Extrusion technology
Production of 0.5-50 kg batches of experimental feeds for fish, pets and other animals using extrusion technology (up to 6 mm pellet size). Extruded feeds are advantageous over cold-pelleted feeds due to higher bulk density, increased durability, shelf life, palatability, etc. Extruded feed has high carrying capacity for oil inclusion, for flavouring and use of enzymes and other additives and allows for greater flexibility in research. One of the challenges when working with novel ingredients is their technical optimization and texturizing using existing technology to obtain acceptable-quality pellets.
FTL will be equipped with Lab-Compounder KETSE 20/40 from Brabender, a twin-screw compact extruder with modular screw configuration and variable speed. The system will log experimental conditions and production data.

Figure 1 KETSE 20/40 extruder/ lab compounder.

Figure 2 Vacuum coater GVM series.

Pelleting

Small sized batches of 100 – 5000 g of pellet fed using a table screw press for production of experimental feeds for fish, insects, mice etc. Such feed batches can also be steam conditioned if needed.

Vacuum coating

Coating of extruded feed with lipids and lipid soluble additives. This step is applicable to extruded fish feeds and pet foods but can also be used on poultry and pig feeds. Depending on pellet structure after extrusion, lipids can be added (4-40%) as well as various liquids. This step allows for addition of heat sensitive products such as enzymes, vitamins etc. Batch size of up to 50 kg will be possible. FTL is equipped with Amandus KAHL gentle coater, with decreased generation of fines.

Drying

FTL can perform oven drying of 1-20 kg batches at 40-105°C.

Grinding

Grinding of dried raw materials with hammer mill and air-swept grinders down to a particle size of 75 µm. This process is applicable to pre-dried materials in small batches of 100 g to 20 kg for experimental feed production.

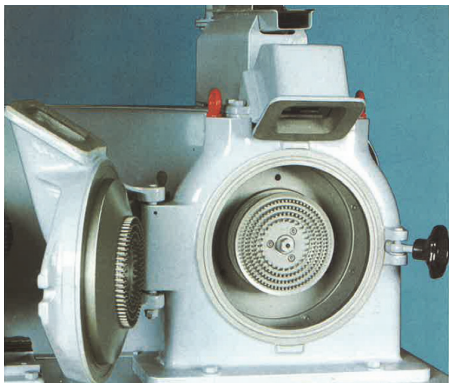

Figure 3 Air swept pulveriser.

Mixing

Mixing of dried bulk materials in batches of 0.1-50 kg.

Pellet quality

Measurements of pellet hardness, pellet durability and other technical properties in relation to chemical composition of the pellet. These properties determine shelf life, sinking speed and palatability of experimental feeds.

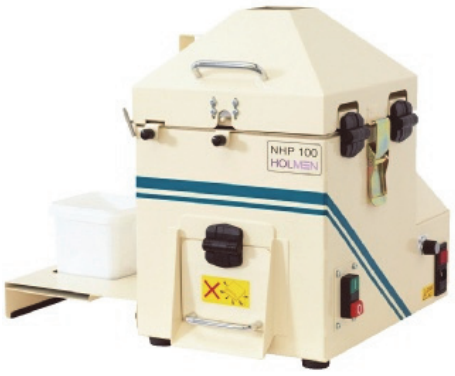

Figure 4 Pellet durability tester. .

Figure 5 Pellet hardness tester.

Collaboration

FTL is open for both internal and external collaboration and an existing network include both SLU-BTC, Umeå, Sweden and NMBU-Fortek, Ås, Norway as possible partners on future projects.

Status

Laboratory is currently under construction and while certain equipment is in place, other is currently on order. The planned official start-up is in autumn 2018.

Grass silage for biorefinery - effect of additives on silage quality and liquid-solid separation in timothy and red clover silages

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Introduction

Timothy and red clover are forages with excellent potential for biomass production under Boreal conditions and they provide versatile properties as raw materials for a green biorefinery. Ensiling timothy and red clover allows green biomass to be processed all year round providing great opportunities for the biorefinery industry, which can create a variety of innovative products. Usually, the first step of a green biorefinery approach is the stratification of liquid and solid fractions by mechanical liquid-solid separation. Yield and composition of liquid and solid fractions vary significantly depending on raw material quality and processing technology.

Silage additives may help to improve fermentation and consequently decrease losses by spoilage. But, they can also modify the characteristics of silage as a raw material for a green biorefinery. According to Rinne *et al*. (2018a), fermentation quality and yield of silage juice were improved by fibrolytic enzymes when used in combination with a formic acid based additive at time of ensiling.

There is a high correlation between silage quality and liquid yield and composition (Franco *et al*., 2018). For instance, greater DM promotes lower liquid yield, which makes it possible to predict the biorefinery potential of silage. Silage production system can be manipulated in order to prepare a feed that best meets the requirements of a particular green biorefinery process. The aim of this experiment was to evaluate the effect of additives on chemical composition and fermentation quality of timothy and red clover silages. Effects of additives and silage raw material quality (plant species and wilting) on the efficiency of the biorefinery process were also evaluated from yield and composition of the liquid fraction.

Materials and methods

The experimental silages were produced in Jokioinen, Finland in June 2016. A mixed timothy and meadow fescue sward was harvested on June 7th and ensiled after a short (4-h; G4) or long (24-h; G24) wilting period. Red clover (RC) was harvested from a pure stand on June 20th and ensiled on June 21st after wilting for 24 h (Table 1).

Treatments used were: i) control without additive (C), ii) formic acid (FA) based additive (AIV 2 Plus, Eastman Chemical Company, Oulu, Finland at 5 l/t) and lactic acid bacteria strains (LAB, Sil-All 4×4+; Microbial Developments, Worcestershire, England at 5 g/t). Details of the additives are in Table 2. All materials were ensiled in cylindrical pilot scale silos with 12 litre capacity using three replicates per treatment. The silos were semi-closed without water-locks and seepage was not drained. Densities were 599, 540 and 816 kg/m^3 for G4, G24 and RC, respectively.

The silos were stored at room temperature with protection from light and opened on September 26th after an ensiling period of 111, 110 and 97 days, respectively, for G4, G24 and RC. Deteriorated silage on surface and bottom of the silo was discarded. Silage was carefully mixed and samples were taken and analysed for chemical composition and fermentation products. The liquid-solid separation was conducted from frozen and de-frosted samples using a pneumatic press (in-house built equipment; Luke, Jokioinen, Finland). A sample of 150 g was put into a mesh bag and squeezed between two piston plates during 2 minutes at 6 bars (× 100 kPa) of pressure. Liquid was quantitatively collected and weighed. Three analytical replicate measurements were made per sample and a mean value was used for statistical analyses. Silage and respective juice fractions were analysed for chemical composition by routine methods as described by Seppälä *et al*. (2016).

Table 1 Chemical composition of timothy and meadow fescue (4 and 24 hours wilting; G4 and G24) and red clover (RC) herbage prior to ensiling

	G4	G24	RC
Dry matter (DM), g/kg	290	298	285
Buffering capacity, g lactic acid/100 g DM	6.40	6.54	10.10
In DM, g/kg			
Ash	82	88	101
Crude protein	98	103	197
Water soluble carbohydrates	153	109	89
Neutral detergent fibre	537	563	334
D-value (g digestible org. matter/kg DM)	700	681	677
Organic matter digestibility	0.762	0.746	0.753
Metabolizable energy, MJ/kg DM	11.2	10.9	10.8

Table 2 Description of additives used in the ensiling experiment

Abbr.	Company	Composition	Concentration	Name	Amount used
FA	Eastman Chemical Company	Formic acid Ammonium formate	76.0 % 5.5 %	AIV 2 Plus	5 l/t
LAB	Microbial Developments	*Lactobacillus plantarum* *Pediococcus acidilactici* *Pediococcus pentosaceus* *Propionibacterium acidipropionici* Alpha-amylase from *Bacillus amyloliquefaciens* Cellulase from *Trichoderma reesei* Beta-glucanase from *Aspergillus niger* Xylanase from *Trichoderma longibrachiatum*	≥1×10^{11} cfu/g ≥4×10^{10} cfu/g ≥4×10^{10} cfu/g ≥2×10^{10} cfu/g ≥3600 BAU/g ≥60 CMCU/g ≥1000 IU/g ≥1500 IU/g	Sil-All 4×4+	5 g/t

Cfu=colony-forming units; BAU=bacterial amylase units; CMCU=carboxymethyl cellulose units; IU=international units.

Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. For the timothy data set, the sums of square was further partitioned into contrasts, with additive and wilting period effects and their interaction. The effect of additives was tested in red clover silages. An overall interaction effect between additive and forage species was also evaluated.

Results and discussion

The wilting period of 24 hours was not effective in order to increase dry matter (DM) concentration of the timothy grass (Table 1). Due to rainy weather, wilting was conducted indoors and it resulted in only a minimal (8 g/kg during 20 h) increase in herbage DM

concentration. However, substantial losses in grass water soluble carbohydrate (WSC) concentration appeared which were reflected in increased ash, crude protein (CP) and neutral detergent fibre (NDF) concentrations and decreased D-value showing that extensive respiration had taken place during the wilting period. Red clover had higher CP concentration than timothy with lower WSC concentration and consequently higher buffering capacity. All three silage batches were rather similar in DM concentration slightly below 300 g/kg.

Dry matter concentration increased with additive inclusion in G4 (Table 3). Formic acid additive resulted in silage with the highest DM concentration but no effluents were detected for any of the treatments, while control and LAB did not differ in G24. No additive effect on DM was observed for RC. There was an interaction between additive and wilting period in timothy silages for pH and ammonia with the highest value for C in G4. LAB resulted in the lowest pH and ammonia concentration in silages produced with G4 and RC.

The interaction between additive and wilting period of timothy resulted in a higher concentration of ash for control treatment (Table 3), which was even more pronounced in G4 than in G24 silages. Ash concentration was lower for additive treatments due to smaller ensiling losses promoted by the additives. Following the same pattern, RC silage without additive gave the greatest ash concentration, while LAB treatment had the lowest concentration. There were no effects of additive, wilting or interactions for CP concentration of either timothy or RC silages. However, for individual averages, LAB treatment decreased CP concentration of silages. Overall comparison of the effects showed greater concentrations of WSC for LAB treatment, indicating that it was more effective in restricting fermentation and, consequently, reducing the use of WSC as a fermentation substrate. There was an interaction of additive and wilting period for ethanol concentration of timothy silages. Additives decreased ethanol concentrations of silages with a stronger effect for G4 than for G24 silages. LAB decreased ethanol concentration of RC silages, while the highest concentration was found for FA.

Table 3 Composition and fermentation quality of timothy and red clover silages treated with additives, and yield and retained compounds of liquid-solid separation

	Timothy										Red clover						
	4 h wilting[1]			24 h wilting[1]			SEM[2]	P-value[3]			24 h wilting[1]			SEM[2]	P-value[3]	SEM[2]	P-value[3]
	C	LAB	FA	C	LAB	FA		Add	Wilt	Add*Wilt	C	LAB	FA		Add		Add*S (24 h)
DM, g/kg	261^{d}	286^{c}	287^{c}	294^{b}	298^{b}	306^{a}	1.5	<0.001	<0.001	<0.001	285	279	287	2.1	0.634	1.9	0.021
pH	4.93^{a}	3.92^{c}	4.06^{bc}	4.21^{b}	4.13^{bc}	4.05^{bc}	0.058	<0.001	0.004	<0.001	4.30^{a}	4.22^{b}	4.27^{ab}	0.014	0.024	0.014	0.012
Ammonia, g/kg N	106^{a}	36^{c}	63^{b}	53^{b}	59^{b}	58^{b}	2.2	<0.001	<0.001	<0.001	38^{a}	26^{b}	37^{a}	0.7	<0.001	1.2	<0.001
Chemical composition, g/kg DM																	
Ash	97^{a}	87^{c}	88^{c}	91^{b}	89^{bc}	88^{c}	0.5	<0.001	0.062	<0.001	106^{a}	103^{b}	105^{ab}	0.7	0.071	0.6	0.901
CP	120^{ab}	115^{b}	117^{b}	119^{ab}	116^{b}	123^{a}	1.3	0.134	0.097	0.056	214^{a}	206^{b}	215^{a}	1.1	0.069	1.3	0.104
Water soluble carbohydrate	13.2^{bc}	69.5^{a}	18.3^{b}	19.5^{b}	17.4^{b}	8.0^{c}	1.94	<0.001	<0.001	<0.001	12.3^{b}	26.1^{a}	6.7^{b}	1.79	0.121	2.01	0.010
Ethanol	19.5^{a}	5.1^{e}	15.9^{b}	9.0^{d}	7.6^{d}	10.9^{c}	0.31	<0.001	<0.001	<0.001	3.3^{b}	2.4^{c}	13.4^{a}	0.13	<0.001	0.13	<0.001
Acids, g/kg DM																	
Formic	0.2^{b}	0.2^{b}	15.1^{a}	0.1^{b}	0.3^{b}	14.4^{a}	0.2	<0.001	0.154	0.513	0.4^{b}	0.4^{b}	17.4^{a}	0.13	<0.001	0.19	<0.001
Lactic	32^{e}	120^{a}	69^{bc}	85^{bc}	93^{b}	66^{d}	3.5	<0.001	0.019	<0.001	124^{a}	111^{b}	65^{c}	1.7	<0.001	1.3	<0.001
Acetic	7.8^{d}	8.7^{d}	19.6^{a}	16.2^{bc}	14.6^{c}	19.0^{ab}	0.7	<0.001	<0.001	<0.001	36.3^{a}	17.6^{b}	21.3^{b}	0.96	<0.001	0.89	<0.001
Propionic	1.0^{a}	0.3^{b}	0.4^{b}	0.3^{b}	0.3^{b}	0.3^{b}	0.1	<0.001	0.002	<0.001	0.4	0.4	0.4	0.02	0.530	0.02	0.777
Butyric	27.9^{a}	0.2^{b}	1.8^{b}	0.7^{b}	0.2^{b}	0.1^{b}	1.6	<0.001	<0.001	<0.001	0.2^{a}	0.2^{a}	0.1^{b}	0.01	0.121	0.23	0.199
Total VFA	36.9^{a}	9.2^{c}	21.8^{b}	17.3^{b}	15.2^{bc}	19.6^{b}	1.70	<0.001	0.002	<0.001	37.1^{a}	18.3^{b}	21.9^{b}	0.97	<0.001	0.79	<0.001
C4_C6	0.17^{a}	0.02^{b}	0.02^{b}	0.06^{ab}	0.02^{b}	0.13^{ab}	0.0	0.016	0.881	0.004	0.22^{a}	0.07^{b}	0.11^{b}	0.010	<0.001	0.023	0.002
Fermentation products[4]	88^{d}	134^{a}	106^{bc}	111^{b}	116^{b}	96^{cd}	2.3	<0.001	0.411	<0.001	165^{a}	132^{b}	101^{c}	1.9	<0.001	1.6	<0.001
LA/TA[5]	0.453^{b}	0.929^{a}	0.758^{a}	0.830^{a}	0.860^{a}	0.771^{a}	0.0382	<0.001	0.004	<0.001	0.770^{b}	0.859^{a}	0.749^{b}	0.0066	0.005	0.0056	<0.001
Liquid from a pneumatic press separation																	
PDM[6], g/kg	254^{c}	275^{b}	270^{b}	285^{a}	288^{a}	289^{a}	1.4	<0.001	<0.001	<0.001	271	271	275	2.2	0.518	1.8	0.564
Yield[7]	0.190^{a}	0.191^{a}	0.169^{a}	0.115^{b}	0.119^{b}	0.108^{b}	0.0092	0.445	<0.001	0.601	0.223	0.221	0.216	0.0062	0.549	0.0066	0.810
DM, g/kg	101^{b}	116^{ab}	118^{ab}	113^{ab}	121^{a}	132^{a}	4.0	0.001	0.008	0.726	119	130	134	3.9	0.031	3.7	0.999
DM retained in liquid[7]	0.076^{a}	0.080^{a}	0.074^{a}	0.046^{b}	0.050^{b}	0.050^{b}	0.0032	0.438	<0.001	0.623	0.097	0.106	0.105	0.0022	0.020	0.0029	0.379
CP, g/kg DM	265^{a}	206^{b}	195^{b}	205^{b}	191^{b}	206^{b}	3.9	<0.001	<0.001	<0.001	235^{a}	211^{b}	218^{b}	2.5	<0.001	3.1	0.019
CP retained in liquid[7]	0.162^{a}	0.137^{ab}	0.116^{b}	0.077^{c}	0.080^{c}	0.079^{c}	0.0052	0.003	<0.001	0.001	0.102	0.105	0.102	0.0016	0.394	0.0046	0.935

[1]C: control treatment without additive; FA: formic acid based additive (AIV 2 Plus, 5 l/t); and LAB: lactic acid bacteria strains (Sil-All 4×4+, 5 g/t). [2]SEM = Standard error of the mean. [3]Add: effect of additive; Wilt: effect of wilting period; Add*Wilt: interaction effect of additive and wilting period; Add*S: interaction effect of additive and species (timothy and red clover) after 24 hours wilting. [4]Total VFA + lactic acid + ethanol. [5]Lactic acid proportion of total acids. [6]Posterior DM of the silages used in the mechanical liquid-solid separation. [7]Proportion of one.
Means within the same row without same superscript differ (P<0.05) separately for timothy (4 and 24 hours wilting) and red clover.

There were interactions between additive and wilting of timothy silages for lactic, acetic, propionic and butyric acids (Table 3). Additives increased lactic acid of G4, while an opposite effect was observed for G24. Additives were efficient in decreasing concentrations of propionic and butyric acids of G4, while no effect was observed for G24. Silages containing additives had higher concentration of acetic acid, the incremental effect being even stronger for G4 than for G24. The joint evaluation of pH, ammonia N in total N and butyric acid clearly demonstrate a worse preservation quality for G4 than for G24, although wilting period was not efficient in increasing DM concentration of the herbage. Instead, it drastically decreased WSC concentration (Table 1). Likely, the microbial composition was positively affected during the wilting period, which favoured a better fermentation quality of the G24 silages in comparison to G4.

There were interactions between additive and forage species after 24 hours wilting for DM concentration and proportion of ammonia-N in total N (Table 3). Additives increased DM concentration of G24, but no effect was observed in RC silages. Additives also decreased ammonia proportion in total N of RC silages, while no improvement was achieved for G24 silages. The interaction between additive and forage species showed increasing concentration of acetic acid for G24 silages treated with additives, while the opposite effect was observed in RC.

A new DM analysis was conducted immediately before the mechanical liquid-solid separation of the de-frosted samples (Table 3), which showed higher values for G24 than for G4, resulting in lower liquid yields for G24 than for G4. There was no effect of additive on liquid yield of G4, G24 or RC silages. However, in a previous experiment (Rinne *et al*., 2018b), FA resulted in a lower liquid yield when using the same separation technique as in the current study. In general, liquid yield is highly dependent on silage DM concentration (Franco *et al*. (2018). Additives increased liquid DM concentration of G4, G24 and RC. Longer wilting period of grass increased liquid DM concentration but as the liquid yield was lower, the proportion of DM retained in the liquid was lower for G24 than for G4. Additives increased liquid DM concentration and proportion of DM retained in liquid for RC silages.

Equations proposed by Franco *et al*. (2018) to estimate liquid yield (liquid yield = 0.834 – 0.0022*DM) and liquid DM concentration (liquid DM = 31 + 0.3346*DM) with silage DM concentration in g/kg were used and results were regressed against observed values of the current study. Correlations were 0.66 ($P<0.001$) and 0.50 ($P=0.008$) between predicted and observed values, with adjusted R^2 of 0.42 and 0.22 for liquid yield and liquid DM concentration, respectively. It proves that the equations to predict the potential of a silage batch for a biorefinery process can be used with some confidence. There was an interaction between additive and wilting period for liquid CP concentration, as it drastically decreased with additive inclusion for G4, but only small decrease was observed for G24. G4 silages resulted in higher proportion of CP retained in liquid than G24. Additives did not affect CP retained in liquid in RC silages.

There was no interaction of additive and forage species for liquid yield, liquid DM concentration, and DM and CP retained in liquid.

Conclusions

The use of formic acid and lactic acid bacteria strains as additives on timothy and red clover forages positively impacted on chemical composition and fermentation quality of the silages.

Chemical composition influenced the liquid yield in liquid-solid separation of timothy silages while no effect of additives on liquid yield was observed. A shorter wilting period resulted in higher crude protein retained in the liquid fraction. Additives increased crude protein retained in liquid for red clover, while no effect was observed for timothy silages.

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References

Franco, M., Winquist, E. & Rinne, M., 2018. Grass silage for biorefinery – A meta-analysis of silage factors affecting liquid-solid separation. Proc. XVIII Intern. Silage Conf., Bonn, Germany, 24-26 July 2018 (in press). https://www.isc2018.de/

Rinne, M., Jalava, T., Stefanski, T., Kuoppala, K., Timonen, P., Winquist, E. & Siika-aho, M., 2018a. Optimizing grass silage quality for green biorefineries. Proc. Europ. Grassl. Fed. General Meet., Cork, Ireland 17-21 June 2018 (in press). www.europeangrassland.org

Rinne, M., Timonen, P., Stefanski, P., Franco, M., Winquist, E. & Siika-aho, M., 2018b. Grass silage for biorefinery – Effects of type of additive and separation method. Proc. XVIII Intern. Silage Conf., Bonn, Germany, 24-26 July 2018 (in press). https://www.isc2018.de/

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M., 2016. Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass Forage Sci. 71, 458-471.

Grass silage for biorefinery - dairy cow responses to diets based on solid fraction of grass silage

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Introduction

There is a growing need to develop alternatives to non-renewable fossil fuel-derived products. A Green Biorefinery involves sustainable processing of green biomass into marketable products and energy. Innovative feed products, volatile fatty acids, amino acids and fibres can be used as such or as raw materials for further processes (Wilkinson & Rinne, 2018). Furthermore, grass contains crude protein (CP), sugars and minerals that can be used in green biorefineries. A constant year-round supply and predictable quality can be achieved by storing grass as silage. Separation of cell wall contents (i.e. press-juice) from plant structural framework (i.e. fibre-rich press-cake) involves the most technical concepts of a Green Biorefinery (McEniry & O´Kiely, 2013). Yield and composition of the liquid and solid fractions varies greatly, depending on raw material quality and processing technology (Franco et al., 2018). Fractionation offers a possibility to produce feedstuffs for both ruminants and monogastric farm animals, and even food for humans.

There is limited information of the impact of fractionation on nutritive value of separated solid fraction in ruminant diets. Increased fibre content, reduced soluble CP and mineral contents simultaneously with decreased digestibility have been reported. As a result, especially at later harvest dates, the use of solid fraction in diets of ruminants has been limited (McEniry & O´Kiely, 2013). Higher milk production with diets containing solid fraction, as compared to silage was reported by Damborg et al. (2017).However, the clover silage used was harvested a week later than the clover used to make the silage for subsequent separation into liquid and solid fractions.

The aim of this experiment was to evaluate the effect of silage solid fraction fed to dairy cows on intake, ruminal fermentation, digestibility, milk yield and milk composition. The hypothesis of the present study is that there is no reduction in milk production in a diet that contains silage solid fraction when compared to the original silage due to increased intake, which would compensate a decreased digestibility of the diet.

Materials and methods

The effects of diets based on silage solid fraction were studied using 24 multiparous Nordic Red cows. Four of the cows were ruminally fistulated. Cows weighed 661 kg (s.d. 78.2) and were 125 days (s.d. 27.7) in milk at the beginning of the experiment. The experimental design was an imbalanced change over design (three diets and two 21-day periods). The cows were kept in a free-stall barn, fed forage at 07:00, 13:00, 16:00 and 18:00 h and milked at 07:00 and 17:00 h in a milking parlour.

All cows were offered a grass silage of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) from primary growth harvested on 21st and 22nd of June 2017. Grass was slightly wilted, precision chopped and ensiled into two separate clamps using a formic acid based additive (AIV 2 Plus Na, Eastman Chemical Company, Oulu, Finland) at a rate of 5 l/t. The same silage was fractionated using a Haarslev twin screw press (more details are given in Stefański et al., 2018). Silage solid fraction was produced every week on two subsequent

days at the beginning of the week and was treated with a formic and propionic acid based preservative (AIV Ässä Na, Eastman Chemical Company, Oulu, Finland) at a rate of 5 l/t mixed with approximately 10 l of water to improve the aerobic stability. No signs of heating of the solid fraction could be detected during the study. The average temperature outdoors during the experiment was -4.8 °C.

Solid fraction was used as a feedstuff for dairy cows and the press juice was given to pigs in a farm scale experiment (results not presented here). The silage solid fraction was fed to the cows using following treatments: original grass silage (F0), 0.75 original grass silage and 0.25 solid fraction (F25) and 0.50 original grass silage and 0.50 solid fraction (F50). The proportions were formulated on a dry matter (DM) basis. The silage was fed using an automatic feeding wagon (TR Feeding robot, Pellon Group Ltd, Ylihärmä, Finland). For F25 and F50, silage and solid fraction were mixed in a TMR wagon. The mixed forage was delivered by hand at the same time as the automatic wagon fed F0. In addition, all cows were given a total of 13 kg/d of a standard concentrate partly from automatic feeders (Pellon Group Ltd, Ylihärmä, Finland) and partly in the milking parlour. Cows were fed forage *ad libitum* and refusals were weighed daily during the last week of both periods. All three forages were sampled daily during the last week of both periods and samples were frozen (-20^{o} C) to create a composite sample. Samples were analysed at Luke laboratory using standard methods.

Cows were milked in a 2 × 6 auto-tandem milking parlour. Milk yield was recorded automatically for every milking. Milk samples were taken on last two days of both periods separately for morning and evening milkings. Analyses of milk fat, protein, lactose and urea were done by an infra-red analyser (Milko-Scan 605; Foss Electric, Hillerod, Denmark) at the laboratory of Valio Ltd. (Seinäjoki, Finland). Yield of milk constituents was calculated as a weighted mean according to milk yield.

Rumen fluid was sampled during the third day in the last week of both periods. Samples were taken 0, 3 and 6 h after feeding from four rumen fistulated cows and analysed for pH, NH_3 and volatile fatty acids (VFA). Two of the cows were on diet F0 and two on diet F50. Faeces of all cows were spot sampled twice a day for 4 days. The acid insoluble ash method was used for diet organic matter digestibility (OMD) measurements.

Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability with dietary treatment as fixed effect and cow as random effect. Linear (P_L) and quadratic (P_Q) effects of addition of silage solid fraction were evaluated using orthogonal polynomial contrasts.

Results and discussion

The feeds used in the current experiment had a typical composition for those types of feeds. Dry matter and neutral detergent fibre (NDF) contents increased with increasing solid fraction, while CP content decreased (Table 1). Fractionation of silage has resulted in similar results also in the experiment of McEniry and O`Kiely (2013). Similar changes have been detected due to delayed harvest date of grass silage (Rinne et al., 1999) but in that case, also the fibre digestibility decreased while in the present study, only the amount of fibre increased. Damborg et al. (2017) found a higher CP concentration and D-value in fractionated solid fraction compared to unfractionated grass-clover. The reason for this was likely a six days earlier harvest time for the fractionated material.

Table 1 Composition of the experimental concentrates and forages with different proportions of silage solid fraction fed to dairy cows

	Concentrate feeds[1]		Silage solid	Silage solid fraction proportion[2]		
	BC	MPC	fraction	F0	F25	F50
Dry matter (DM; g/kg)	870	865	432	210	242	284
In DM (g/kg)						
Ash	76	79	42	69	63	57
Crude protein	219	224	106	144	133	122
Neutral detergent fibre	237	193	711	589	611	614
Metabol. energy (MJ/kg DM)	12.1	12.5	10.7	10.8	10.8	10.7
AAT[3] (g/kg DM)	124	122	76.2	81.1	79.8	78.9
PBV[4] (g/kg DM)	43.5	51.0	-7.8	22.8	14.0	5.2

[1]BC, basal concentrate, MPC, concentrate offered in the milking parlour
[2]F0 (original grass silage), F25 (0.75 original grass silage and 0.25 solid fraction) and F50 (0.50 original grass silage and 0.50 solid fraction). Proportions defined on DM basis
[3]Amino acids absorbed from the small intestine (Luke, 2018)
[4]Protein balance in the rumen (Luke, 2018).

Cows fed F25 managed to increase intake despite of an increased fibre content of the diet. Cows fed F50 were unable to further compensate, which resulted in a quadratic effect on DM intake (P_Q=0.001; Table 2). An increase in silage NDF resulted in a reduction of DM intake in cows fed silages prepared at 1-week intervals (Rinne et al., 1999), but with progressing growth also the digestibility of fibre decreased which explains the intake responses. In the experiment of Damborg et al. (2017) cows fed solid fraction from clover silage had higher intake compared to the cows fed grass-clover silage. According to Damborg et al. (2017), the mechanical extraction treatment may result in enhanced ruminal degradation. This effect may partly explain the increased total DM and forage intake of F25 and F50 in the present experiment. Diet OMD decreased quadratically (P_Q=0.001) with increasing silage solid fraction proportion, but numerically the differences were small. There were no differences in concentrate intake between the diets.

There was a linear decreasing effect of increasing silage solid fraction on energy corrected milk (ECM) production (P_L = 0.043; Table 2). Cows fed F50 had a lower ECM production than cows fed only silage while cows fed F25 managed to maintain a similar production level as F0, but the quadratic effect did not reach significance (P_Q=0.161). Reduction in silage digestible organic matter (D-value) has been reported to reduce milk and ECM production (Huhtanen & Nousiainen, 2012). There was a linear decreasing effect of the increasing silage solid fraction to the production of milk fat (P= 0.050) and protein (P= 0.020). Rinne et al. (1999) found a substantial, curvilinear decrease in daily milk protein and fat outputs when maturity of the grass ensiled increased.

There were no differences in milk composition between diets in the current study, though higher milk protein concentration has been associated with higher digestibility of silages (Huhtanen & Nousiainen, 2012). This may have been because fibre quality of the solid fraction was the same as in the silage as opposed to a quality decrease of grass fibre with increasing maturity. In terms of milk production efficiency, using original silage resulted in highest values (P_Q<0.001) for kg ECM/kg DM, metabolizable energy (ME) and even nitrogen use efficiency.

Ammonia N concentration and pH in rumen fluid were similar among experimental diets (Table 3). There were no interactions between the diet in the proportions of volatile fatty acids at 0, 3 and 6 hours after feeding. This indicates similar rumen conditions for F0 and

Table 2 Intake, organic matter digestibility, milk production and milk production efficiency of dairy cows fed different proportions of silage solid fraction

	Silage solid fraction[1]				Statistical significance[2]	
	F0	F25	F50	SEM	Lin	Quad
Feed intake (kg dry matter (DM)/day)						
Total	23.9	26.0	25.7	0.19	<0.001	<0.001
Forage	12.7	14.8	14.6	0.18	<0.001	<0.001
Concentrate	11.2	11.2	11.1	0.04	0.299	0.160
Nutrient intake per day (kg)						
Crude protein	4.3	4.5	4.2	0.02	<0.001	<0.001
Organic matter	22.3	24.6	23.8	0.13	<0.001	<0.001
Neutral detergent fibre	10.0	11.7	11.6	0.08	<0.001	<0.001
Metabolizable energy (MJ)	275.7	300.3	288.7	2.46	0.002	<0.001
Organic matter digestibility	0.724	0.717	0.719	0.0424	<0.001	<0.001
Production per day						
Milk (kg)	37.2	37.5	36.2	0.38	0.088	0.114
Energy corrected milk (ECM, kg)	39.8	39.9	38.4	0.42	0.043	0.161
Fat (g)	1706	1706	1643	20.4	0.050	0.244
Protein (g)	1326	1332	1276	13.5	0.020	0.090
Lactose (g)	1620	1633	1586	18.1	0.214	0.210
Milk composition (g/kg)						
Fat	46.0	45.5	45.6	0.37	0.461	0.956
Protein	35.7	35.5	35.4	0.16	0.124	0.997
Lactose	43.5	43.5	43.8	0.10	0.102	0.341
Urea (mg/100ml)	32.7	33.7	32.2	0.650	0.608	0.146
Efficiency of milk production						
Nitrogen use efficiency[3]	302	290	299	2.3	0.410	0.002
ME efficiency[4]	0.601	0.538	0.548	0.0052	<0.001	<0.001
kg ECM/kg DM intake	1.66	1.51	1.52	0.014	<0.001	<0.001

[1]F0 (original grass silage), F25 (0.75 original grass silage and 0.25 solid fraction) and F50 (0.50 original grass silage and 0.50 solid fraction). Proportions defined on DM basis.
[2]Lin = linear effect of the amount of silage solid fraction, Quad = quadratic effect of the amount of silage solid fraction
[3]N excreted in milk / N intake
[4]Metabolizable energy (ME) excreted in milk / (ME intake – ME for maintenance).

F50. Replacing 0.5 of silage with silage solid fraction increased the proportion of acetic acid (P = 0.020) and a tended to decrease the proportion of butyric acid (P = 0.056). The proportion of rumen acetate has been found to increase with increasing fibre concentration due to increased maturity of ensiled grass (Rinne et al., 1999). This change in the proportion of acetic acid in the current study probably results from an increased amount of NDF in the F50 treatment.

Table 3 Rumen fermentation of dairy cows fed different proportions of silage solid fraction

	F0[1]	F50	SEM	Statistical significance
pH	6.09	6.12	0.075	0.777
Ammonia N (mmol/l)	7.67	6.02	0.520	0.155
Total volatile fatty acids (mmol/l)	133.5	136.1	4.54	0.726
Proportions of volatile fatty acids				
Acetic acid	0.659	0.667	0.0008	0.020
Propionic acid	0.178	0.180	0.0024	0.561
Butyric acid	0.125	0.119	0.0557	0.056

[1]F0 (original grass silage) and F50 (0.50 original grass silage and 0.50 solid fraction). Proportions defined on a DM basis.

Conclusions

There was an increase in DM intake and a reduction in ECM production as a response to increasing proportion of silage solid fraction in the diet of dairy cows. Thus, the silage solid fraction was not as good as the original silage for high producing dairy cows, but the magnitude of the effects was relatively small. Farm scale biorefinery could be an alternative to improve usability of silage. Silage solid fraction could be used as a feed for heifers or dry cows and the nutrient rich silage juice could be used for dairy cows to fortify total mixed rations. Amino acids included in the liquid faction could increase the amount of intestinally absorbable amino acids if liquid flow was sufficient to enable a high proportion of rumen escape. Alternatively, if there is a higher added value for silage liquid in pig feeds or other innovative uses, the production potential of silage solid fraction is only marginally reduced for use in ruminant production.

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References

Damborg, V., Jensen,S. & Weisbjerg, M., 2017. Dairy cows do not consider pulp from green protein extraction a waste stream. Biovalue Ann. Meet., Foulum, Denmark, September 2017.

Franco, M., Winquist, E. & Rinne, M., 2018. Grass silage for biorefinery – A meta-analysis of silage factors affecting liquid-solid separation. XVIII Intern. Silage Conf., 24-26 July 2018, Bonn, Germany (in press). www.isc2018.de

Huhtanen, P. & Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Livest. Sci. 148, 146-158.

Luke, 2018. Feed tables and nutrient requirements, Natural Resources Institute Finland (Luke). www.luke.fi/feedtables.

McEniry, J. & O´Kiely, P., 2013. The estimated nutritive value of three common grassland species at three primary growth harvest dates following ensiling and fractionation of press-cake. Agric. Feed Sci. 22, 194-200.

Rinne, M., Jaakkola, S., Kaustell, K., Heikkilä, T. & Huhtanen, P., 1999. Silages harvested at different stages of grass growth *v.* concentrate foods as energy and protein sources in milk production. Anim. Sci. 69, 251-263.

Stefański, T., Franco, M., Savonen, O., Jalava, T., Winquist, E. & Rinne, M. 2018. Grass silage for biorefinery – Separation efficiency and aerobic stability of silage, solid and liquid fractions. Proc of the 9th Nordic Feed Sci. Conf., Uppsala, Sweden, June. 2018 (in press).

Wilkinson, J.M. & Rinne, M., 2018. Highlights of progress in silage conservation and future perspectives. Grass Forage Sci. 73, 40-52.

Forages – a local protein source for growing pigs

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Introduction

Forages efficiently utilise the short growing season in the Nordic countries and can produce high yields. Protein crops such as rapeseeds, faba beans or soybeans require a mild climate and good growing conditions. There is an increasing interest in utilising forages as a protein source for e.g. growing pigs. The juice fraction of forages consists mainly of plant cell content is rich in proteins, water-soluble carbohydrates, minerals, organic acids and lipids (Kamm & Kamm, 2014), and can be easily digested. Typically, 30 to 50% of the dry matter (DM) and 40 to 60% of the crude protein are recovered in the juice fraction (Hermansen et al., 2017). Juice dry matter content can vary considerably (3.6 to 32%, O'Keeffe et al., 2011), but typical values vary between 5 and 7%. Several pilot-scale green bio-refineries have been established and have develop a wide spectre of bio-products but so far, bio-refining of forages has not been commercially implemented (Xiu & Shahbazi, 2015; Kamm et al., 2016). Small-scale bio-refining systems that require minimum processing and transport may be a viable alternative to integrated bio-refineries. However, experiences on storage and feeding fresh forage juice to growing pigs on a commercial scale is scarce.

The aim of the project was to assess the effects of including forage juice preserved with formic acid in the diet of growing pigs on growth rate and meat quality on a commercial farm. Gaining experience in production, storage and feeding of forage juice on farm level were also important goals.

Materials and methods

Animal feeding and management

A commercial farm located in Central East Norway (60˚N, 11˚E), producing conventionally fed slaughter pigs, hosted the study. A total of 160 crossbred piglets were blocked according to biological litter (litter size on average 15, SD 3.0), (biological) sex and weight (average live weight 43 kg, SD 5.5, and average age 82.6 days, SD 2.9), and allocated randomly to eight groups into four test and four control groups of 20 animals each. Each group was housed in a separate pen. The pigs were liquid-fed according to a feed curve with increasing feed intake per pig per day. The Control group was offered a diet with a protein rich (16% crude protein) commercial concentrate feed mixture mixed with water. The Test group was offered a diet with 10% forage juice and 90% of an adjusted concentrate feed mixture on DM basis, all mixed with water to the same liquid content as the diet offered in the Control group (20% DM). The Test mixture was created to ensure that the two diets were isonitrogenous and isoenergetic with similar amino acid composition, based on analyses of forage juice produced in a previous pilot study (Mutsaers, 2016). The contribution of major ingredients and chemical composition of the two concentrate mixtures are in Table 1. The concentrate mixtures were produced by Felleskjøpet Agri, Norway. The percentage of soybean meal was 4.9 and 7.6 in the Test and Control diets, respectively. Feed consumption was recorded at two

times in two adjacent pens per group during a 58-day period. Forty pigs of the same sex, 20 in each pen, had a common feeding trough. All animals were weighed three times at the start, mid-way, and at the end of the period (Table 2). After the feeding experiment was finished (day 58 and until the day of slaughter), both groups were offered the Control diet. The length of this period was 4, 13 or 21 days to compensate for differences in body weight at the end of the feeding experiment. This is normal procedure in the Norwegian pig industry, due to the demand for uniform carcasses.

Live weight at the day of slaughter was estimated by dividing slaughter weight by a constant carcass percentage of 68. The pigs were finished at an average of 73 kg (SD 4.2) slaughter weight, with an associated live weight of 107 kg.

Lean meat percentage, slaughter weight (n=75 pigs in each group) and a score of stomach ulcers (n=30) were measured at a commercial slaughterhouse (Nortura, Rudshøgda, Norway). This data was registered at an individual level and connected to the records from the feeding experiment. Some extra measurements were done for pigs slaughtered the first week, four days after the feeding experiment ended. Meat and fat samples for those pigs were transported to a pilot plant at Animalia meat research institute (Oslo, Norway) where some meat and fat quality analyses were conducted. Fat colour (n=30) was measured using a colorimeter (Minolta Chroma Meter CR-400), and intramuscular fat percentage (n=3) was analysed with a Near Infrared spectroscopy equipment (Foodscan, FOSS, Denmark) (Gjerlaug-Enger, et al., 2010). In addition, fatty acid composition of subcutaneous fat (n=5) was analysed by Eurofins (Moss, Norway).

Effects of the Test diet on feed conversion ratio, average daily weight gain, lean meat percentage, mortality and stomach ulcers were analysed statistically using a GLM and the mixed model procedures in SAS (SAS, 2011). The model included overall mean, pig starting weight, effect of block, effect of treatment and error term (double pen, 40 pigs).

Grass juice production

The first cut from 2.3 ha of organically managed ley (80% timothy and meadow fescue, 20% red clover) located at the pig farm was harvested by a flail harvester, transported by a forage wagon to the farm and fed via a conveyor belt to a RhineTech screw press (Arnhem, Netherlands). At harvest, the grasses were in the phenological stage of heading. The work was conducted over a three-day period in mid June and the dry matter content of the crop averaged 18%. The press screw had a capacity of 8 tonnes per hour. Fresh forage juice was pumped into 1000-L polyethylene containers. An acid based additive (75% formic acid, 8% sodium formate) was applied, partially at the flail harvester, and partially into the juice containers. The pulp was baled and wrapped with stretch film by use of an Orkel MC850 Compactor (Fannrem, Norway), and sold to a nearby dairy farm.

Three weeks after the feeding study had started, two samples of juice were collected from the containers. The samples were kept frozen (-20°C) until chemical analyses were performed at SYNLAB/ALcontrol Laboratories, Stjørdal, Norway.

Table 1 Relative proportion of major ingredients and chemical composition of the feed mixtures (calculated) and the grass juice (n=2)

	Feed mixture		Forage juice
	Test	Control	
Main ingredients, g/kg			
Barley	403	444	-
Oat	284	250	-
Faba beans	80	70	-
Soy, extracted	55	76	-
Rape seed meal	102	90	-
Dry matter, g/kg	866	871	44
Chemical composition, g/kg of DM			
Crude protein	160	161	204
Ash	44	45	191
Crude fat (ether extract)	58	48	161
Crude fibre	62	61	25
Mono- and disaccharides	NA[1]	NA	78
Lysine	11.1	10.4	10.2
Threonine	7.4	7.2	9.0
Tryptophane	2.3	2.2	2.4
Methionine+Cysteine	7.0	6.8	BDL[2]
Phosphorus	45	45	15
Potassium	64	65	61

[1]Not analysed. [2]Below detection limit (14.3 g/kg of DM).

Results and discussion

A total yield of 52000 L of forage juice with a DM content of 5% was achieved. Roughly estimated, juice output was 20% on a DM basis. Both yield and DM content were lower than reported from other studies such as Hermansen et al. (2017). The reason for the moderate juice yield may have been due to the relatively late stage of plant development at harvest (Mutsaers, 2016), rainfall during harvest (giving low DM%, but not low yield), and possibly related to the equipment used. The concentration of fat in the juice was higher than in other studies (Houseman et al., 1976; Tenorio et al., 2016). Fermentation quality of the juice was not documented by analyses. According to measurements with Lacmus paper, pH was approximately 3.0. After three months of storage in the containers, mould progressively developed in the upper layer of the juice. In order to avoid mould, the containers were carefully emptied under restricted mixing. The smell of the juice offered to the pigs was fresh, and mycotoxin analyses warranted that the juice could be used without risk to animal health. No signs of appetite loss were observed. However, a decision was made to end the feeding experiment after 58 days even though not all pigs had reached target slaughter weight.

At the start of the feeding experiment, pigs in Test and Control group were 43.0 and 42.9 kg, respectively, and average live weight at the end of the experiment was 94.8 and 96.1 kg for the Test and Control group, respectively.

Daily live weight gain of the pigs was moderate in this study (on average 933.5 g/day for growth from test start to slaughter day (SD 100.0). Average level of finisher growth in Norway was 996 g/day in 2016 (Norsvin, 2016), with lower start weight (32.4 kg) and higher slaughter weight (81.6 kg) compared with our experiment. This difference may have been due to the relatively high initial weight of the piglets at the start of the experiment and

restricted space relative to the number and size of animals in the pen. Pigs were given larger pens halfway into the finisher period, but problems with tail biting indicated that the animals might have been stressed. Mortality was 2.5% for both groups, which is low, but slightly higher than the average reported on national level for Norwegian pig production for the period 2012-2016 (1.9%) (Norsvin, 2016).

Average daily weight gain did not differ between groups during the first 17 days, but from day 18 to 58 it was lower ($P = 0.05$) for the Test-diet compared to the Control-diet. From day 58 to slaughter, all pigs received the Control-diet, and no significant differences between the groups were found (Table 2). According to Barber et al. (1979), forage juice could replace up to 3.5% of the dietary protein in the control feed without negative effect on weight gain. Preserved with HCl and sodium metabisulphite, and heated to 85°C for a few seconds the forage juice (pH 3) could be stored for several weeks with negligible loss in that study.

Feed conversion rates, calculated for the 58-day period were similar to average records in Ingris (2012-2015: 23.8 MJ/kg body weight gain) and did not differ between groups. Nor did meat percentage differ between the groups. However, the omega-6:omega-3 ratio of the subcutaneous fat was significantly more favourable in the Test-group compared to the Control-group. Forages have a high proportion of the omega-3 fatty acid alpha-linolenic acid, which is most likely the reason for the changed ratio. Average number of gastric ulcers was 1.5, indicating good stomach health in both groups.

Table 2 Growth rate, body weight at end of the study, slaughter weight, meat quality and product quality of pigs fed a wet pig feed (Control) or an isonitrogenous and isoenergetic Test feed including 10% forage juice on dry matter basis (n=2)

	Test	Control	Difference	SEM[1]	*P*-value
Feed conversion ratio, MJ/kg body weight gain[2]	24.3	23.2	1.1	0.97	NS
Average live weight gain, g/day					
Day 1 to 17	808	801	7	18.7	NS
Day 1 to 58	892	917	-25	10.9	0.11
Day 18 to 58	927	965	-38	13.5	0.05
Day 58 to slaughter[3]	1090	1148	-58	49.1	NS
Mortality, %	2.5	2.5	0	-	NS
Meat and fat quality					
Lean meat, %	60.6	60.5	0.1	0.28	NS
Omega-6:omega-3 ratio	8.58	9.69	-1.11	0.12	<0.001
Stomach ulcer, number	1.53	1.45	0.08	0.26	NS
Intra muscular fat (NIR), %	2.08	1.69	0.39	0.16	NS
Fat colour L (whiteness)	77.1	77.2	-0.10	0.24	NS
Fat colour a (redness)	3.72	3.35	0.37	0.19	NS
Fat colour b (yellowness)	5.29	5.15	0.14	0.13	NS

[1]Standard error of means; [2]Constant carcass portion: 68%; [3]From day 58 to slaughtering all pigs were fed the Control diet.

Forage juice could be successfully produced and was well preserved for storage up to three months. However, the study revealed that development of mould is a challenge that must be solved in order to store grass juice for longer periods.

Despite a moderate inclusion level of forage juice in the Test-diet, a reduction in daily weight gain was observed between day 18 and 58 (P=0.05). This may be explained by a lower feed

value than anticipated. However, during the first 18 days, weight gain was not negatively affected. This indicates that the nutritional value may have been depreciated over time. The observation of mould may be an indicator of decreasing quality, though not documented. Feeding well-preserved forage juice requires further research.

Only one sample (three replicates) of the pulp silage was analysed chemically. However, it indicated good feeding value for dairy cows (32% DM, 14.6% crude protein, 62.7% NDF, 74.0% OMD, pH 5.2, NEL 6.36 MJ/kg of DM).

Conclusions

The inclusion of 10% forage juice (on DM basis) in a wet feed diet did not affect daily live weight gain in growing pigs, but reduced live weight gain with 38 g/day in finishers. Feed conversion rate did not differ between the groups, whereas the omega-3:omega-6 ratio was more beneficial for human nutrition in fat from pigs fed forage juice than from pigs fed concentrate feed only. Improved preservation methods for forage juice are needed.

References

Barber, R.S., Braude, R., Mitchell, K.G., Partridge, I.G. & Pittman R.J., 1979. Value of lucerne juice and grass juice as sources of protein for the growing pig. Anim. Feed Sci. Techn. 4, 233-262.

Gjerlaug-Enger, E., Aass, L., Ødegård, J. &Vangen, O., 2010. Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. Animal, 4, 11, 1832-1843.

Hermansen, J.E., Jørgensen, U., Lærke, P.E., Manevski, K., Boelt, B., Jensen, S.K., Weisbjerg, M.R., Dalsgaard, T.K., Danielsen, M., Asp, T., Amby-Jensen, M., Sørensen, C.A.G., Jensen, M.V., Gylling, M., Lindedam, J., Lübeck M. & Fog E., 2017. Green biomass - protein production through bio-refining. DCA Report No. 093, February 2017.

Houseman, R.A., 1976. The utilization of the products of green-crop fractionation by pigs and ruminants. Proc. Nutr. Soc. 35, 213-220.

Kamm, B. & Kamm, M., 2004. Principles of biorefineries. Appl. Microbiol. Biot. 64, 137-145.

Kamm, B., Schönicke, P. & Hille, Ch., 2016. Green biorefinery - Industrial implementation. Food Chem. 197, 1341-1345.

Mutsaers, W., 2016. Protein fra norsk gras som erstatning for soyaprotein i svinefôr. Norsk landbruksrådgivning, report. 46 p. Available at https://innlandet.nlr.no/media/ring/28541/Protein%20fra%20gras%20til%20grisefor%20web%20.pdf.

Norsvin, 2016. Ingris Årsstatistikk 2016. 40 p. Available at https://norsvin.no/Media/Public-Media/Files/Ingris-AArsstatistikk-2016.

SAS Institute Inc., 2011. SAS/STAT® 9.3 User's Guide. Cary, NC.

Tenorio, A.T., J Gieteling., de Jong, G.A.H., Boom, R.M. & van der Goot, A.J., 2016. Recovery of protein from green leaves: Overview of crucial steps for utilisation. Food Chem. 203, 402-408.

Xiu, S. & Shahbazi, A., 2015. Development of Green Biorefinery for Biomass Utilization: A Review. Tr. Ren. Energy. 1, 4-15.

Techniques to measure ruminal protein degradation – a review

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Introduction

A common goal of all modern ruminant feed evaluation systems is to estimate microbial and feed amino acid (AA) uptake by the animal under a set of given conditions. Important information required to estimate feed AA contribution includes protein degradation rate, outflow rate and intestinal digestibility of undegraded proteins. Most ration formulation models such as CNCPS (Sniffen et al., 1992) and NorFor (Volden, 2011) are static, based on rumen dynamics concepts and, hence, require nutrient degradation rates. Simple time-independent models are fitted to degradation curves to estimate rate constants for use as feed parameter inputs. The validity of these models are often questionable but will not be further examined here. Instead, actual methods for measuring protein degradation are reviewed with particular reference to their shortcomings. Focus will be on observed and perceived problems.

Accuracy of protein degradation assays and in vivo measurements

No "gold" standard exist to estimate accuracy of protein degradation. The nearest we could wish for are in vivo estimates. In vivo measurements of feed protein flow to the duodenum have been made by a variety of methods (e.g. Lebzien et al., 1996, Huhtanen et al., 2014). But so far, there are no reliable techniques for estimating vivo protein degradation rates. Crude protein in a diet contains multiple fractions with different degradation and passage characteristics. Additional problems consist of separating post-ruminal bacterial protein from feed protein, uptake of small peptides and AAs from the rumen and that flow from the rumen is not easily monitored.

Rumen ammonia levels in vivo will indicate crude differences in degradability among protein sources. But, levels are affected by microbial growth as well as by uptake and flow from the rumen which are difficult to correct for. A possible alternative was proposed by Dewhurst et al (2001) by use of H_2S concentrations in rumen headspace gas. The problems of this method differ from those associated with rumen ammonia levels and will be discussed below in connection with in vitro H_2S measurements.

Criteria for true microbial protein degradation

The aim of estimating protein degradation is to enable prediction of feed AA flow to the small intestine. As soon as the protein has been hydrolysed in the rumen and absorbed by the microflora, it should be regarded as degraded (Ahvenjärvi et al., 2009) and be unavailable for passage out of the rumen. A small proportion may, however, be excreted back into the rumen fluid (Broderick and Craig, 1989) but could probably be ignored. If we settle for a two-step system (hydrolysis and absorption) to define degradation, accurate methods must encompass these steps. In a study of in vitro fermentation of casein, Broderick and Craig (1989) identified three extracellular N pools (protein, peptides and AAs) in bacteria, one intracellular (AAs) and one total ammonia pool. Later, a model based on these data was made by Udén (2000) showing that degradation rate of casein to peptides was similar to microbial uptake of peptides. This means that uptake cannot be ignored.

Many methods assume that all N which is soluble in rumen buffer or rumen fluid can be regarded as 100% rumen degradable (Krishnamoorthy et al, 1983). Appreciable amounts of soluble proteins escape rumen fermentation and even free AAs can leave the rumen at a level

of 10% (Volden et al., 1998). Therefore, accurate measurements of protein degradation will need to fulfil the criteria stated earlier as closely as possible in order to be accurate.

Measuring degradation in porous bags

Porous bags have been used in situ by e.g. Mehrez and Ørskov (1977). Loss of N from the bag over time is used to estimate degradation rate as all losses are assumed to have an infinite rate of degradation. Attempts to correct for particular loss of protein have been made by e.g. Madsen et al. (1995) but have not been widely applied. The bag technique is the dominating method for estimating rumen protein degradability even though it is labour intensive, cannot handle soluble proteins, has inherent problems with microbial N contamination of bag residues and the environment inside the bag may also differ from the rumen itself. Buffer soluble N may be as high as 80% of total N in peas and lupines (Hedqvist and Udén, 2006) and microbial N contamination requires the use of ^{15}N to correct rumen undegraded residues (Krawielitzki et al., 2006; Kamoun et al, 2014; González et al., 2018). This becomes particularly important when analysing low-protein feeds. However, Machado et al. (2013) has proposed an exponential equation to estimate microbial contamination. It is based on time in the rumen and crude protein (CP) content of the feed and may be able to replace the use of ^{15}N but does not solve any of the other problems with the method. However, the method does not fulfil criteria for true degradation.

Measuring degradation in vitro

Enzymatic degradation

Solubility in buffer and detergents were used originally in the CNCPS model by Sniffen et al. (1992) to separate crude protein into five fractions. These fractions were linked with their enzymatic degradation rates in vitro, derived from the method developed by Krishnamoorthy et al. (1983) and has constituted the basis for protein evaluation in CNCPS (Sniffen et al., 1992). Low cost and simplicity were attractive features with this system but the similarity between fungal enzymatic and bacterial protein degradation is questionable. Solubility is measured by filtration after enzymatic degradation as an estimate of degradation which doesn't account for remaining soluble proteins, peptides and AAs. And, no bacterial uptake of peptides and AAs is accounted for, which means that it does not fulfil the criteria above.

Bacterial degradation

Several methods have employed rumen bacteria. Inhibitors of bacterial protein synthesis (but not hydrolysis) was used by Broderick (1987) in his so called inhibited in vitro (IIV) system. Bacterial release of AAs and ammonia were used as measure of degradation, which reasonably well fulfils the criteria mentioned earlier. Incubations up to 6 h were used for rate estimates of log transformed data. However, exponential functions have been difficult to fit (Udén and Broderick, unpublished), which indicates time-dependent rates. The reason for this is likely caused by a continuous loss of microbial activity due to bacterial deaths.

An ingenious in rumen vitro method was developed by Raab et al. (1983) which theoretically fulfils criteria above. Degradation is estimated from release of ammonia and total gas using graded levels of carbohydrates. The method requires several levels of carbohydrates and multiple time measurements to estimate rates of degradation. With increasing carbohydrate level, ammonia concentrations decrease as more ammonia is used for bacterial growth whereas more gas is produced from carbohydrate fermentation. By extrapolating to zero gas production, ammonia release at zero bacterial metabolism is estimated. In its original form, it is an expensive, complex and time consuming method unless it can be automated. Improvements to this method have been made by Karlsson et al. (2009) and Lorenz et al. (2011) by continuous ammonia measurements and by removable of protozoa to reduce

bacterial recycling, respectively. Huhtanen (unpublished) has criticized the method. One important issue was that, as the ratio of ammonia release to gas production will differ with length of incubation due to an increasing microbial population, ammonia estimates at zero gas production will not be correct. Further work should possibly be able to overcome this and other shortcomings.

Edmunds et al (2012) estimated utilizable CP (uCP) by a modified Hohenheim gas test where samples are incubated for two time periods in vitro with rumen fluid and excess ammonia. Residual non-ammonia N (NAN) is recorded and blank corrected. Effective uCP is then calculated from the NAN slopes, zero hour intercepts over the two incubation times and from chosen passage rates (kp; /h) as:

Effective uCP (g/kg DM) = Intercept + Slope*Ln(1/kp),

Derivation of the equation was not explained by the authors, however. Inserting in sacco derived rumen undegradable protein and metabolizable energy values into an empirical equation (Eq. 9) from Lebzien et al. (1996) showed a relatively good correspondence with uCP data. This equation of Lebzien et al. (1996) was based on a large number of duodenal measurements. Edmunds et al (2012) rightly commented that it is difficult to judge accuracy of this method in the absence of a "gold" standard. One, often overlooked, problem with this and other in vitro methods is blank corrections. Ammonia production from buffer and rumen inocula is unlikely to be similar in flasks with and without substrate, due to more microbial recycling and lysis of cells in blanks (Lorenz et al., 2011).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis has been used in a number of studies (e.g. McNabb et al, 1994; Messman and Weiss, 1994) to study the continuous decrease (and increase) of specific feed proteins (13 to 82 kDa) after incubation in rumen fluid. No degradation rates were estimated by Messman and Weiss (1994). However interesting, this type of method cannot be regarded to fulfil the criteria for protein degradation, as stated above, as the complete metabolism of feed proteins was not monitored.

High-speed centrifugation and TCA was used to isolate soluble proteins from bacterial and insoluble feed proteins after in vitro rumen incubations which made it possible to measure their disappearance in solution (Hedqvist and Udén, 2006). The method used only a total of 30 ml of fluids and a buffer:filtered rumen fluid ratio of 1:2. An advantage of the method is that it does not require use of inhibitors of protein synthesis, which should allow bacterial growth, as opposed to the IIV method. However, only protein hydrolysis was used as a measure of degradation and an unexpected rapid disappearance also occurred before 30 min. A rumen in situ experiment was also part of this study. Soluble proteins (66 g) were pulse dosed into the rumen and soluble protein levels recorded over time. Results were confusing as approximately 85% of the soluble proteins could not be recorded minutes after dosing.

Inspired by the previously mentioned study, a macro in vitro system with 5 to 7.5 kg whole rumen contents and 2 L of buffer was designed by Udén (2013). The objectives were to create an in vitro environment as similar to in vivo conditions as possible to measure disappearance of soluble proteins. Due to the large amount of fermentable matter, relative to buffer, fermentation time was limited to approximately 3 h. Also, only 8 vessels/run was possible to manage in this study. A series of experiments revealed that recovery of casein after 1 min of incubation increased asymptotically with dose (400 to 1600 mg TCA-N /kg rumen content). This indicated some form of adsorption of soluble protein to bacteria (Nugent and Mangan, 1981; Wallace, 1985) or to other fractions of the rumen content, similar to the experiences by Hedqvist and Udén (2006). At 2ºC, initial disappearance was however negligible. In contrast, initial recoveries of egg protein were not affected by levels used (800 to 3700 mg total N/kg

rumen content) and degradation proceeded more or less linearly within the 3-h incubation. Neither method for estimating degradation of soluble proteins fulfil the above mentioned criteria of true degradation.

Sulphur AA contain the major proportion of plant sulphur. They are metabolised in the rumen predominately to H_2S, and to a lesser degree also to methyl sulphide and di-methyl sulphide (Dewhurst et al, 2001). Hydrogen sulphide is found both in rumen fluid and gas but contrary to ammonia, it is quickly released as gas due to a pKa value of 7.0, compared to 9.2 for ammonia. It has been proposed as a replacement for ammonia release into rumen liquor for studying protein degradation. Reasons have been that gas measurement are less invasive during in vivo studies (Dewhurst et al, 2001) and for both in vivo and in vitro studies, measurements are simpler and can be automated.

Peer reviewed publications of in vitro studies have not been found but one MSc study has been presented by Häll-Larsson (2004). In this study, the author examined the effects of species and varieties on in vitro hydrogen sulphide production. The main finding of this study was that lower hydrogen sulphide levels were seen for *Lotus corniculatus* as compared to meadow fescue, ryegrass, tall fescue and timothy. Häll-Larsson (2004) suggested that the cyanogenic properties and presence of tannins in *L. corniculatus* contributed to the lower levels seen. Rumen microbes are known to detoxify hydrogen cyanide to form thiocyanate but this process requires H_2S and the tannins may have reduced degradation of sulphur AAs.

Examination of publications on in vivo measurements of H_2S in ruminal headspace gas reveals aspects important for the potential efficacy of a gas in vitro application. Dewhurst et al (2001), Dewhurst et al (2007) and Fonseca et al. (2013) have reported H_2S profiles of pulmonary release from cows. Dewhurst et al (2001) found that appearance in rumen gas over time paralleled fluid ammonia levels, which in turn developed in opposite direction to ammonia in rumen gas as a consequence of a slowly increasing pH during the measurement period. In the second study, Dewhurst et al (2007) measured H_2S concentrations in rumen headspace gas after: i) iso-S additions of Na_2SO_4, cysteine and methionine added into the rumen, ii) after supplying graded levels of cysteine into the rumen and iii) cows had been grazing white clover or perennial ryegrass. Cysteine was the only S form that resulted in a large increase in H_2S, but the level declined rapidly after a few hours. The cysteine level was twice as high in white clover compared to perennial ryegrass but the hydrogen sulphide concentration in rumen headspace gas was negligible for the white clover treatment. Dewhurst et al (2007) attributed this to cyanogenic compounds in white clover, responsible for this remarkable difference.

In the study of Fonseca et al. (2013), the effects of maize gluten feed (MGF), sunflower meal (SFM) and soybean meal (SBM) were compared with respect to hydrogen sulphide concentrations in rumen headspace gas in non-lactating cows. A much higher concentration was seen for MGF followed by SFM and SBM. Batches of MGF with different levels of heat damage were also compared. Both experiments suggest that H_2S in headspace gas is more representative to the very early degradation of protein and is more comparable to differences in in-sacco wash losses than disappearance over longer times as measured by the in-sacco method.

In spite of the fact that the H_2S method fulfils the criteria for degradation, stated earlier, it seems that there are several reasons why the H_2S method may not work for measuring protein degradation in vitro. Rumen microbes will incorporate sulphur AAs into protein and the level of available carbohydrates should affect release of H_2S into rumen fluid and headspace gas. Data from Fonseca et al. (2013) shows a very early (1 to 2 h) H_2S peak, which seems to indicate that the balance of H_2S release and uptake of sulphur AAs is highly positive in the

beginning of the fermentation but rapidly decreases almost to zero within 3 to 4 h. This is undoubtedly a serious problem and may require an approach similar to the one described earlier, suggested by Raab et al (1983). As non-ionized hydrogen sulphide is volatile with a pKa value of 7.0, this means that release rate from rumen fluid will depend on pH. In addition, cyanogenic plants may also be difficult to analyse.

Conclusions

This review has examined a number of methods to estimate protein degradation in porous bags, enzymatically and in vitro by: the IIV technique, the Raab method, the Hohenheim gas test, gel electrophoresis, H_2S liberation and TCA precipitation of soluble N. It is obvious that neither of the present methods fulfil the criteria for true ruminal degradation and/or criteria for correct measurements. A correct method must measure protein, which has been degraded to the extent that it is no longer available for passage as protein, peptide or AA out of the rumen. This means that protein disappearance measured by e.g. filtration or gel electrophoresis does not suffice and that appearance of end-products in the form of NH_3 and H_2S must be possible to correct for microbial uptake. In light of this, this author can see only one slight chance of a future success in assay development. Success will depend on the possibility for correcting end-product release for microbial uptake. It seems that a modification of the Raab method could have a chance to work. Measuring H_2S in headspace gas instead of NH_3 in the fluid may simplify the technique. And, if microbial S uptake over time could be estimated, standard corrections could be applied.

References

Ahvenjärvi, S., Stefanski, T. & Huhtanen, P., 2009. In vitro method for determining the ruminal degradation rate of rapeseed meal protein using 15N isotope labelled ammonia nitrogen. Anim. Feed Sci. Technol. 153, 88–100.

Broderick, G. A., 1987. Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. Br. J. Nutr. 58, 463-475.

Broderick, G. A. & Craig, W. M., 1989. Metabolism of peptides and amino acids during in vitro protein degradation by mixed rumen organisms. J. Dairy Sci. 72, 2540-2548.

Dewhurst, R.J., Evans, R.T., Mottram, T.T., Spanel, P. & Smith, D., 2001. Assessment of rumen processes by selected-ion-flow-tube mass spectrometric analysis of rumen gases. J. Dairy Sci. 84, 1438-1444.

Dewhurst, R.J., Kim, E.J., Evans, R.T., Cabrita, A.R.J. & Fonseca, A.J.M., 2007. Effects of dietary sulphur sources on concentrations of hydrogen sulphide in the rumen head-space gas of dairy cows. Anim. 1, 531–535.

Edmund, B., Südekum, K.-H., Spiekers, H, Schuster, M. & Schwarz, F.J., 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106–113.

Fonseca, A.J.M., Cabrita, A.R.J., Pinho, L.A.O. E., Kim, J. & Dewhurst, R.J., 2013. Effects of protein sources on concentrations of hydrogen sulphide in the rumen headspace gas of dairy cows. Anim. 7, 75–81.

González,J. Mouhbi, R., Guevara-González, J.A. & Arroyo, J.M., 2018. Effects of correcting in situ ruminal microbial colonization of feed particles on the relationship between ruminally undegraded and intestinally digested crude protein in concentrate feeds. J. Sci. Food Agric. 98, 891–895.

Hedqvist, H. & Udén, P., 2006. Measurement of soluble protein degradation in the rumen. Anim. Feed Science Technol. 126, 1-21.

Häll-Larsson, K., 2004. Effects of different forages on production of hydrogen sulphide in a rumen in vitro system. MSc thesis No. 196, Department of Anim. Nutr. Managem., Swedish Univ. Agr. Sci., Uppsala, Sweden, 39 pp.

Huhtanen, P., Bayat, A., Krizsan, S.J. & Vanhatalo, A., 2014. Compartmental flux and in situ methods underestimate total feed nitrogen as judged by the omasal sampling method due to ignoring soluble feed nitrogen flow. Br. J. Nutr. 111, 535–546.

Kamoun, M., Ammar, H., Théwis, A., Beckers, Y., France, J. & López, S., 2014. Comparison of three 15N methods to correct for microbial contamination when assessing in situ protein degradability of fresh forages. J. Anim. Sci. 92, 5053–5062.

Karlsson, L., Hetta, M., Udén, P. & Martinsson, K., 2009. New methodology for estimating rumen protein degradation using the in vitro gas production technique. Anim. Feed Sci. Technol. 153, 193–202

Krawielitzki, K., Schmidt, T., Voigt, J., Kowalczyk, K. & Gabel, M., 2006. Dynamics of microbial contamination of protein during ruminal in situ incubation of feedstuffs. J. Anim. Feed Sci. 15, 313–328.

Krishnamoorthy, U., Muscato, T.V., Sniffen, C.J., Van Soest, P.J., 1982. Nitrogen Fractions in Selected Feedstuffs. J. Dairy Sci. 65, 217-225.

Lebzien, von P., Voig, J., Gabel, M. & Gadeken, D., 1996. Zur Schatzung der Menge an nutzbarem Rohprotein am Duodenum von Milchkuhen. J. Anim. Physiol. Anim. Nutr. 76, 218-223.

Lorenz, M.M., Karlsson, L., Hetta, M. & Udén, P., 2011. Recycling of microbial N and estimation of protein degradation by in vitro gas production. Anim. Feed Sci. Technol. 170, 111– 116.

Machado, P.A.S., Valadares Filho, S.C., Detmann, E., Santos, S.A., Valadares, R.F.D., Ducatti, C., Rotta, P.P. & Costa e Silva, L.F., 2013. Development of equations to estimate microbial contamination in ruminal incubation residues of forage produced under tropical conditions using ^{15}N as a label. J. Anim. Sci. 91, 3836–3846.

Madsen, J., Hvelplund, T., Weisbjerg, M.R., Bertilson, J., Spörndly. R., Olsson, I., Harstad, O.M., Volden, H., Tuori, M., Varvikko, T., Huhtanen, P. & Olafsson, B., 1995. The AAT/PBV protein evaluation system for ruminants. A revision. Norwegian J. Agric. Sci., Suppl. No 19, l-37.

McNabb, W.C., Spencer, D., Higgins, T.J. & Barry, T.N., 1994. In vitro rates of rumen proteolysis of ribulose-1,5-bisphosphate carboxylase (Rubisco) from lucerne leaves, and of ovalbumin, vicilin and sunflower albumin 8 storage proteins. J. Sci. Food Agr. 64, 53–61.

Mehrez, A. Z. & Ørskov, E. R., 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb.) 88, 645–650.

Messman, M.A. & Weiss, W.P., 1994. Use of electrophoresis to quantify ruminal degradability of protein from concentrate feeds. Anim. Feed Sci. Technol. 49, 25-35.

Nugent, J.H.A. & Mangan, J.L., 1981. Characteristics of the rumen proteolysis of fraction 1 (18S) leaf protein from lucerne (Medicago sativa L.). Br. J. Nutr. 46, 39–58.

Kamoun, M., Ammar, H., Théwis, A., Beckers, Y., France, J. & López, S., 2014. Comparison of three 15N methods to correct for microbial contamination when assessing in situ protein degradability of fresh forages J. Anim. Sci. 92, 5053–5062.

Raab, L., Cafantaris, B., Jilg, T. & Menke, K.H., 1983. Rumen protein degradation and biosynthesis 1. A new method for determination of protein degradation in rumen fluid in vitro. Br. J. Nutr. 50, 569-582.

Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G. & Russel, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562-3577.

Udén, P., 2013. Degradation of two soluble proteins – casein and egg protein by a macro in vitro method. J. Anim. Physiol. Anim. Nutr. 97, 656–665.
Volden, H., 2011. NorFor - The Nordic Feed Evaluation System, EAAP Publ. no. 130. Wagening Acad. Publ., Wageningen, the Netherlands.
Volden, H., Velle, W., Harstad, O. M., Aulie, A. & Sjaastad, V., 1998. Apparent ruminal degradation and rumen escape of lysine, methionine, threonine administered intraruminally in mixtures to high yielding cows. J. Anim. Sci. 76, 1232–1240.
Wallace, R.J., 1985. Adsorption of soluble proteins to rumen bacteria and the role of adsorption in proteolysis. Br. J. Nutr. 53, 399–408.

Microbial protein and rumen undegraded protein are not additive

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Introduction

New feed protein evaluation systems started to evolve in the 1970's and 80's replacing crude protein (CP) or digestible CP with metabolisable protein (MP) that estimates the amount of amino acids (AA) absorbed from the small intestine. The MP systems take into account requirements of rumen degradable protein (RDP) of microbes and AA requirements of the host animal. When rumen microbial requirements are met, supply of MP can only be increased by feeding more rumen undegraded protein (RUP). Although the MP systems are theoretically more correct than systems based on total or digestible CP, predicting milk protein yield has seldom been better than predicting it from energy intake, and differences in tabulated MP values have not reflected differences in performance (e.g. hay vs. silage from the same sward, untreated vs. treated protein supplements). This means that systems often fail to reach the main objective of feeding values: correct relative and absolute ranking based on productive value. The possible reasons for this is discussed in this review

Responses to rumen undegraded protein

Santos et al. (1998) reviewed 127 comparisons from 88 lactation trials published from 1985 to 1997. The studies included comparisons of the effects of replacing soybean meal (SBM) with high RUP sources, such as heat-treated or chemically treated SBM, maize gluten meal, distillers grains, brewers grains, blood meal, meat and bone meal, feather meal, or blends of these sources. In these studies, milk yields were higher ($P<0.05$) in 17% of the comparisons. Fish meal and treated SBM accounted for most of the positive effects on milk yield from RUP whereas maize gluten meal resulted in mostly negative results. In the analysis of Ipharraguerre and Clark (2005), average milk yield response to different RUP sources was about 1.0% in 165 comparisons with only treated SBM increasing milk yield. Milk protein yield response was on average 1% in 125 comparisons without significant improvements with any RUP source.

Huhtanen et al. (2011) compared marginal milk yield and milk protein responses to incremental CP intake derived from untreated (N = 120 diets) or treated rapeseed meal (RSM; N = 82) diets. Marginal increases to incremental CP intake were similar for untreated and treated rapeseed meal (RSM; 136 and 133 g/kg CP intake, respectively). Corresponding responses to SBM at 98 g/kg CP intake was lower than corresponding responses to RSM ($P<.0.01$).

In a meta-analysis by Huhtanen and Hristov (2009), milk protein yield response was about 5 times greater for microbial MP compared with feed MP both in North American and North European datasets (1734 diets). Two reasons can be suggested for this: 1) increases in microbial MP are associated with similar relative increases in metabolisable energy (ME) intake that is the main driver of milk production and 2) because the differences in feed MP

intake are overestimated. Analysis of omasal flow data (Broderick et al., 2010) indicated that, in addition to overestimation of the differences in RUP intake, the NRC (2001) system underestimated differences in microbial MP intake. In a re-analysis of omasal flow data, milk protein yield response was 3.6 times greater for microbial MP than for feed MP when supplies were estimated according to the NRC (2001) system, but only 1.8 times greater when the MP estimates were based on omasal flow data. Microbial MP predicted milk protein yield at least as well as total MP, and energy intake in terms of total digestible nutrients (TDN) was at least as good a predictor of milk protein yield as MP (Huhtanen and Hristov, 2009). Marginal production responses to incremental MP derived from a reduced ruminal protein degradability were only 60 – 80 g/kg, i.e. only about 10% of the default value (0.67) in the NRC (2001) protein evaluation system.

These four meta-analyses, based on large datasets, indicate that benefits of decreasing ruminal protein degradability to increase supply of RUP, are much smaller than expected, especially when degradability is reduced by chemical or physical (heat) means.

Potential of RUP

Post-ruminal casein infusion could be expected to result in a maximal milk protein yield response to increased supply of RUP since a post-ruminal infusion of protein completely avoids ruminal degradation, true digestibility of casein is complete and its AA composition is ideal in terms of His, Met and Lys. In the meta-analysis of Huhtanen and Hristov (2010), transfer efficiency of casein into milk protein was 0.285, i.e. much lower than default values in various protein evaluation systems (e.g. 0.67 in NRC, 2001). Transfer efficiency was higher (0.32 vs. 0.18) when calculated MP balance was negative rather than positive (Martineau et al., 2016). Marginal responses to RSM supplementation are rather high compared to casein infusions, in the light of RSM protein being partially degraded in the rumen, not completely digestible in the intestines and with an AA composition less ideal than that of casein. Compared with post-ruminal casein infusion, infusions of SBM (Rogers et al., 1984) or a soya protein isolate (Chamberlain and Choung, 1993) increased milk protein yield, but relative responses were smaller than for casein infusion (0.57 and 0.30, respectively) in their study. With cows fed red clover – grass silage, relative milk protein yield response with ruminal casein infusion was 0.33 of that with duodenal casein infusion (Khalili and Huhtanen, 2002). From infusion studies, it can be concluded that increased post-ruminal supply of high quality protein has a positive effect on milk production, but even in ideal circumstances (casein infusion), expected production responses are less than half of default values of MP utilization above maintenance for milk protein production in current feed protein evaluation systems.

Why are production responses to increased RUP supply poor?

Milk and milk protein production are mainly driven by ME intake. Dietary concentrations of ME and microbial MP were much more strongly correlated than were ME and feed MP (R^2 = 0.58 vs. 0.04 in the updated Finnish dataset (Huhtanen and Nousiainen, 2012; N = 1285 diets). Therefore, intake of microbial MP and ME are strongly correlated, whereas Feed MP (RUP) intake can be increased e.g. by heat treatment without an increase ME intake. In the same dataset, the regression of milk protein yield on total MP intake and ratio of feed MP/total MP were evaluated. The coefficient for the latter was highly significant. In the bivariate model, ME intake = Microbial MP + Feed MP, the regression coefficient of microbial MP was 4-fold the value for feed MP (131 vs. 32 MJ/kg MP, respectively). It can

be concluded that differences in "productive value" of microbial and feed MP is largely related to different associations to energy intake.

Overestimation of the ranges in ruminal protein degradability can be another reason for the small marginal production responses to incremental MP from increased RUP supply. Results of the meta-analysis of omasal sampling data (Broderick et al., 2010) indicated that the observed range in RUP supply between the diets within a study was smaller than that predicted by the NRC (2001) system. In most protein evaluation systems, effective ruminal protein degradability (EPD) is estimated using in situ methodology that is flawed because of several inherent problems, including: 1) the assumption that proteins, peptides, and amino acids in the soluble fraction are completely degraded, 2) a physical restriction of feeds within the bag from microbial interaction and digestion, and 3) an imprecise quantitation of microbial contamination of undigested residues (Broderick and Cochran, 2000). In addition, errors result from particles losses from the bags and use of incorrect kinetic models in calculating EPD from degradation parameters. Difficulties in standardization of procedures and large between-laboratory variation (Madsen and Hvelplund, 1994) are further concerns about the method.

In the evaluation of feed protein systems using a constant EPD value for calculating MP supply differences among diets within a study were better predicted than determined EPD values (Tuori et al., 1998). Consistent with this, the German system that calculates MP intake from ME and urea-free CP performed better than many more complicated systems in a comparison based on 72 diets (Schwab et al., 2005). Certainly, there are differences in EPD among diets, but a flawed methodology can result in greater errors than using constant EPD values. In situ EPD of grass silages was predicted with high precision (R^2>0.70) from concentrations of NDF, CP and N solubility (Yan and Agnew, 2004). Using their equations for calculating EPD values and, consequently, MP concentrations of silage, resulted in a greater prediction error of milk protein yield than using a constant EPD for all silages irrespective of their composition (Rinne et al., 2009). Constant values for forage EPD were used earlier in the Nordic AAT-PBV system in Sweden.

Variance component of random slope was greater for models predicting milk protein yield from MP intake than from only bacterial MP or energy intake (Huhtanen and Hristov, 2009). In addition to random variation in the dependent variable which increases variance of the random slope, errors in RUP component of MP related to the in situ method can explain greater slope variance of MP compared to energy content. For example, underestimating the differences in EPD among diets within a study reduces differences in MP and, consequently increases the slope of protein yield regressed upon MP. Vice versa, overestimation of differences in MP decreases the slope value.

In the analysis of a dataset with 379 treatment means by a mixed model regression analysis with random slope and intercept, DM intake (DMI) and ME intake were better predictors of milk protein yield in terms of smaller AICC than the MP-A system (Table 1). In the MP-A system, EPD values were based on in situ values and MP calculations with an equation taking into account different energy value for rumen microbes of different substrates. Much larger coefficient of variation of the slope for MP intake compared with DM or ME intake suggests that the RUP component increases variance for the slope of protein yield on MP intake. Most likely this is because differences in EPD do not reflect the true productive value. Random slope variance was much smaller in the MP-B system, which uses a constant EPD value for

forages, takes into account digesta flow and production studies in determining tabulated EPD values. Strong negative correlation between slope and intercept probably explains the greater intercept variance for the MP-A system. Omitting random slope from the model increased random variance much more for the MP-A system (67%) than for the other models (13-27%) indicating that feed-MP from different sources is not "a uniform currency" in the MP-A system. Even with a fixed regression model, ME intake was a better predictor of milk protein yield than MP-A (root mean squared error: 66.1 vs. 81.2; R^2: 0.82 vs. 0.73, respectively).

Table 1 Variance components of milk protein yield predicted from dry matter intake (DMI), metabolizable energy intake (MEI) or metabolizable protein intake (MPI) (N = 379 diets)

	Variance component				AICC[1]	SD[2]	Slope	SD/Slope
	Intercept	Interaction	Slope	Residual				
DMI	59471	-2960	165	688	3854.6	12.8	51.0	0.25
MEI	41629	-163	0.73	645	3817.9	0.85	4.05	0.21
MP-A[3]	74934	-36837	21093	648	3899.6	145	303	0.48
MP-B	34548	-14972	7911	402	3688.6	89	398	0.22

[1]Akaike's information criteria; the smaller the better; [2]SD = standard deviation (square root of the slope variance); [2]SD = standard deviation; [3] MP-A and MP-B are two different metabolisable protein systems.

Microbial protein synthesis

Most current protein systems assume that microbial and feed MP are additive. In the analysis of Santos et al. (1998), high RUP diets resulted in decreased microbial protein synthesis in 76% of the comparisons. Duodenal flow of non-microbial non-ammonia N (MNNAN; mainly feed N) was numerically greater in most of comparisons, but total NAN flow increased only in 5 out of 24 comparisons when SBM was replaced with high RUP sources ($P<0.05$). Mean NAN flow was 480 and 490 g/d for SBM and high RUP diets, respectively.

The results of a later analysis by Ipharraguerre and Clark (2005) are in good agreement with Santos et al. (1998). Duodenal flow of NMNAN increased about 25% with high RUP supplements, but microbial NAN flow decreased 7% ($P<0.05$) and total NAN flow increased only 6%, and increases in duodenal flow of Lys and Met did not reach statistical significance. The authors discussed that a shortage of energy, AA, peptides, or ammonia in the rumen can depress growth of ruminal bacteria when RDP sources are replaced with RUP supplements.

Results from the study by Broderick and Reynal (2009) do not give support for ammonia limiting microbial synthesis when high RUP sources are fed. They formulated diets containing the same amount of RDP and RUP (NRC, 2001) by gradually replacing solvent extracted SBM with lignosulphonate treated SBM and urea. Microbial and total NAN flow decreased with incremental levels of urea in the diet (Table 2). Efficiency of microbial N synthesis decreased with urea level but, because ruminal ammonia-N concentration also increased, a shortage of ammonia N did not explain this.

Replacing crimped barley on a DM basis with heat-treated RSM (Expro) in a grass silage based diet markedly increased feed N flow to the omasum, but recovery of increased feed N flow as total NAN flow was only about 60% due to reduced microbial N synthesis (Krizsan et al., 2017). Consistently with Broderick and Reynal (2009), ruminal ammonia N concentration increased with increasing intake of a high RUP supplement. In a production study (Gidlund et al., 2015) milk protein yield response on increased level of Expro-RSM supplementation was lower than the mean response to incremental CP from RSM supplementation (Huhtanen et al., 2011) and in a direct comparison, no differences in milk production were observed

between untreated and RSM and Expro-RSM (Huhtanen and Heikkilä, 1996). Disappointing production responses to Expro and other treated RSM can be due to overestimation MP concentration due to reduced microbial synthesis, and probably also due to smaller difference in EPD due to a greater escape of soluble NAN from untreated RSM.

Table 2 The effect of replacement of solvent extracted soybean meal (SBM) with urea and lignosulphonate treated SBM in isonitrogenous diets (same RDP and RUP). Adapted from Broderick and Reynal (2009)

	RDP from urea, % of DM				
	0	1.2	2.4	3.7	P-value Linear
DMI[1], kg//d	23.7	22.0	23.9	22.5	0.64
Milk yield, kg/d	39.3	38.6	38.5	36.0	<0.01
Protein yield, kg d	1.27	1.22	1.21	1.17	<0.01
N intake, g/d	570	551	561	534	0.08
NAN flow, g/d	604	562	573	509	<0.01
Microbial N, g/d	440	363	374	342	<0.01
Microbial N, g/ kg OMTDR[1]	29.3	28.6	24.4	24.5	0.05
Rumen ammonia N, mg/100 ml	8.2	9.3	10.3	10.7	<0.01
Milk urea N, mg/100 ml	6.8	7.5	8.1	9.1	<0.01

[1]DMI = dry matter intake; [2]OMTDR = organic matter truly digested in the rumen.

The study with ^{15}N labelled silage N fractions (Ahvenjärvi et al., 2018) indicates that soluble NAN fractions (AA, peptides) can be better N sources for rumen microbes than ammonia N. Together with escape of soluble NAN and uptake to microbial N, recovery of the ^{15}N dose as silage soluble NAN was about 30% greater than recovery of ^{15}N ammonia in the form of NAN flow form the rumen. Broderick and Reynal (2009) referred to several in vitro and in vivo studies, in which urea supplements have decreased efficiency of microbial N synthesis compared to RDP from true protein.

Voigt and Piatkowski (1991) proposed the following equation to adjust microbial protein synthesis for ruminal protein degradability:

$$\text{MPS (g/kg DOM)} = [(185 - 1.31 \times \text{RUP}) \times \text{DOM}],$$

where, RUP = rumen undegraded protein (% of CP) and DOM = digestible OM (kg/kg DM). With values of 170 g CP/kg DM and 0.7 kg DOM/kg DM, calculated recovery of increased RUP as protein flow to the small intestine is 0.46. Decrease in MPS is greater than decrease in ruminally digested OM with reduced protein degradability. Using this equation, predicted milk protein yield within study was shown to be better than in other systems evaluated (Tuori et al., 1998).

Feed intake

It is well known that including high quality protein supplements in diets of dairy cows increases feed intake (e.g. Oldham, 1984). Therefore, feed intake responses to different protein supplements could be used as an indirect indicator of the supple of "productive protein". It was earlier suggested (Oldham, 1984) that improved diet digestibility is the reason for the positive effects of protein supplementation on feed intake. However, greater intake effects of post-ruminal than ruminal casein infusions (Khalili and Huhtanen 2002) and of duodenal protein infusion compared with ruminal protein or glucose infusions (Faverdin et al., 2003) suggest that g that part of the response is related to metabolic effects, possibly

mediated via improvements in the ratio of AA to energy at tissue level. In line with this, fishmeal (Chamberlain et al. 1992) and rapeseed expeller (Shingfield et al. 2001) supplements elicited greater intake responses than feather meal and wheat gluten meal or urea in cows given grass silage-based diets. Feather meal is poor in histidine and methionine, whereas wheat gluten is poor in lysine and methionine. More recently, Puhakka et al. (2016) reported markedly low DM intake in cows fed faba bean supplements (poor in methionine) compared to those fed isonitrogenous RSM supplements. According to a meta-analysis of Huhtanen et al. (2011), RSM supplements increased feed intake more than SBM supplements. In all these studies, increased milk yield, probably resulting from increased and/or more balanced AA supplies, was associated with increases in feed intake. Similar effects have seldom been seen in studies investigating effects of treatments to reduce ruminal protein degradability. Therefore, it could be hypothesized that treatments to reduce ruminal protein degradability do not influence supply of "productive protein".

Conclusions and future perspectives

Although current protein systems are theoretically more comprehensive than earlier systems based on CP or digestible CP. The equations and variables included in the new models can theoretically be justified, but lack of accurate and precise analytical methods to determine key parameters have delayed progress in estimating MP supply, and especially prediction of production responses to changes in MP supply. In addition to flawed methodology to determine RUP, it has not been fully realized that productive values of microbial and feed MP are not equal and that current microbial protein and RUP estimates are not additive. There is plenty of evidence based on large datasets from both of the two latter factors. It is unlikely that any progress can be made by fine-tuning the in situ method by introducing different correction methods, or by introducing new elements in the models. Vice versa, it seems that the more complicated the models are, the worse they perform in predicting production responses – the ultimate goal of feed evaluation. When energy intake predicts milk protein yield at least as well as MP, very little progress has been made in estimating true MP value of the diet. To make better progress, it might be better to take a step backwards and start developing new protein systems from a simple equation: MP = a × MEI + b × CPI (urea free) and thereafter introduce new elements; e.g. having three different categories for EPD. Another important factor, not included in the current systems, is the effect of protein quality on efficiency of microbial protein synthesis. There is plenty of evidence that soluble NAN is a better N source for rumen microbes than ammonia N and that increased RUP decreases efficiency of microbial protein synthesis more than a decrease in rumen fermentable substrate. It is also important that when revising or including new elements in system, the impact of these elements should be evaluated by large datasets from production experiments. It is important to evaluate that also the cows – not only the model – respond to changes in model parameters.

References

Ahvenjärvi, S., Vaga, M., Vanhatalo, A. & Huhtanen, P., 2018. Ruminal metabolism of grass silage soluble N fractions. J. Dairy Sci. 101, 279-294.

Broderick, G.A. & Cochran, R.C., 2000. In vitro and in situ methods for estimating digestibility. In: Feeding in Systems and Feed Evaluation Models (Eds. M.K. Theodorou & J. France) CABI Publishing, CAB International, Wallingford, UK. pp. 53–85.

Broderick, G.A., Huhtanen, P., Ahvenjärvi, S. Reynal, S.M.& Shingfield, K.J., 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle - A meta-analysis. J. Dairy Sci. 93, 3216–3230.

Broderick, G.A. & Reynal, S.M., 2009. Effect of source of rumen degraded protein on production and ruminal metabolism in lactating dairy cows. J. Dairy Sci., 92, 2822-2834.

Chamberlain, D.G., Choung, J. J. & Robertson, S., 1992. Protein nutrition of dairy cows receiving grass silage diets: effects of feeding a protein supplement of unbalanced amino acid composition. J. Sci. Food Agric. 60, 425-430.

Choung, J.-J. & Chamberlain, D.G., 1993. The effects of abomasal infusions of casein or soya-bean-protein isolate on the milk production of dairy cows in mid-lactation. Br. J. Nutr. 69, 103-115.

Faverdin, P., M'hamed, D. & Vérité, R., 2003. Effects of metabolizable protein on intake and milk production of dairy cows independent of effects on ruminal digestion. Anim. Sci. 76, 137-146.

Gidlund, H., Hetta, M., Krizsan, S., Lemosquet, S., Huhtanen, P., 2015. Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage-based diets. J. Dairy Sci. 98, 8093-8106.

Huhtanen, P. & Heikkilä, T., 1996. Effects of physical treatment of barley and rapeseed meal in dairy cows given grass silage-based diets. Agric. Food Sci. Finl. 5, 399-412.

Huhtanen, P., Hetta, M. & Swensson, C., 2011. Evaluation of canola meal as a protein supplement for dairy cows: a review and meta-analysis. Can. J. Anim. Sci. 91, 529-543.

Huhtanen, P. & Hristov, A.N., 2010. Effects of energy and protein supply on milk protein yield responses in dairy cows. In: G. Matteo Grovetto (Ed). Energy and Protein Metabolism and Nutrition. EAAP Publ. 127, 287-298.

Huhtanen, P. and Hristov, A.N., 2009. A meta-analysis of the effects of protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J. Dairy Sci. 92, 3222–3232.

Huhtanen, P., & Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Livest. Sci. 148, 146–158.

Ipharraguerre, I.R. & Clark, J.H., 2005. Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J. Dairy Sci. 88, E22-E37.

Khalili, H. & Huhtanen, P., 2002. Effect of casein infusion in the rumen, duodenum or both sites on factors affecting forage intake and animal performance of dairy cows fed red clover-grass silage. J. Dairy Sci. 85, 909-918.

Krizsan, H., Gidlund, H., Fatehi, H. & Huhtanen, P., 2017. Effect of supplementation of heat-treated canola meal on ruminal nutrient metabolism. J. Dairy Sci. 100, 8004–8017.

Madsen, J. & Hvelplund, T., 1994. Prediction of in situ protein degradability in the rumen. Results of a European ringtest. Livest. Prod. Sci. 39, 201–212.

Martineau, R., Ouellet, D.R., Kebreab, E. & Lapierre, H., 2016. Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel meta-analysis. J. Dairy Sci. 99, 2748–2761.

NRC (National Research Council), 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy of Science, Washington, DC.

Oldham, J. D., 1984. Protein energy relationships in dairy cows. J. Dairy Sci. 67, 1090-1114.

Puhakka, L., Jaakkola, S., Simpura, I., Kokkonen, T. & Vanhatalo, A., 2016. Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed

intake, nutrient digestion, and milk production in cows fed grass silage-based diets. J. Dairy Sci. 99, 7993-8006.
Rinne, M., Huhtanen, P. & Nousiainen, J., 2009. Effects of silage effective protein degradability and fermentation acids on metabolizable protein concentration: a meta-analysis of dairy cow production experiments. J. Dairy Sci. 92, 1633–1642.
Rogers, J.A., Clark, J.H., Drendel, T.R. & Fahey, G.C., Jr., 1984. Milk production and nitrogen utilization by dairy cows infused postruminally with sodium caseinate, soybean meal or cottonseed meal. J. Dairy Sci. 67, 1928-1935.
Santos, F.A.P., Santos, F.E.P., Theurer, C.B. & Huber, J.T., 1998. Effects of rumen-undegradable protein on dairy cow performance: A12-year literature review. J. Dairy Sci. 81, 3182–3213.
Schwab, C.G., Huhtanen, P., Hunt, C.W. & Hvelplund, T., 2005. Nitrogen Requirements of Cattle. In: Interactions between Cattle and the Environment. R. Pfeffer and A. Hristov (Eds.), CAB International. pp. 13-70.
Shingfield, K.J., Jaakkola, S. & Huhtanen, P., 2001. Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. Anim. Sci. 73, 541-554.
Tuori, M., Kaustell, K. & Huhtanen, P., 1998. Comparison of the protein evaluation systems of feed for dairy cows. Livest. Prod. Sci. 55, 33–46.
Voigt, J. & Piantkowski, B., 1991. Models for estimation of non-ammonia nitrogen supply to the small intestine and to the balance of nitrogen in the rumen of dairy cows. Proc. 6th Int. Symp. Prot. Metab. Nutr. Vol 2, 364-366.
Yan, T. & Agnew, R.E., 2004. Prediction of nutritive value of grass silages: II. Degradability of nitrogen and dry matter using digestibility, chemical composition and fermentation data. J. Anim. Sci. 82, 1380–1391.

In vivo nutrient digestibility of a protein fraction extracted from macroalgae *Saccharina latissima* in sheep

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Introduction

Future projections estimate an increase of import soya in Norway by 35% in 2050 compared to 1961-1990 levels due to increasing demand for food and protein feeds (Özkan Gülzari *et al*., 2017). Macroalgae species (also known as seaweed) possess a wide range of application areas in practice, as functional foods and animal feed additives, pharmaceuticals, cosmetics and cosmeceuticals (Garcia-Vaquero and Hayes, 2016). Given that Norway lacks domestically produced protein-rich feed sources, resulting in import of soya, macroalgae may provide a potential source of protein in ruminant nutrition (Chapman *et al*., 2015).

There exist three types of macroalgae species, namely red, brown and green algae. They contain various amounts of complex carbohydrates and polysaccharides such as alginates and laminarin in brown algae; agars, xylans and porphyrans in red algae; and sulphated galactans in green algae (Makkar *et al*., 2016). *Saccharina latissima,* a brown algae species common in Norway, has gained importance and popularity in human diets and is a potential source of protein for animal diets (Chapman *et al*., 2015). Brown seaweed species may contain low to moderate amounts of crude protein and high concentrations of potassium, sodium and iodine. However, the chemical composition will vary greatly depending on species and seasonal conditions (Molina-Alcaide *et al*., 2017). While red macroalgae is widely distributed and found only in small sizes, brown macroalgae species are abundant, large in size and easy to cultivate. *Porphyra* spp., red algae with a bright pink colour, are rich in protein (up to 347 g/kg DM (Molina-Alcaide *et al*., 2017) and are the most commonly used among other macroalgae, e.g. nori in Japanese sushi (Makkar *et al*., 2016). *In vitro* organic matter digestibility of *S. latissima* was reported as high as 97% in sheep rumen fluid (Makkar *et al*., 2016). However, before *S. latissima* can be used as a protein source in feeds, the high salt content needs to be reduced, a process that also increases the protein concentration. A previous *in vitro* trial by Ramin *et al*. (2017) found that organic matter digestibility and utilisable protein concentration increased as a result of increased dietary proportion of a protein-enriched fraction of *S. latissima*. However, there seem to be a lack of studies investigating the *in vivo* digestibility of seaweed species, and the extent to which they may provide comparable nutrient characteristics to soybean meal (SBM) for ruminants. To fill this gap, we have compared *in vivo* protein, organic matter and fibre digestibility of a diet supplemented with a protein-enriched fraction of *S. latissima* with those of an unprocessed red macroalgae *Prophyra* spp. and SBM.

Materials and methods

This study was conducted in accordance with the regulation for use of animals in experiments, adopted by the Norwegian Ministry of Agriculture and Food, and approved by the Ethics Commission on Animal Use by the Norwegian Food and Safety Authority, application number (FOTS ID) 8838 on 31.08.2017. It complies with the EU Directive 2010/63/EU on the use of experimental animals, which was incorporated into the Agreement on European Economic Area in May 2015.

An *in vivo* digestibility trial was conducted with four weathers of Norwegian White Sheep, 30 months of age and 80-88 kg live body weight, by using four rations in a 4 x 4 Latin square. Restricted feeding with four different diets, consisted of a control diet without any protein

supplement (Control), and the control diet supplemented with protein-enriched fraction of *S. latissima* (SW1), *Porphyra spp.* meal (SW2; CoDo International limited), or extracted SBM (Champion Soya pellets, Felleskjøpet, Norway), respectively. The protein diets were planned to be isonitrogenous and isoenergetic and to meet the maintenance energy requirement of adult rams (INRA, 2007). To increase the palatability, the protein feed was mixed with a fixed amount of molasses at time of feeding. The control group also received the same amount of molasses. Chemical composition of ingredients and diets and diet formulation are in Table 1 and Table 2, respectively.

Each period consisted of an eight-day adaptation in individual pens followed by a seven days in individual metabolism crates for daily collection of urine, faeces and feed refusals. Frozen faecal samples were coarsely ground and freeze dried. Freeze-dried samples were weighed immediately and then again after 24 hours storage in room temperature. The faeces and feed samples were ground to pass a 1-mm screen using a Tecator Cyclotec Sample Mill®. Frozen urine samples were thawed before subsampling and stored at 4ºC until further chemical analyses. Faeces and feed samples were analysed for dry matter (DM), ash, Kjeldahl nitrogen (N), ash free neutral detergent fibre (aNDFom) and acid detergent fibre (ADF) whilst urine samples were analysed for DM and N.

Data were analysed by Proc Mixed in SAS (SAS Institute, 1999–2000) for a 4 × 4 Latin square design according to the following model:
$Yijkl = \mu + Pi + Tj + sk + Eijk$,
where, Yijkl = dependent variable, μ = overall mean, Pi = effect of period i, Tj = effect of diet j, sk = effect of sheep k, and Eijk = residual error. Period and diet were considered fixed effects. The same model was used for the nitrogen digestibility of the individual protein sources, with three treatments (SW1, SW2 or SBM) and four periods. In two out of four periods, the animals refused to eat some of the SW1 supplement. Data from these sheep were excluded from the analysis. Differences between least squares means of response variables were estimated with Tukey's test. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. All reported values are least squares means.

Results and discussion

Nitrogen content was higher in SW2 and SBM than SW1 and control. Amino acids were found in highest concentration in the diet containing SBM followed by SW2 and SW1, respectively (Table 1) and ash content was highest in SW1. In two of the four periods, animals refused to consume SW1. The reason for the low intake was likely due to its high

Table 1 Chemical composition of individual feeds (g/kg DM unless specified)

	Hay		Molasses		SW1		SW2		SBM	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ash	43	0.5	132	0.4	221	2.7	87	3.7	77	19.1
Kjeldahl-N	13.3	0.33	10.9	0.09	35.8	0.66	60.9	0.50	82.1	3.18
Amino acids	57	6.1	20	1.9	163	4.0	282	2.4	468	25.5
Crude fat	10.5	0.79	2.0	0.34	18.5	3.32	2.5	1.06	16.3	4.3
aNDFom	615	8.6	-	-	408	25.1	431	13.5	182	24.0
ADF	364	5.0	-	-	352	8.5	66	7.0	90	5.7
Crude fibre	308	4.4	-	-	152	2.1	36	6.1	64	3.9
Organic matter	957	0.5	868	0.44	779	2.7	913	3.7	923	19.1
Gross energy, MJ/kg DM	19.0	0.08	15.7	0.03	15.7	0.05	18.8	0.07	19.5	0.51

SW1: *S. latissima* extract; SW2: *Porphyra spp.*; SBM: soybean meal; aNDFom: amylase treated ash-free neutral detergent fibre; ADF: acid detergent fibre.

content of ash.

Total N intake was similar in diets containing SW2 and SBM and higher than that of SW1, which was found higher than the control (Table 3). Digestibility of DM, OM, aNDFom, ADF and CF was not different among diets. Nitrogen digestibility of diets containing SW2 and SBM was similar and higher than those of control and SW1 (p=0.002). Despite the lower N intake in SW1 than SW2 and SBM, a higher ratio of N excreted in faeces in SW1 than in SBM may be attributed to that the amino acids in SW1 are less degraded in the rumen or less digested in the intestines compared to SBM.

Table 4 shows that N digestibility of the protein feeds, calculated by difference, was 74.2, 88.6 and 96.9% for SW1, SW2 and SBM, respectively. It is important to note that these ratios reflect the exclusion of two periods where some of the SW1 was not consumed by the animals.

Table 2 Formulation, chemical analysis and nutritive value of experimental rations

	Diet[1]			
Item	Control	SW1	SW2	SBM
Composition (g DM)				
Hay	961	799	854	888
Protein feed[1]	0	162	108	72
Molasses	39	39	38	40
Sum	1000	1000	1000	1000
Chemical analysis (g/kg DM)				
Organic matter	953	925	949	951
Kjeldahl-N	13.2	16.8	18.3	18.1
Amino acids	56	73	80	85
aNDFom	591	557	571	559
ADF	349	347	318	330
Crude fibre	296	271	267	278
Gross energy[2]	18.9	18.3	18.8	18.9

[1]SW1: *S. latissima* extract; SW2: *Porphyra spp*.; SBM: soybean meal; [2]Gross energy analysed by bomb calorimetry of diet ingredients.

Table 3 Effect of diet on total digestibility (least square means)

	Diet[1]				SEM[2]	P-value
	Control	SW1	SW2	SBM		Diet
n	4	2	4	4		
Intake DM, g/d	1104	1105	1139	1094	0.36/0.55	
Faecal excretion DM, g/d	352	360	354	337	10.6/16.2	0.613
Intake N, g/d	14.5	18.6	20.9	19.8	0.07/0.11	
Faecal excretion N, g/d	6.6^{b}	8.3^{a}	7.5^{ab}	6.8^{b}	0.17/0.26	0.014
Digestibility %						
Dry matter	68.1	67.4	68.9	69.1	1.0/1.5	0.723
Organic matter	69.6	68.9	70.5	70.7	0.96/1.46	0.697
Nitrogen	54.5^{b}	55.4^{b}	64.2^{a}	65.6^{a}	0.87/1.34	0.002
aNDFom	69.0	70.4	70.3	69.3	1.22/1.86	0.850
ADF	64.1	62.4	60.8	62.4	2.00/3.06	0.724
Crude fibre	68.0	66.1	65.5	67.5	1.54/2.36	0.675

[1]SW1: *S. latissima* extract; SW2: *Porphyra spp*.; SBM: soybean meal; [2]SEM: standard error of the mean. The first figure is for Control, SW2 and SBM and the second figure for SW1.

Table 4 Nitrogen digestibility of the protein sources in relation to that of control diet calculated by difference (Least square means) n=2 for SW1 and 4 for SW2 and SBM.

Item	Protein source[1]			SEM
	SW1	SW2	SBM	
N digestibility %	74.2[c]	88.6[b]	96.9[a]	2.28/2.43

[1]SW1: *S. latissima* extract; SW2: *Porphyra spp*.; SBM: soybean meal.

Conclusions

Despite that *S. latissima* is abundant in nature and easy to cultivate, there appears to be challenges in using it in the form used in the present study as a protein supplement in ruminant nutrition. Its high ash content and low N digestibility contributes to this. Therefore, our conclusion is that SW1, in its current form, is not suitable as a protein feed in ruminant nutrition. The properties of the protein in processed SW1 appears to reduce microbial degradation of N and amino acid absorption in the intestines, resulting in a greater excretion of N in faeces than the other protein sources. Further work should focus on improving processing of macroalgae since removal of salt and heat treatment during may adversely affect protein properties.

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References

Chapman A.S., Stévant P. & Larssen W.E., 2015. Food or fad? Challenges and opportunities for including seaweeds in a Nordic diet. Bot. Mar. 58, 423-433.

Garcia-Vaquero M. & Hayes M., 2016. Red and green macroalgae for fish and animal feed and human functional food development. Food. Rev. Int. 32, 15-45.

INRA, 2007. Alimentation des bovins, ovins et caprins - Besoins des animaux - Valeurs des aliments - Tables INRA 2007, Versailles, 307 pp.

Makkar H.P.S., Tran G., Heuzé V., Giger-Reverdin S., Lessire M., Lebas F. & Ankers P., 2016. Seaweeds for livestock diets: A review. Anim. Feed Sci. Tech. 212, 1-17.

Molina-Alcaide E., Carro M.D., Roleda M.Y., Weisbjerg M.R., Lind V. & Novoa-Garrido M., 2017. In vitro ruminal fermentation and methane production of different seaweed species. Anim. Feed Sci. Tech. 228, 1-12.

Özkan Gülzari Ş., Åby B.A., Persson T., Höglind M. & Mittenzwei K., 2017. Combining models to estimate the impacts of future climate scenarios on feed supply, greenhouse gas emissions and economic performance on dairy farms in Norway. Agr. Sys. 157, 157-169.

Ramin M., de Oliveira Franco M., Roleda M., Aasen I., Hetta M. & Steinshamn H., 2017. Effect of extracted seaweed protein fractions on estimated utilizable crude protein, methane emission and fermentation parameters an in vitro evaluation. Proc. 8th Nordic Feed Sci. Conf. pp. 65-70.

In vitro ruminal digestion and methane production from different products of microcrystalline cellulose

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Introduction

Cellulose is a large component of dietary fibre and one of the most abundant natural materials. It is a long-chain carbohydrate polymer composed of repeating β-D-glucose units. Structurally, parent cellulose has both amorphous and crystalline regions. Plant materials rich in α-cellulose (woody sources and cotton linters) are mainly used as raw material for production of micro scale crystalline material because the cellulose strands are structurally arranged (Chukwuemeka & Okhamafe, 2012; Osong et al., 2016; Thoorens et al., 2014). By chemical, mechanical and biological means, it is possible to isolate the crystalline regions of cellulose and to produce a number of functional ingredients in the form of cellulose crystals with variable shape, size and lignin levels. One of them is microcrystalline cellulose (MCC) (Habibi et al., 2010).

Native lignin is indigestible by animals. In contrast to native lignin, purified lignin does not represent a barrier to digestion in monogastric animals (Baurhoo et al., 2008). During isolation, physical and chemical linkages that exist between native lignin and other plant biopolymers are broken. However, there are chemical differences between native and commercially isolated (purified) lignin, such as molecular weight, changes in ratio of functional groups and configuration of the molecule. Commercial purified lignin is produced as a by-product of the paper industry, separated from wood by chemical pulping processes. These structural changes are likely to introduce antimicrobial properties that typically do not occur in native lignin. For this reason, purified lignin may possess biological properties not characteristic of native lignin (Baurhoo et al. 2008). Antimicrobial activity of various phenolic compounds has been well recognized (Hartley & Akin, 1989) and it is possible that purified lignin originating from different industrial processes has similar antimicrobial properties in animal nutrition.

MCC has been studied as a functional ingredient in foods and has been shown to provide positive effects on gastrointestinal physiology and for being hypolipidemic. The techno-functional and nutraceutical properties of MCC are influenced by the physicochemical structure of the material which is defined to a varying extent by the source of raw material and processing conditions (Nsor-Atindana et al., 2017). There are a limited number of references in the literature on the effect of MCC on performance and health of monogastric animals and no published reports of MCC as a feed component for ruminants have been found by the authors. Additionally, it was observed in Luke, Jokioinen, that no bacterial, yeast and mould growth could be seen on wet MCC samples during prolonged storage. The objective of this study was, therefore, to determine in vitro ruminal digestion, methane production and antimicrobial activity of 7 different products of MCC and one lignin product.

Materials and methods

Preparation of MCC products

Seven MCC and one lignin product were studied. The MCC samples varied in particle size, lignin and moisture concentration (Table 1) and were produced by XAMK/FiberLaboratory using the AaltoCell™ technique. This technique is based on two patented inventions by Aalto University, Espoo, Finland (Dahl et al., 2011a, b). These inventions allow new production process concepts for implementation in mass production of MCC in an economic and environmentally feasible way in a chemical pulp mill. In this study, softwood kraft pulp was used as a raw material for MCC. The lignin concentration of the product is known to depend on from where in the kraft pulp process the material is taken to the MCC process. White MCC's were prepared from bleached softwood kraft pulp and lignin rich MCC was prepared from pulp taken just after the pulp cooking process. The preparations of MCC were carried out under mild acid hydrolytic conditions in a continuous digester. Temperature in the digester was 160 ºC and the delay time varied between 35 and 45 min. In the hydrolytic process, pH was adjusted to 1.9 with H_2SO_4. The solid residue and hydrolysis liquid were separated and washed by a Horizontal Belt Filter machine Nanopar Oy, Puumala, Finland). After the washing stage, MCC was dried in an infrared dryer and micronization was conducted by the Mikropulva Ltd. mechanical milling equipment.

In vitro ruminal digestion and methane production

Three levels of inclusion (10, 20 and 30 %) based on dry matter (DM) content were used in this study. Three separate runs were conducted. Each run contained vessels with inoculum, standard, basal diet and vessels with basal diet partially replaced with different MCC products. The experiment was conducted using standard in vitro method for studying degradation rates of feeds in the Luke laboratory based on gas production described by Rinne et al. (2016). A modification of the method enabled samples of head space gas to be taken for methane measurements.

Table 1 Chemical composition of the microcrystalline cellulose (MCC) fractions and a lignin product

Sample name	MCC A1	MCC A2	MCC A4	MCC B1	MCC B2	MCC B3	MCC C1	Lign-S
Moisture	Dry	Wet	Dry	Dry	Wet	Dry	Wet	Dry
Colour	White	White	White	Brown	Brown	Brown	Brown	Brown
Size, µm	100 - 150	30 - 50	30 - 50	30µ- 50	30 - 50	100 - 150	25-80	30 - 50
Dry matter, g/kg	952	286	974	954	305	825	172	460
In dry matter, g/kg								
Ash	6	3	4	3	1	1	2	3
Crude protein	4	5	4	3	6	5	11	56
WSC	3	1	3	10	6	8	0	8
NDFom	976	1005	967	986	991	984	993	726
ADF	945	964	940	916	973	949	970	734
ADL	60	3	7	32	39	29	56	523
Cellulose	885	961	933	884	934	920	914	212
Hemicellulose	31	41	27	70	18	35	23	-9
Cellulase solubility, g/kg organic matter	434	503	395	473	404	379	269	339

WSC= water soluble carbohydrates, NDFom = ash-free neutral detergent fibre, ADL = acid detergent fibre, ADL = acid detergent lignin, Cellulose = ADF - ADL, Hemicellulose = NDFom – ADF.

To collect representative gas samples for determination of methane concentration during the first 24 h of incubation, vessel size was increased from 250 ml to 500 ml. After 4 h of incubation, gas was collected from the head space by a 60 ml syringe to lower the pressure to prevent inhibiting fermentation. After 24 h of incubation, the gas from the syringe and headspace gas were combined, mixed and sub-sampled (1 ml). After that, the head space gas was released. All runs were conducted for 72 h. Each experimental vessel contained 0.5 g of sample DM and moist samples were incubated without drying. The basal diet contained grass silage and concentrates in a 1:1 proportion on DM basis. Both basal feed ingredients were dried and ground through 1-mm screen. The grass silage was the standard used in the in vitro procedure and the concentrate was the standard concentrate used in Luke Jokioinen dairy herd. Ash contents were 89 and 71, crude protein 147 and 192, ash-free neutral detergent fibre 537 and 243 g/kg in DM for grass silage and concentrates, respectively.

Gas samples were analysed by gas chromatography for CH_4 concentration. Before chemical analyses, feed samples, MCC and lignin products were dried at 60°C until dry. The DM concentration of the samples was determined by drying at 105°C for 20 h. Ash was determined by ashing at 600°C for 2 h, CP content was analysed using a Dumas-type N analyser (Leco FP-428 nitrogen analyser, Leco Corporation, St Joseph, USA) and water soluble carbohydrates according to Somogyi (1945). Neutral detergent fibre (NDFom) concentration was analysed according to Van Soest et al. (1991) and acid detergent fibre according to Robertson and Van Soest (1981). Acid detergent lignin (ADL) was analysed using the AOAC Method 973.18. Amylase was not included in the fibre analyses (no starch in the samples) and results are presented as ash-free. Pepsin-cellulase solubility was analysed according to Nousiainen et al. (2003). The results are presented in Table 1.

Antimicrobial activity

The antimicrobial activities of the MCC and lignin samples against *Escherichia coli* and the yeast *Candida albicans* were evaluated by in vitro bioassays. The modified disk diffusion method (Välimaa et al. 2007, Balouri et al. 2016) was used, because the samples did not dissolve into the following solvents: ultrapure water (milli-Q), 99.5% ethanol (Etax Aa, Altia Industrial, Finland) or DMSO (Applichem) at room temperature or at 30°C. *Escherichia coli*, KBAK 1610/15 078 br (*E.coli* 1610) and *Escherichia coli*, KBAK 1603/15 01 br (*E.coli* 1603) were obtained from the National Institute for Health and Welfare (THL), Finland. The *Candida albicans* ATCC 10231 yeast strain was received from the American Type Culture Collection (Microbiologics, USA).

For a test, the microbial strains (stored at -80°C) were first cultured on a solid medium. Bacterial strains were cultured on Tryptone Soya agar (TSA, Lab M) at 35°C for 24 hours and the yeast strain on Potato Dextrose Agar (PDA, Lab M) at 25°C for 48-72 hours . Then the strains were pre-cultured. One colony from each plate was taken into 5 ml of Tryptone Soya Broth (TSB, Lab M) or PDB and incubated for 24 hours, shaking at 200 rpm and 35°C or for 48-72 hours shaking at 150 rpm and 25°C, respectively.

Thereafter, the cells were washed. After centrifugation (15 min, 5200 g, 4°C), the supernatant was removed and the pellet suspended in 1xPBS (Difco). This washing step was repeated. For the growth inhibition tests, the bacterial cell suspension was adjusted to the concentration of $1.5*10^8$ cfu (colony forming units)/ml and the yeast cell suspension at a concentration of $1*10^6$ cfu/ml determined by absorbance at OD 600nm (Dynamica, HALO DB-20S UV-VIS Spectrophotometer) and confirmed by plating on TSA or PDA. Prior to adding the test

samples on the Mueller-Hinton agar (acumediq, Neogen) plates, the microbial cell suspensions were spread onto the plates.

The MCC and lignin samples were used as follows: the four dry samples were mixed with water (sterile Milli-Q, 21°C) to obtain a paste. The samples in paste and pulp (no water added) forms were placed on agar plates by use of a sterile cork borer (Ø approximately 6 mm). The pellet form samples were placed on the agar plates as such. Sterile Milli-Q was used as a negative control and streptomycin (0.1 mg/ml, Sigma-Aldrich Finland) and nystatin (1.9 mg/ml in DMSO from Sigma) as positive controls for bacterial and yeast cells, respectively. After incubation, *E.coli* 1610 and *E.coli* 1603 at 35°C for 24 h and the *C. albicans* ATCC 10231 at 25°C for 72 h, diameters of the inhibition zones around the samples/filters were measured and photographed.

Statistical analysis

Results of the in vitro gas production were analysed using SAS GLM procedure. Linear and quadratic effects of MCC inclusion were evaluated using orthogonal polynomial contrasts. Pairwise comparisons were made by Tukey test. Since quadratic effects were not significant, only linear effects are presented below. Significance was assumed at $P< 0.05$

Results and discussion

In vitro ruminal digestion and methane production

In this study, inclusions levels were greater than what would be used in animal feeding. The rationale for this was to find out if responses existed and whether they were dose sensitive. Total gas production for all MCC products and Lignin S at 10% inclusion levels was quite similar when calculated per unit organic matter and did not differ from basal diet total gas production ($P>0.05$). The lignin product at 20 and 30% inclusion levels had lower total gas production ($P< 0.05$) than the basal diet and MCC products (Table 2). Figure 1 presents the effect of the tested product on relative gas production. Inclusion of Lignin S decreased total gas production compared to the basal diet by 10, and 16% for the 20, and 30% of inclusion levels, respectively. Native lignin is indigestible by animals. In contrast to native lignin, purified lignin does not represent a barrier to digestion in monogastric animals (Baurhoo et al., 2008). Reports on effects of lignin or purified lignin on animal performance are limited (Baurhoo et al., 2008) Lignin has low ruminal degradability and inclusion of lignin in the diet decreased total gas and methane production of the whole diet. In this study, Lignin S performed as expected in decreasing total gas and methane production and may be treated as a negative control. Increasing levels of MCC A1, MCCA2, MCC A4, and MCC B2 linearly increased total gas production (Table 2). In contrast, an increasing inclusion of Lignin S decreased linearly total gas production ($P=0.004$).

None of the treatments affected total methane production ($P>0.05$; Table 2) compared to the basal diet. Effects of the tested products on relative methane production are in Figure 1. None of the products reduced methane production. Similar to total gas production, increasing level of Lignin S decreased linearly methane production ($P=0.029$).

Also the ratio of methane to total gas decreased linearly with increased level of inclusion for MCC A1, MCC A4, MCC B1, and MCC B3 (Table 2).

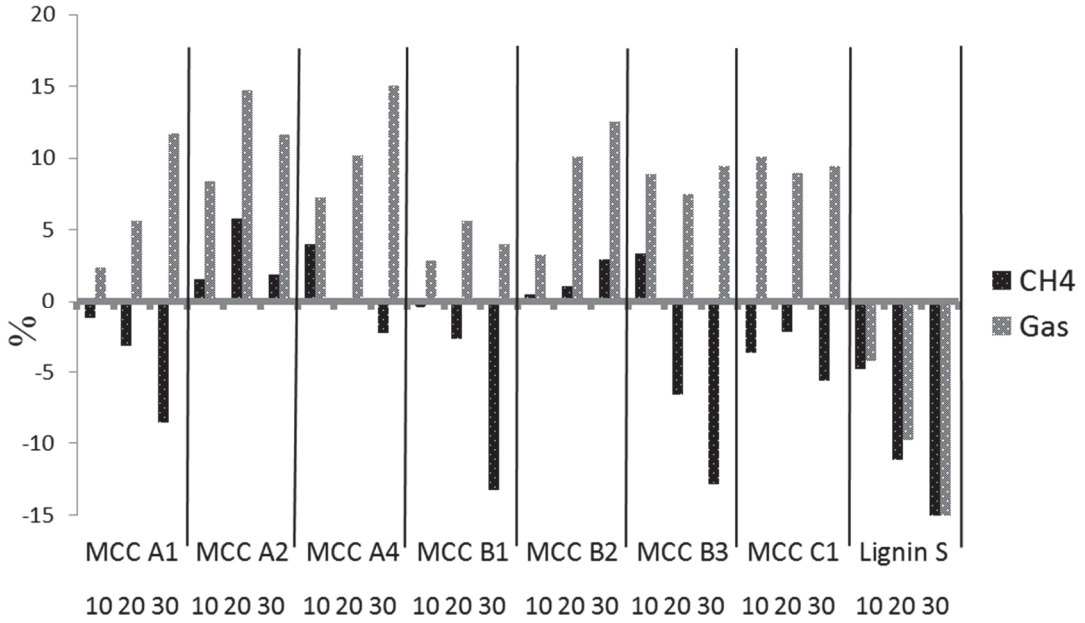

Figure 1 Effect of microcrystalline cellulose (MCC) fractions and Lignin S products at 10 to 30% inclusion on gas and CH_4 production relative to the basal diet.

The results suggest that there is no dramatic difference between the studied products in terms of total gas and methane production potential. All MCC products increased numerically the total gas production compared to basal diet suggesting that the products may have higher digestibility than the basal diet and may provide additional energy to the host animal if included in the diet. The higher level of MCC inclusion increased on average total gas production by 11% when expressed on an organic matter basis. The basal diet also contained more crude protein and crude fat, which have lower gas production potentials than carbohydrates and this should, at least partly, have contributed to the differences. Based on in vitro cellulase solubility, digestibility of the MCC fractions were very low. A typical value for grass silages would be 757 g/kg OM (Huhtanen et al., 2006) and for MCC samples it was on average only 400 g/kg OM. Results are not directly comparable as non-fibre components of the forage sample are totally soluble in that analysis and they represent on average about half of forage DM. If digestion of non-fibre components is assumed to be 100%, cellulase solubility of NDFom in typical forages is ca 500 g/kg OM which is not much higher than for the MCC samples. This shows that traditional feed analysis methods need to be modified for untypical samples such as MCC.

Antimicrobial activity

The objective of this study was to screen for antimicrobial activity of MCC and lignin products against *E. coli* 1610 and *E. coli* 1603 bacterial strains and *C. albicans* ATCC 10321 yeast strain. Since the samples could not be dissolve, their antibacterial activities were tested using a modified disk diffusion method. The results show that none of the MCC and lignin samples had antimicrobial activity against any of the bacterial or yeast cell cultures as measured by the modified disk diffusion method (data not shown).

Table 2 Effect of inclusion of the microcrystalline cellulose (MCC) products and Lignin Son total gas and CH_4 production

Name	Level of inclusion, % in DM	Total gas production, ml/g OM	Linear effect of MCC	CH_4 production, mg/g OM	Linear effect of MCC	CH_4 / Total gas	Linear effect of MCC
Basal diet		346 [b, a, c]		41.9 [a]		0.121	
MCC A1	10	354 [b, a, c]	P_L=0.008	41.4 [a]	P_L=0.271	0.117	P_L=0.006
	20	366 [b, a]		40.6 [a]		0.111	
	30	387 [a]		38.3 [a]		0.099	
MCC A2	10	375 [b, a]	P_L=0.005	42.5 [a]	P_L=0.699	0.114	P_L=0.177
	20	397 [a]		44.3 [a]		0.112	
	30	386 [a]		42.6 [a]		0.110	
MCC A4	10	371 [b, a]	P_L=0.001	43.6 [a]	P_L=0.657	0.117	P_L=0.018
	20	381 [a]		41.8 [a]		0.110	
	30	398 [a]		40.9 [a]		0.103	
MCC B1	10	356 [b, a, c]	P_L=0.295	41.7 [a]	P_L=0.096	0.117	P_L=0.012
	20	365 [b, a]		40.7 [a]		0.111	
	30	360 [b, a]		36.3 [a]		0.101	
MCC B2	10	357 [b, a, c]	P_L=0.002	42.1 [a]	P_L=0.713	0.118	P_L=0.142
	20	381 [a]		42.3 [a]		0.111	
	30	389 [a]		43.1 [a]		0.111	
MCC B3	10	377 [b, a]	P_L=0.059	43.3 [a]	P_L=0.056	0.115	P_L=0.002
	20	372 [b, a]		39.1 [a]		0.105	
	30	379 [b, a]		36.5 [a]		0.097	
MCC C1	10	381 [a]	P_L=0.056	40.3 [a]	P_L=0.542	0.106	P_L=0.069
	20	377 [b, a]		41.0 [a]		0.109	
	30	379 [b, a]		39.5 [a]		0.104	
Lignin S	10	331 [b, a, c]	P_L=0.004	39.9 [a]	P_L=0.029	0.120	P_L=0.866
	20	312 [b, c]		37.2 [a]		0.119	
	30	291 [c]		35.0 [a]		0.120	
SEM		12.24		2.65		0.0064	

[b, a, c] Treatments with the same letter are not different across all treatment and levels (P>0.05).

Conclusions

Traditional feed analyses revealed that fibre concentration of MCC was high but readily digested by rumen microbes. In the antimicrobial activity tests, none of the products, including lignin, showed any antimicrobial activity. Based on these analyses, all fractions seemed suitable as feed fractions for ruminants. In vivo trials are needed to confirm the effects of MCC on feed intake, rumen fermentation and diet digestion under production conditions.

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References

Balouiri, M., Sadiki, M. & Ibnsouda, S.D., 2016. Methods for in vitro evaluating antimicrobial activity: A review. J. Pharmaceut. Biomed. 6, 71–79.

Baurhoo, B., Ruiz-Feria, C. A., & Zhao, X., 2008. Purified lignin: Nutritional and health impacts on farm animals—A review. Anim. Feed Sci. Technol. 144, 175–184.

Chukwuemeka, A & Okhamafe, A.O., 2012. Physicochemical, spectroscopic and thermal properties of microcrystalline cellulose derived from corn cobs. Int. J. Recycl. Org. Waste Agric. 1, 2–7.

Dahl, O., Vanhatalo, K., Parviainen, K., 2011a. A novel method to produce microcellulose. FI patent 2011050526

Dahl, O., Vanhatalo, K., Parviainen, K. & Svedman, M., 2011b. A novel method to produce microcellulose. FI patent 2011050527

Habibi, Y., Lucia, L. A., & Rojas, O. J., 2010. Cellulose nanocrystals- chemistry, self-assembly, and applications. Chem. Rev. 110, 3479–3500.

Hartley, R. D., & Akin, D. E., 1989. Effect of forage cell wall phenolic acids and derivatives on rumen microflora. J. Sci. Food Agr. 49, 405-411.

Huhtanen, P., Nousiainen, J., & Rinne, M., 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15, 293–323.

Nousiainen, J., Rinne, M., Hellämäki, M., & Huhtanen, P., 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Technol. 103, 97 – 111.

Nsor-Atindana, J., Chen, M., Goff, H. D., Zhong, F., Sharif, H. R., & Li, Y., 2017. Functionality and nutritional aspects of microcrystalline cellulose in food. Carbohyd. Polym. 172, 159–174.

Osong, S. H., Norgren, S. & Engstrand, P., 2016. Processing of wood-based microfibrillated cellulose and nanofibrillated cellulose, and applications relating to papermaking–A review. Cellulose 1, 93–123.

Rinne, M., Kautto, O., Kuoppala, K., Ahvenjärvi, S., Willför, S., Kitunen, V., Ilvesniemi, H., & Sormunen-Cristian, R., 2016. Digestion of wood-based hemicellulose extracts as screened by in vitro gas production method and verified in vivo using sheep. Agr. Food Sci. 25, 13-21.

Robertson, J.B., & Van Soest, P. J., 1981. The detergent system of analysis and its application to human foods. In: James, W.D.T. and Theander, O. (eds.). The Analyses of dietary Fibre in Foods. New York, NY, Marcell Dekker. pp. 123-158.

Somogyi, M., 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160, 61 – 68.

Thoorens, G., Krier, F., Leclercq, B., Carlin, B. & Evrard, B., 2014. Microcrystalline cellulose, a direct compression binder in a quality by design environment—A review. Int. J. Pharmaceut. 473, 64–72.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583 – 3597.

Välimaa A. L., Honkalampi-Hämäläinen, U., Pietarinen, S., Willför, S., Holmbom, B. & von Wright, A., 2007. Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms. Int. J. Food Microbiol. 115, 235–243.

Total digesta mean retention time in dairy cows with different abilities to consume large quantities of roughage

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Introduction

Increasing proportion of forage in diets to dairy cows is highly interesting due to improved animal health and also for economic benefits to the farmer if the cow maintains a high milk production. Feeding cows with forage will take advantage of cows' ability to convert non-human edible products to products edible for humans.

Effects of diet seem to be different between individual cows and it has been shown that cows have different capacities to eat high amount of roughage (Patel, 2012).This difference can have various causes such as difference in size, age, lactation state and intake of concentrates, but these factors explain only parts of the variation. Other contributing factors may be differences in passage rate, microbial community composition and/or microbial diversity, which in turn can impact on feed utilization. It is generally known that passage rate of digesta increases with increased feed intake. Passage rate is also affected by fibre content as well as particle size (Tafaj *et al.,* 2005; Teimouri Yansari & Primohammadi, 2009). If particles stay in the reticulo-rumen for a short amount of time, cattle's endogenous enzymes in duodenum may not get access to nutrients that are trapped inside non-fermented fibre, and nutrients may be lost in faeces (Fox *et al.,* 2004; Huhtanen *et al.,* 2006;). On the other hand, if retention time in the rumen is long, passage rate will reduce intake, resulting in decreased productivity. Time and efficiency of digestion in the rumen are therefore crucial for nutrient uptake as well as animal productivity.

Feed intake seems to increase passage rate (Huhtanen 2006), but it might also be that increased passage rate actually explains roughage intake. This may be of importance to consider regarding ability of the dairy cow to ingest and digest large quantities of high quality roughage when fed a low concentrate diet, something, which may also be considered with respect to milk production and the environmental impact of the cow. The aim of this study was to investigate passage rate in relation to forage intake.

Material and method

Animals and experimental design

The present passage rate study was a part of a forage intake capacity (FIC) study at the Swedish University of Agricultural Sciences at Lövsta, Uppsala, Sweden. All handling of animals was approved by the Uppsala Ethics Committee for Animal Research, Sweden (Dnr C 99/16). In total, 39 cows (Holstein (HO) and Swedish Red breed (SR)) were included in the FIC study over one whole lactation period. Cows were randomly divided into two treatment groups fed with two different levels of concentrates and forage *ad libitum*. The passage rate study included 14 cows (7 HO and 7 SR) in mid lactation (median lactation week =15 (range =12-18)), when cows had a fix concentrate allowance of 5.25 and 10.5 kg DM respectively. Four of the selected cows had a "high concentrate diet" (HCD) and 10 of the selected cows had a "low concentrate diet" (LCD). The passage rate study periods took place during seven days at four different sessions, in May, June, August and October in year 2017. Each cow was included in one study session only.

All cows were housed in a free-stall barn with an automatic milking system (DeLaval VMS™; DeLaval, Tumba, Sweden); milk yield was recorded during all milkings. The cows were equipped with neck transponders and had access to separate concentrate blends in two concentrate feeding stations (DeLaval, Tumba, Sweden) and concentrates were also fed in the milking station. Three kg of concentrate was accessible per day in the robot, while the rest of the concentrate was provided in concentrate feeding stations. Silage was fed *ad libitum* in forage mangers placed on weigh cells (BioControl, Rakkestad, Norway). Silage and concentrates were fed separately to all cows throughout the period and were available at all hours. The grass silage was a first cut and the theoretical chop length was 20 mm. Forage intake was measured as the difference between weight when the cow entered the manger and when she was leaving the manger. Chemical composition of the feeds are in Table 1.

Table 1 Chemical composition of grass silage (n=10) and concentrate (standard deviations are presented in brackets)

Feed	Grass silage[1]	Concentrate[2]
DM, g/kg	446 (33.4)	880
Ash, g/kg DM	88 (4.6)	91
CP, g/kg DM	155 (7.8)	164
aNDFom, g/kg DM	490 (11.1)	442
OMD[3], % of OM	79.3 (2.14)	

[1]Grass silage had pH 4.3 and ammonia nitrogen 50 g per kg total N, [2]values of chemical composition came from the manufacturer. The concentrate consisted of (on dry matter basis) 56.6 % unmolassed sugar beet fibre, 12.0% wheat bran, 9.4% wheat middlings, 7.0% heat treated rapeseed meal Expro®; 7.0% distiller's dried grains Agrow feed™ 90, 2.6% vegetable fat AkoFeed®Cattle, 2.3% limestone, 2.0% molasses of sugar beet, 0.9% sodium chloride, 0.2% mineral premix. [3]OMD was calculated as 0.9∗VOS – 2, according to Lindgren (1983).

Feed sample collection and analysis

Samples of silage (~1 L/day) and concentrates (~0.2 L/day) were collected 5 d/week during measurement periods and pooled into one sample per each 2-week period. Silage samples were immediately frozen at -20°C. Analyses of dry matter (DM) was performed at 60°C (NorFor, 2011), ash and crude protein according to EC No. 152/2009, neutral detergent fibre (aNDFom) was assayed with a heat-stable amylase and expressed exclusive of residual ash (Chai and Udén, 1998), organic matter digestibility *in vitro* (VOS) (Lindgren, 1979).

Milk Yield, Feed Intake, Drinking and Body Weight

Milking, feeding and water drinking data were collected during sampling period and summarized into one average value per cow. A scale registered weights of cows before entering AMS.

Feed markers and faecal sampling

Chromium mordanted fibre fraction from the roughage was prepared by following method of Udén *et al.* (1980). Each cow was given the equivalent of 2 g of Cr. Faecal sampling occurred at 0, 8, 12, 16, 20, 24, 28, 32, 36, 44, 52, 60, 68, 76, 84, 92, 100, 108,116, 124, 132, 140, 148, 156, 164 h. Exact time was noted and used further on to correct sampling time per cow. After each sampling session, faecal samples were distributed on Petri dishes and kept at -20°C until further analysis. Samples were put in a -80°C freezer for ≥16 h, followed by freeze drying (72 h) and milling (1-mm sieve). All selected samples were sent to ALS Global (Luleå, Sweden) for Cr analysis. Analysis was performed by inductively coupled plasma sector field mass spectrometry (ICP-SFMS). Reported Cr concentrations was further related to exact time of sampling per cow and fitted using Excel Solver to a curve based to equation:

$$Cr_{curvefit} = A \cdot \frac{\mathrm{k1}}{k1 - k2} \cdot \left(e^{-k_1 \cdot (t-L)} - e^{-k_2 \cdot (t-L)}\right)$$

where, $Cr_{curvefit}$ = chromium concentration; A = estimated marker concentration in Pool 1; k_1 = estimated passage rate from Pool1 to Pool 2 (h^{-1}); k_2 = passage rate from Pool 2 (h^{-1}); t =time (h) and L = lag phase (h) (Udén and Sutton, 1994)

Total mean retention time (h) was calculated as:

$$TMRT = \frac{1}{k_1} + \frac{1}{k_2} + L$$

Rumen passage rate for forage aNDFom was calculated according to Equation 7.5 in NorFor model (NorFor, 2011):

$$r_{kpNDFr} = 0.4803 + \frac{0.78 \cdot 1.937}{1 + \left(\frac{\mathrm{NDFI}}{\mathrm{BW} \cdot 7.484}\right)^{-3.198}}$$

where, r_kpNDFr = ruminal passage rate of roughage aNDFom (%/h); NDFI = daily total aNDFom intake (g/d); BW = cow body weight (kg).

Estimation of mean retention time in rumen (h) based on passage rate estimated by the NorFor model was calculated as:

$$MRT = \frac{1}{(r_{kpNDFr} \cdot 100)}$$

Statistical analyses

Statistical analyses were performed with SAS (SAS 9.3 Institute Inc., Cary, NC, 2008). Proc MEANS were used for means, min and max values within treatment group. TMRT values were subjected to the MIXED procedure by using the model:

Yij = ForageDMI_BW + $\mathrm{Treatment}_i$ + $(\mathrm{ForageDMI\_BW} \times \mathrm{Treatment})_i$ + e_{ij}

where, the terms are continuous effect of forage DMI per kg of BW and fixed effect of treatment group ($_j$ = 2) and e_{ij} is the random error. Least square means were calculated using LSMEANS/PDIFF. All effects were declared significant at $P<0.05$.

Results

Values of feed intake, water intake and passage rate are in Table 2.

Table 2 Forage dry matter intake (DMI), concentrate DMI, total DMI, total neutral detergent fibre (aNDFom) intake, drinking water intake, total mean retention time (TMRT), body weight and milk production in cows with different concentrate allowances. Values are means (range) in kg/day, if nothing else stated

	Low concentrate (N=10)	High concentrate (N=4)
Forage DMI	21.0 (17.3-25.9)	16.8 (15.9-18.2)
Concentrate DMI	5.1 (4.8-5.3)	10.4 (10.3-10.6)
Total DMI	26.2 (22.5-29.5)	27.3 (26.3-28.7)
Total aNDFom intake	12.6 (10.8-14.2)	12.9 (12.4-13.6)
Water intake	115 (90-147)	120 (107-139)
TMRT, hours	53.1 (46.7-64.0)	48.9 (46.7-50.9)
Body weight, kg	728 (650-884)	677 (627-765)
Milk yield	35.1 (27.3-40.0)	40.0 (35.0-46.6)

There was no linear relationship between forage DMI per kg BW and TMRT (P=0.94). In addition, TMRT was not affected by concentrate allowance (P=0.99): LSmeans 52.8 ± 2.1 and 48.3 ± 9.4 hours for LCD and HCD, respectively.

Passage rate predicted by NorFor was on average 52.6 h, and measured average passage rate was 51.3 h. However, variation in observed values was larger (46.7-64.1 h) than variation predicted by NorFor (51.8-53.8 h).

Discussion

In this material, there was no relationship between forage intake and TMRT. Longer retention time for forage compared to concentrate has been observed (Colucci *et al*., 1990). A decreased forage:concentrate (F:C) ratio increases rumen retention time, but this seems to be more prevalent at low feed intake levels (Colucci *et al*., 1990; Huhtanen and Jaakkola, 1993). In this study, intake levels were high and no relationship between TMRT and F:C ratio. However, there was a numerical difference between concentrate allowance and TMRT, although TMRT was not correlated with forage intake within the LCD group.

It could have been expected that forage intake should have increased fibre content of the diet, but the concentrate used was based on by-products and aNDFom concentration was similar to the forage aNDFom, resulting in no difference in aNDFom intake related to forage intake. Total aNDFom intake was not related to TMRT. It seems that TMRT was not related to any of the estimated parameters. Further evaluation will be performed on feed digestibility, rumen microbial structure, and methane production.

Mean values of ruminal MRT prediction by the NorFor model and observed mean value of TMRT based on Cr-curves were similar. There was a small difference in MRT, which might have been related to ruminal and total retention times of aNDFom, although this might play a minor role in comparison with observed values since fermentation in hindgut plays a minor role in fibre digestion compared to rumen (Lund *et al*., 2006). Lund *et al.* (2006) assumed that on average, 0.82 of total tract retention time is due to pre-duodenal retention and 0.18 to lower gut retention. Variation in MRT predicted by NorFor was much lower compared to the observed variation, indicating that there are individual differences that are not accounted for in the NorFor model.

References

Chai, W. & Udén, P., 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. Anim. Feed Sci. Tech. 74, 281-288.

Colucci, P.E., Macleod, G.K., Grovum, W.L., McMillan, I. & Barney, D.J., 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73, 2143-2156.

EC No 152/2009. Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union L54/1, 1-130.

Fox, D., Tedeschi, L., Tylutki, T., Russell, J., Van Amburgh, M., Chase, L., Pell, A. & Overton, T., 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112, 29–78. https://doi.org/10.1016/j.anifeedsci.2003.10.006

Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R. & Nørgaard, P., 2006. Digestion and passage of fibre in ruminants. In Ruminant physiology: digestion, metabolism and impact of nutrition on gene expression, immunology and stress (pp. 87-135). Wageningen Press, Wageningen, the Netherlands.

Jaakkola, S. & Huhtanen, P., 1993. The effects of forage preservation method and proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. Grass Forage Sci. 48, 146-154.

Lindgren, E. 1983. Re-calibration of the VOS method for determining energy value in ley forage. Department of Animal Nutrition and Management, SLU, Sweden, 4 pp. (in Swedish).

Lindgren, E., 1979. The Nutritional value of roughages determined in vivo and by other laboratory methods. Report 45. Department of Animal Nutrition, SLU, Sweden. 61pp (in Swedish).

Lund, P., Weisbjerg, M.R. & Hvelplund, T., 2006. Passage kinetics of fibre in dairy cows obtained from duodenal and faecal ytterbium excretion: effect of forage type. Anim. Feed Sci. Technol. 128, 229-252.

NorFor, 2011. NorFor - The Nordic Feed Evaluation System. Ed: H. Volden. EAAP publication No. 130. Wageningen Academic Publishers, the Netherlands.

Patel, M. 2012. Effects of increasing proportion of high-quality grass silage in the diet of dairy cows. PhD thesis, SLU No. 80.

Udén, P., Colucci, P.E. & Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J. Sci. Fd. Agric. 31, 625-632.

Udén, P. & Sutton, J.D., 1994. Retention of Cr-labelled grass hay and silage in different segments of the gastrointestinal tract of dairy cows. Livest.Prod.Sci.37, 297-309.

Tafaj, M., Kolaneci, V., Junck, B., Maulbetsch, A., Steingass, H. & Drochner, W., 2005. Influence of fiber content and concentrate level on chewing activity, ruminal digestion, digesta passage rate and nutrient digestibility in dairy cows in late lactation. Asian Australas. J. Anim. Sci. 18, 1116–1124. https://doi.org/10.5713/ajas.2005.1116

Teimouri Yansari, A., & Primohammadi, R. Effect of particle size of alfalfa hay and reconstitution with water on intake, digestion and milk production in Holstein dairy cows. Anim. 3, 218–27. https://doi.org/10.1017/S1751731108003546.

Volden, H. 2011. Feed calculations in NorFor. In NorFor-The Nordic Feed Evaluation System (pp. 55-58). Wageningen Academic Publishers, the Netherlands.

The influence of milk yield, body weight and parity on feed intake by dairy cows

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Introduction

Accurate prediction of dry matter intake (DMI) is important since it is a primary factor affecting animal performance. However, because of the complexity of factors controlling feed consumption, accurate prediction of daily DMI is difficult. Generally, milk yield has a large influence in prediction equations for DMI (Bertilsson & Burstedt, 1983; NRC, 2001; Volden *et al*., 2011). Most models proposed for predicting voluntary feed intake of lactating dairy cows were developed empirically by applying multiple linear regression techniques (Forbes, 1995). These models generally include milk yield and body weight as predictors. Several studies (Fuentes-Pila *et al*., 1996; Roseler *et al*., 1997) have showed lack of accuracy of existing feed intake prediction equations when challenging them with independent datasets. In early lactation, dairy cows generally compensate for inadequate DMI in relation to the requirement of nutrients for milk production by entering a state of negative energy balance and mobilization of body tissues. Milk production and DMI are apparently partly uncoupled in this period. No prediction adjustments to the DMI for parity is required as long as appropriate body weight and milk production data are used (NRC, 2001). However, management factors such as milking frequency and length of the previous dry period has a large influence on milk production but do not affect DMI (Remond *et al*., 1999; O'Hara *et al*., 2018). These factors are generally not taken into account in prediction models for DMI. It is generally accepted that size of dairy cattle has increased concurrently with an increase in milk yield. However, apparently no genetic correlation between body weight (BW) and energy supply exists (Vandehaar *et al.*, 2014).

Our aims of this study was to investigate: i) the relationship between milk yield and DMI during the early phase of lactation and ii) how body weight and parity are related to DMI and milk yield. We also aimed to investigate the ability of the NRC's model to predict DMI of Swedish red (SR) and Swedish Holstein (SH) cows - both on an individual and group basis.

Materials and methods

Data from two different animal experiments were used in the present study. In Study I, multiparous cows of SR (n=43) and SH (n=34) were blocked by breed and parity and then randomly allocated to two different treatments: a traditional dry period length of 8 weeks or a short dry period length of 4 weeks. After calving, all cows were managed and fed according to the same routines. Lactating cows were fed silage and concentrates separately. They received 5 kg dry matter (DM) concentrate/day after parturition. Starting 3 days after parturition, the amount was gradually increased by 0.4 kg DM/day to a maximum level of 15 kg DM/day. Silage was provided *ad libitum* and individual silage intake of the lactating cows was recorded using BioControl feed bunks (BioControl, Rakkestad, Norway). Body weight (BW) was automatically recorded (AWS 100, DeLaval International, Tumba, Sweden) after each milking and a weekly average was calculated for week 1, 4, 8 and 12 postpartum. Milking was carried out in an automatic rotary system (AMR™, DeLaval International AB, Tumba, Sweden) twice a day at fixed times. For detailed information about the experimental

design, see Andrée O'Hara *et al*. (2018). In the present study, only data from lactation week 1 to 12 are used.

In Study II, 85 cows were allocated to a low concentrate ration. Breeds were SH (n = 36) and SR (n = 49). Concentrate was gradually increased with 0.4 kg DM per day in both groups, from 2.6 kg DM at calving to 4.4 kg DM/day to multiparous cows (n=40) and 3.5 kg DM/day to primiparous cows (n=45). Concentrate and silage were provided as described above. Body weight was recorded and cows were milked as described above. At each milking, milk yield was recorded. Data generated during the period from 2 weeks until 6 weeks after parturition are included. The statistical software Minitab 18 was used to create linear regressions.

Results and discussion

Regression analyses indicated positive relationship between DMI and yield of energy corrected milk (ECM) in cows fed a limited amount of concentrate and silage *ad libitum* during the first six weeks after parturition (Figure 1 upper panel). However, there was no such relationship in primiparous cows. The results may be regarded as controversial since no prediction adjustments of DMI for parity is thought to be required as long as appropriate BW and milk production data are used (NRC, 2001). In Figure 1, lower panel, the relationship between DMI and ECM in early lactation (Week 1 and 2) and mid-lactation (Week 11-12) is shown for multiparous cows. No relationship between DMI and ECM was observed for any of the two stages of lactation. The results indicate that the generally accepted relationship between ECM and DMI was masked by large individual variations in milk yield and DMI.

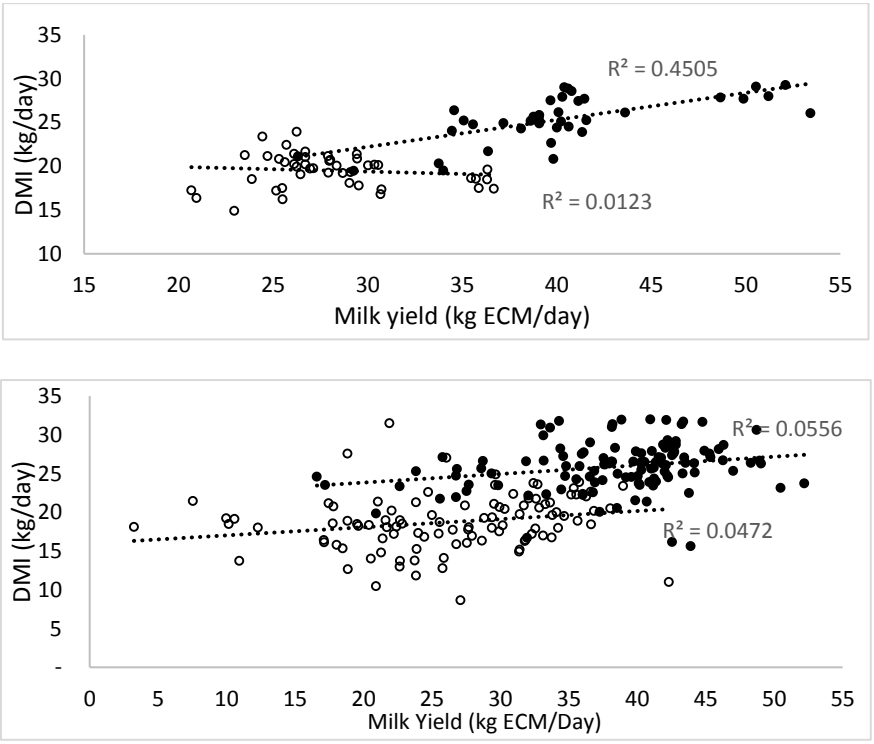

Figure 1 Milk yield and dry matter intake. Upper panel: Primiparous cows N=45 (open circles) and multiparous cows N= 40 (closed circles) during the first 6 weeks after calving. Lower panel: multiparous cows N=74, open circles: Week 1 and 2, closed circles: Week 11 and 12.

Most intake prediction models include adjustments for BW. Over the past 50 years, the BW of dairy cattle has increased concurrently with increasing milk yield per cow. However, BW does not seem to correlate with milk yield (Vandehaar *et al*., 2016). In line with this observation, ECM yield did not correlate with BW in the present study. Body weight explained 1% or less of observed variation in milk yield both among primiparous and multiparous cows and in early lactating and mid-lactating cows (Figure 2).

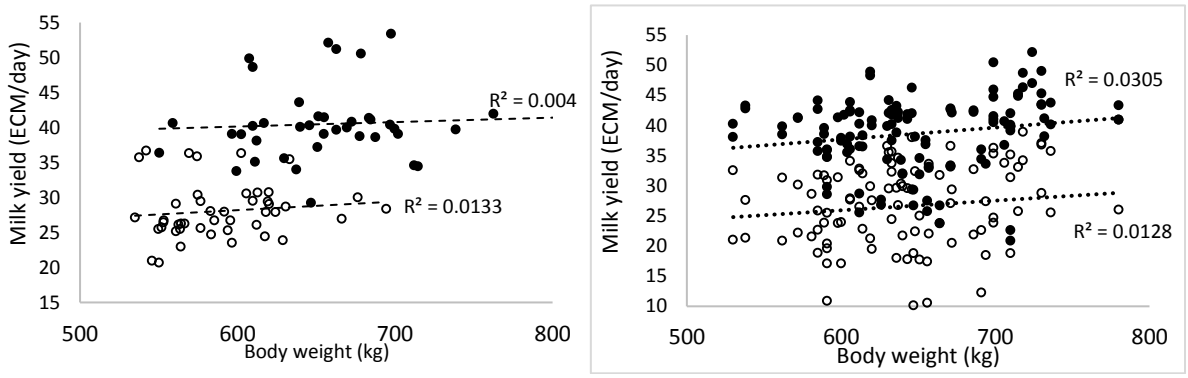

Figure 2. Body weight in relation to milk yield during the first six weeks of lactation. Left panel: Primiparous cows N=45 (open circles) and multiparous cows N= 40 (closed circles). Right panel: multiparous cows N=74, open circles: Week 1 and 2, closed circles: Week 11 and 12 of lactation.

Higher BW should, therefore, not be desirable, especially since heavier cows require more energy for maintenance. It could be argued that BW was influenced by the amount of adipose tissue. But, in this study there was no relationship between BW and body condition score (data not shown).

Prediction of voluntary feed intake is a most important determinant of production efficiency. In the present study, we have tested the relationship between observed and predicted intake by using the NRC model:

$$\text{DMI (kg/day)} = (0.372 \times \text{ECM}) + (0.0968 \times \text{BW}^{0.75}) \times (1 - e^{(-0.192 \times (\text{WOL} + 3.67))}),$$ (NRC, 2001)

where, WOL=week of lactation.

As shown in Figure 3, DMI of both SH and SR cows were fairly well predicted by the NRC model on group level during the 12 first weeks of lactation. This model is based entirely on Holstein cows and this preliminary study indicates that it might also predict DMI for a group of SR cows.

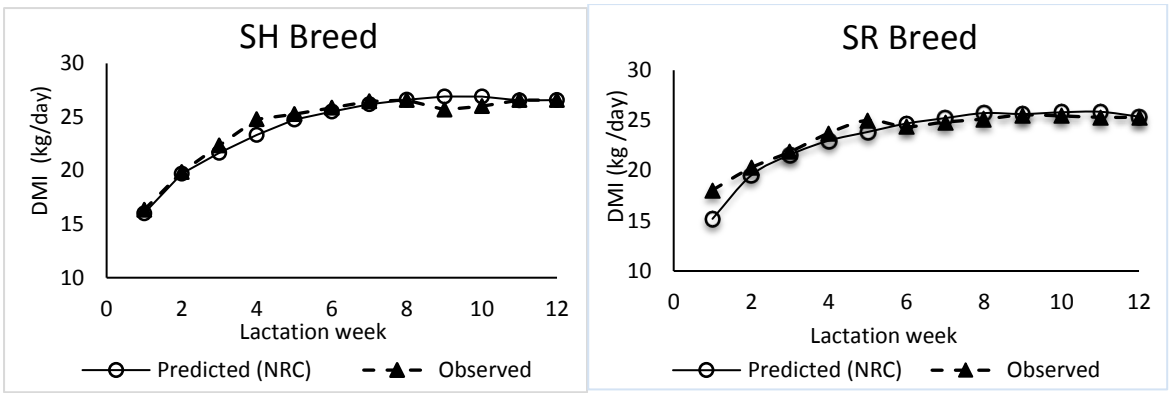

Figure 3 Predicted (NRC, 2001) *vs* observed dry matter intake during the first 12 weeks after calving in multiparous cows of two different breeds (N=74 cows).

Figure 4, left panel, shows the relationship between predicted (NRC, 2001) and observed DMI in primiparous and multiparous cows during their first 6 weeks of lactation. Within parity, there was no correlation between predicted and observed DMI (P>0.05) and the prediction model explained only 10 and 7% of observed variation in DMI for primiparous and multiparous cow, respectively. The NRC model, as virtually all other prediction models for DMI in dairy cows, is largely based on milk yield and assume a standardized rate of utilization of body tissues which in turn is related to week of lactation. However, rate of mobilization of body tissues in individual cows might also be influenced by, for instance, management (Remond *et al*., 1999; Andrée O'Hara *et al*., 2018) and body condition (Rukkvamsuk *et al*., 1999; Agenäs 2003). To some extent, individual variation in efficiency of feed utilization might further reduce the relationship between predicted and observed DMI in individual cows (Vandehaar *et al*., 2016). However, it cannot be excluded that errors in observed DMI and milk yield did not contribute to the poor relationship between predicted and observed DMI especially among primiparous cows.

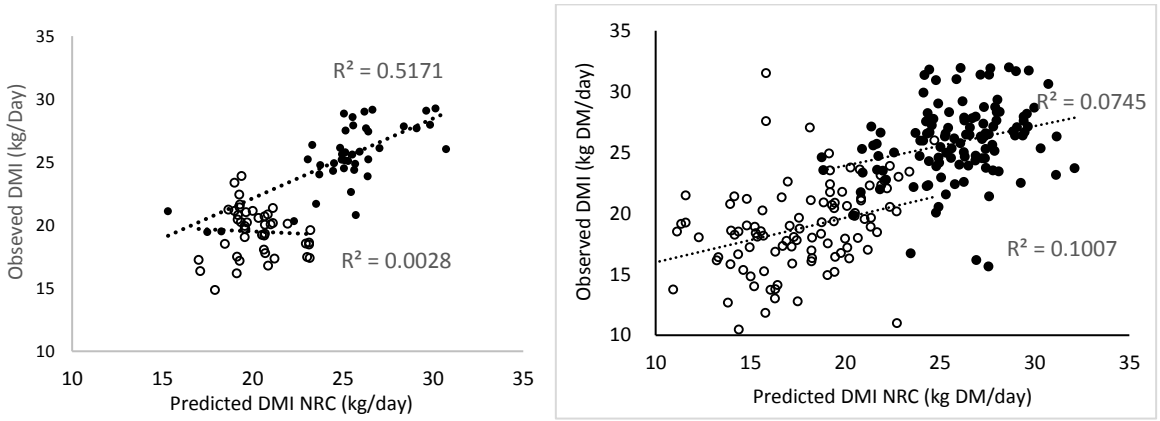

Figure 4 Observed *vs* predicted dry matter intake. Left panel primiparous cows (N=45) and multiparous cows N= 40 during Week 2 to 6. Right panel: Week 1 and 2 (open circles) and Week 11 and 12 (filled circles) after calving; N=74 cows.

Conclusions

Results showed that milk yield was poorly related to dry matter intake especially in early lactating primiparous cows and, it is reasonable to assume that the capacity to utilize body resources as precursors for milk synthesis differed greatly among individual animals. Milk yield did not show any relationship with body weight and results suggest that increased size of dairy cows, as a consequence of breeding for increased milk production, should be regarded as a negative side effect which increases feeding costs, methane production and which will also require larger and more expensive animal facilities. The NRC model apparently predicts DMI both in SR and SH cows on group level. However, DMI of individual cows was poorly predicted.

References

Agenäs, S, Burstedt, E &Holtenius, K., 2003. Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. J Dairy Sci. 86, 870-882.

Andree O'Hara, E., Omazic, A., Olsson, I. & Holtenius K., 2018. Effects of dry period lengthon milk production and energy balance in two cow breeds. Animal 12, 508-514.

Bertilsson, J. & Burstedt, E., 1983. Effects of conservation methods and stage of maturity upon the feeding value of forages to dairy cows. Forage intake and effects of concentrate/forage ratio. Swedish J. Agric. Res. 1, 189-200.

Forbes, J. M. 1995. Voluntary Food Intake and Diet Selection in Farm Animals. CAB international, Wallingford, UK.

Fuentes-Pila J., DeLorenzo M. A., Beede D. K., Staples C. R. & Holter J. B., 1996. Evaluation of equations based on animal factors to predict intake of lactating Holstein cows. J. Dairy Sci. 9, 1562-1571.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington DC, US

Remond, B., Coulon, J. B., Nicloux, M. & Levieux. M. 1999. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. Ann. Zootech. 48, 341-352.

Roseler, D. K., Fox, D. G., Pell, A. N. & Chase, L. E., 1997. Evaluation of alternative equations for prediction of intake for Holstein dairy cows. J. Dairy Sci. 80, 864-877.

Rukkwamsuk, T., Kruip, T. A. & Wensing, T., 1999. Vet. Q. 21, 71-77.

VandeHaar, M. J., Armentano. L. E., Weigel, K., Spurlock, D. M, Tempelman, R. J & Veerkamp, R., 2016. Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. J. Dairy Sci. 99, 4941-4954.

Vandehaar, M., Lu, D., Spurlock, L. E., Lu, Y & Tempelman R. 2014. Phenotypic and genetic correlation among milk energy output, body weight, and feed intake, and their effects on feed efficiency in lactating dairy cattle. J. Dairy Sci. 97, (E-suppl.), 80.

Volden, H., Nielsen, N. I., Åkerlind, M., Larsen, M., Havrevoll, Ö. & Rygh, A. J., 2011. Prediction of voluntary feed intake. In: NorFor – The Nordic feed evaluation system, EAAP Publication No 130.

Dynamic feeding of dairy cows in automatic milking systems

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Introduction

There are several ways to estimate feeding requirements of dairy cows: calculating the amount of concentrate based on expected roughage intake and actual milk yield or according to a standard lactation curve together with expected roughage intake. Modern technology enables both milk yield and roughage consumption to be recorded for each individual on a daily or even hourly basis, which provides opportunity for better fine-tuning of feeding rations. This concept is called dynamic feeding (Meijer & Peeters, 2010, André, 2011). If concentrate allocation is based on actual roughage intake and milk production, overfeeding can be avoided and weight fluctuations limited, while profitability can be improved.

In this study, we investigated whether a dynamic approach to dairy cow feeding can improve the balance between silage intake and milk yield, by utilising automatically generated data from and in the automatic milking system. The hypothesis was that a dynamic feeding approach will enable equally high production from a lower feed input, due to better balance between input and output.

Materials and methods

The study included in total 64 Norwegian Red cows. Cows were of 1st, 2nd and >2 parities (2.3±1.4, range 1-6) and participated in the study during DIM 5-125. A commercial compound concentrate (Formel energi premium 70; FK Agri, Lillestrøm, Norge) was allocated according to either a static or a dynamic feeding regimen, using early (31 May-1 June) or late (9th June) harvested silage, resulting in four treatment groups in total. Cows were grouped according lactation number and calving date and randomly assigned to each of the four treatments. Static feeding regimens were based on standard ratios provided by NorFor (Volden, 2011, 2017), aiming for 305-d milk yields of 7500, 8500 and 9000 kg ECM for 1st, 2nd and >2nd lactation cows, respectively. In the dynamic feeding regimen, concentrate allocation was calculated based on maintenance and actual milk production requirements and intake of silage. Concentrate allocation in the dynamic feeding regimen was revised weekly.

Cows were housed in a loose housing system with FeedFirst™ cow traffic, concrete slatted floors and rubber mats with sawdust bedding in cubicles. Cows were provided silage ad lib in automatic feeding troughs placed on weight cells (BioControl, Rakkestad, Norge), water was provided ad lib and concentrate in feeding stations. Milking was performed in a DeLaval VMS™ (DeLaval International AB, Tumba, Sweden). Intake of silage and milk yield were automatically recorded at each feeding occasion and milking, respectively. Body weight was recorded after each milking (BioControl, Rakkestad, Norge). For nutritional values of the feeds, see Table 1.

Table 1 Nutritional values of feeds used (±SD), g/kg dry matter if not otherwise specified

	Early harvest	**Late harvest**	**Concentrate**
Dry matter (g/kg feed)	261±23.5	359±10.2	878
Ash	75±2.5	62±1.9	78
Crude protein	151±6.9	127±5.8	277
Crude fat	31±1.9	27±1.9	65
aNDFom[1]	579±7.1	614±7.6	179
iNDF[2] (g/kg NDF)	134±59	206±32	206
Starch			289
Rest fraction	164±7.4	173±4.7	162
Sugar			87
Lactic acid	64.0±2.8	38.5±9.2	
Acetic acid	25.0±0.0	17.0±8.5	
pH	4.43±0.2	4.3±0.1	
Ammonia (g/kg N)	60.0±2.8	36.5±4.9	64
OMD[3] (%)	80.3±1.6	71.9±5.6	
NEL20[4] (MJ/kg DM)	6.75±0.0	6.18±0.0	7.53
AAT20[5]	80.5±2.1	77.0±2.8	163
PBV20[6]	42.5±2.1	9.0±24.0	41

[1]aNDFom=ash corrected and amylase treated NDF; [2]iNDF=indigestible NDF; [3]OMD=Apparent total digestibility of organic matter; [4]NEL20= Standard feed value for NEL at 20 kg dry matter intake (DMI); [5]AAT20= Standard feed value for AAT at 20 kg DMI; [6]PBV20= Standard feed value for PBV at 20 kg DMI.

Due to large daily variation in individual feed intake and milk yield, Legendre polynomials were used for non-linear modelling of data prior to statistical analysis. Data was analysed using PROC MIXED in SAS 9.4. The model included fixed effects of feeding regime, harvest time, lactation group and their interactions, as well as an effect of breeding index.

Results and discussion

Static feeding increased total energy intake (P=0.036) and tended to increase total dry matter intake at DIM 25, but had no effect later in the lactation (Table 2). At DIM 25, static cows also had higher production measured as kg ECM with 32.2±0.69 vs 29.4±0.73 kg ECM, respectively (P=0.042) and had higher consumption of concentrate with 10.3±0.29 vs 9.1±0.26 kg, respectively (P=0.0071). There were no differences in energy balance between the groups at DIM 25-125 and no differences in feed efficiency were found. Harvest time had no impact on energy balance, feed efficiency, total energy or total dry matter intake. However, at DIM 25, there was a tendency for higher energy intake from roughage in groups fed early harvested silage with 84.05±3.85 vs 69.24±3.93 MJ, respectively (P=0.0539) and a tendency for higher concentrate intake for cows consuming late harvested silage with 10.3±0.29 vs 9.3±0.28 kg, respectively (P=0.0680).

Table 2 Energy balance, feed efficiency, total energy intake and total dry matter intake at DIM 25, 50, 75, 100 and 125 for cows fed early or late harvested silage in a dynamic or static feeding regimen

		Early harvest		**Late harvest**			**p-values**		
	DIM	Dynamic	Static	Dynamic	Static	SE	Harvest	Feeding	Harvest* Feeding
Energy balance (%)	25	101.9	104.4	99.6	102.7	4.79	0.651	0.446	0.952
	50	107.4	109.5	104.8	107.9	4.79	0.657	0.448	0.944
	75	109.5	112.0	107.2	110.3	4.80	0.649	0.448	0.950
	100	107.9	110.5	105.7	108.8	4.79	0.651	0.441	0.948
	125	111.4	113.8	109.1	112.2	4.79	0.654	0.444	0.945
Feed efficiency (kg ECM per kg DM)	25	1.49	1.51	1.54	1.45	0.087	0.912	0.592	0.584
	50	1.44	1.49	1.48	1.40	0.089	0.769	0.848	0.525
	75	1.44	1.48	1.47	1.47	0.12	0.911	0.801	0.841
	100	1.39	1.44	1.42	1.36	0.15	0.895	0.945	0.748
Total energy intake (MJ)	25	136.7	154.5	133.0	142.0	7.62	0.252	**0.036**	0.594
	50	147.5	154.1	136.8	148.6	7.28	0.230	0.115	0.738
	75	147.9	144.0	129.6	144.6	7.70	0.218	0.349	0.263
	100	142.8	141.1	122.7	147.0	10.33	0.453	0.167	0.252
	125	122.9	129.7	127.0	141.0	11.95	0.483	0.265	0.780
Total dry matter intake (kg)	25	19.4	21.8	19.6	20.9	1.18	0.721	0.057	0.648
	50	21.0	21.7	20.2	22.1	1.10	0.812	0.131	0.601
	75	21.2	20.4	19.3	21.7	1.16	0.768	0.364	0.202
	100	20.6	19.9	18.4	22.2	1.56	0.999	0.203	0.200
	125	17.9	18.7	19.2	21.3	1.66	0.207	0.249	0.725

Lactation number had a large impact on almost all measured variables, as older cows have a higher intake capacity, higher milk yield and higher body weight. These differences were all expected. The range in lactation number was large, and cows were therefore grouped into three lactation groups: 1st lactation, 2nd lactation and older cows. This is also in accordance with the template lactation curves provided in NorFor, which are divided into the same three categories.

The model used for allocating concentrate in this study was fairly simple compared to other models (André, 2011). In addition to body weight and milk yield, individual roughage intake was recorded on a daily basis. However, growth was not taken into account when concentrate was allocated to cows in the dynamic groups, for neither 1st or 2nd lactation cows. Despite this, there was no difference in energy balance between treatment groups and no interaction between treatment and lactation group. This indicates that cows in the dynamic feeding regimen had no problems meeting their needs for growth. Over all, energy balance was positive in all groups during the major part of the lactation, which resulted in weight gain and an increasing trend in BCS from all treatments. This may indicate that the requirements for the Norwegian red breed are difficult to estimate based on the measures included. The Norwegian red is a dual-purpose breed, which may reflect on the effects of concentrate allocation relative to milk yield.

When adapting concentrate allocation according to observed milk yield rather than feeding a set amount of concentrate to enable a higher milk production, there is a risk of not using the maximal milk yield potential of the cow, due to underfeeding of concentrates. Feeding level is an important factor in determining milk yield, together with milking frequency, milking efficiency and genetic potential. However, if there were a feeding level effect, this would likely have been reflected in a treatment difference, since the majority of the cows in the study did not produce as expected also on the static treatments.

An issue with using day-to-day estimates to allocate feeding, is that several estimates cannot be measured on a day-to-day basis. Analysis of milk composition and feed composition is time and cost demanding and results may not be available until weeks after samples are taken. This introduces a factor of error in the calculations. Many of the daily recording also have a large day-to-day variation, such as weight, feed intake and milk yield. For these recordings, seven-day sliding averages were used for each change in concentrate allocation, to avoid rapid and unrepresentative changes in feeding. An algorithm was also used to sort out clear outliers as regards to body weight and roughage intake.

An inherent problem with the feeding troughs used was that cows steal feed from each other, affecting recorded intakes, both for the cow that steal and for the one that is stolen from. There was a higher frequency of silage stealing from cows on the late harvest time, compared to early harvest time (data not shown), which could be due to a higher preference for the early harvested silage. However, stolen amounts of concentrate can be assumed to have been marginal, compared to daily intake, and was therefore assumed not to have been of importance (H. Kismul, personal communication).

Lack of differences in concentrate consumption, milk production or total energy intake indicates that there was no difference in energy supply between the treatment groups. This might have been related to a too small data material, too short measuring periods or lack of positive effects of the dynamic feeding regimen.

A second experiment is on-going and will continue until 220 DIM. In this experiment, there is no factor of harvest time and treatments are limited to static or dynamic feeding. First lactation cows are also excluded, and a higher goal for 305-d milk yield is used for both 2nd lactation and older cows.

Conclusions

Harvest time and feeding regimen had no effect on energy balance or feed efficiency, but cows fed according to a dynamic feeding regimen had lower total energy intake, total dry matter intake, total concentrate intake and ECM production at DIM 25. However, no differences were found later in the lactation.

References

Meijer, R. & Peeters, K.. 2010. The use of precision dairy farming in feeding and nutrition. In: Proc. 1st N. Am. Conf. Precision Dairy Manag., 20–23 March 2010, Toronto, Canada.

Andre, G. 2011. Adaptive models for operational use in dairy farming – Increasing economic results utilising individual variation in response. PhD thesis. Wageningen University, Wageningen, the Netherlands.

Volden, H., 2011. NorFor - The Nordic Feed Evaluation System, Volden H. (ed.), Wageningen Academic Publ.

Volden, H., 2017. Equation changes since NorFor 2011 (EAAP no. 130). http://www.norfor.info/files/pdf-dokumenter/Artikler/Equation_changes_since_the_NorFor_book_2011.pdf

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Technical note: Particle size distribution analysis using sieving aids

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Introduction

Mechanical disruption of intact grain kernels is needed for allowing access of digestive enzymes to the endosperm. Rolling and grinding is typically used for mechanical processing of kernels. Description of particle size distribution obtained by mechanical processing is needed to evaluate sufficiency of the process. Sieving is often used to analyse particle size distributions. Agglomeration of sample material on test sieves is a known problem and is typically solved by use of various sieving aids that keep sample material moving while sieving (Stark and Chewning, 2012; Retch brochure, 2013).

The objective of this study was to implement a standardized sieving procedure for dry concentrate feeds in the department laboratory.

Materials and methods

Meals of wheat and maize were produced using a hammer mill (P50SP, President, Herning, Denmark) fitted with a 2 mm screen as described by Razzaghi et al. (2016). Particle size distribution analysis of grain meals was determined in two runs using a sieve shaker (AS200 control, Retsch GmbH, Haan, Germany) and 8 circular sieves + pan (200 mm diameter test sieves, Retsch GmbH) with a 4000, 2000, 1000, 500, 250, 125, 63, and 45 µm opening test sieves. A 10 min shaking interval procedure was used with 10 second intervals and 2 mm amplitude. As agitators, 40 light and hollow plastic balls were put on all sieves finer than 1000 µm (Figure 1).

Figure 1 Sieving agitators (40 light and hollow plastic balls) as used on all test sieves finer than 1000 µm

As dispersing agent, 0.5 g of SiO_2 sieving agent (SSA-58, Gilson Company Inc., Lewis Center, Ohio, USA) per 100 g of sample was gently mixed into the sample. The SiO_2 was assumed to distribute similarly to feed particles on test sieves as SiO_2 attach to all particles.

The geometric mean diameter (Dgw) and standard deviation (Sgw) of particle size distribution were calculated according to ASABE (2013). Statistical analysis was performed by meal using the Mixed procedure of SAS with a model containing aid (none, agitator, or agent) as fixed effect. Least significant means are presented. Means were separated using the Tukey-Kramer least significance test ($P \leq 0.10$). Significance was declared when $P \leq 0.05$.

Results and discussion

For wheat meal, no visual agglomeration on test sieves appeared and no differences in amount of meal on the 250 µm and larger test sieves were observed (Table 1 and Figure 2). In spite of no visual agglomeration of meal, both agitators and the dispersing agent made more sample material move through test sieves 125 and 63 µm. Hence, more sample material was observed on the 45-µm test sieve as well as in the pan as compared to no particles on the 45-µm sieve and pan when no aids were used. For wheat, there appeared to be no difference between using agitators or dispersing agent in particle size distribution.
Appearance of particles on the finest test sieves with either sieving aid was not reflected in differences in calculated geometric mean diameter (Table 1). Yet, standard deviation for particle size distribution increased when sample material was spread over all test sieves from either sieving aid.

Table 1 Particle size distribution (g/kg), and geometric mean diameter (Dgw) and standard deviation (Sgw) of wheat meal as affected by use of no aid, agitators (light plastic balls) or dispersing agent (amorphous SiO_2)

	Aids				
Item	None	Agitator	Agent	SEM	*P*-value
>1000 µm	72	60	71	4.2	0.24
500 to 1000 µm	397	410	407	9.9	0.67
250 to 500 µm	255	263	247	5.7	0.27
125 to 250 µm	161[a]	130[b]	123[b]	3.9	0.01
63 to 125 µm	113[a]	71[b]	86[b]	3.5	0.01
<63 µm	0.9[a]	65[b]	65[b]	5.6	0.01
Dgw[1], µm	394	371	373	11	0.37
Sgw[1], µm	2.21[a]	2.41[b]	2.45[b]	0.023	0.01

[1]Calculated according to ASABE (2013); [a,b,c] signify that means differ within row ($P \leq 0.10$).

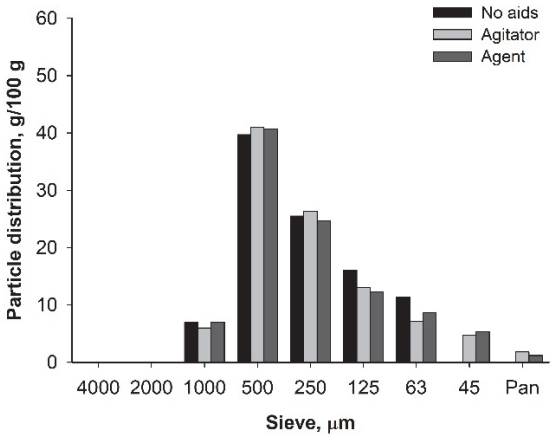

Figure 2 Particle size distribution of wheat meal (hammer milled to pass a 2-mm screen) as affected by use of agitators (light plastic balls) and dispersing agent (amorphous SiO_2). Each bar represents the mean of duplicate determinations.

For maize meal, visual agglomeration of sample material on test sieves clearly appeared on 500, 250, and 125 µm test sieves (Figure 3), when no sieving aids were used. Use of agitators prevented visual agglomeration on test sieves 500 and 250 µm, but agglomeration on the 125 µm test sieve was still visually evident. SiO_2 dispersing agent prevented visual agglomeration of maize meal on any test sieve.

Figure 3 Agglomeration of maize meal on 500, 250, and 125 µm test sieves when no aids were used.

The visual observations of sample material agglomeration were clearly reflected in the particle size distributions when sieving aids were used (Table 2 and Figure 4). The amount of sample material on 500 and 250 µm test sieves was reduced and most sample material was observed on the 63 and 45 µm test sieves as well as in the pan with an agent present. Indeed, a greater amount of dispersing agent might make even more particles move through to the finest test sieves, but that was not tested in the current work.

Table 2 Particle size distribution (g/kg), and geometric mean diameter (Dgw) and standard deviation (Sgw) of maize meal as affected by use of no aid, agitators (light plastic balls) or dispersing agent (amorphous SiO_2)

	Aids				
Item	None	Agitator	Agent	SEM	*P*-value
>1000 µm	26[a]	10[b]	18[c]	0.2	<0.01
500 to 1000 µm	526[a]	256[b]	244[b]	5.5	<0.01
250 to 500 µm	395[a]	278[b]	255[b]	6.0	<0.01
125 to 250 µm	38[a]	308[b]	166[c]	3.3	<0.01
63 to 125 µm	12[a]	134[b]	179[c]	8.7	<0.01
<63 µm	0.2[a]	14[b]	137[c]	1.4	<0.01
Dgw[1], µm	508[a]	280[b]	229[c]	4.1	<0.01
Sgw[1], µm	1.61[a]	2.10[b]	2.59[c]	0.015	<0.01

[1] Calculated according to ASABE (2013); [a,b,c] signify that means to differ within row ($P \leq 0.10$).

The described effects of agitators and dispersing agent were reflected in decreased calculated geometric mean diameters from 508 to 280 µm with agitators and a further decrease to 229 µm with dispersing agent, equivalent to a 81 and 122% overestimation without sieving aids, respectively (Table 2). Likewise, the standard deviation in particle size distribution increased the more the sample material was spread over all test sieves.

These results for wheat and maize are in good agreement with the observations by Stark and Chewning (2012). For sieving easy flowing meals like that of wheat, use of agitators was sufficient to make sample material distribute to all test sieves; however, as a uniform procedure is preferred for routine laboratory analysis, general use of the dispersing agent is recommended.

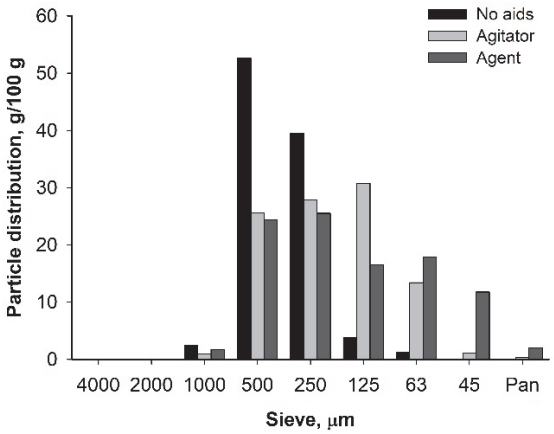

Figure 4 Particle size distribution of maize meal (hammer milled to pass a 2-mm screen) as affected by use of agitators (light plastic balls) and dispersing agent (amorphous SiO_2). Each bar is the mean of duplicate determinations.

Conclusions

It is recommended to routinely use the SiO_2 dispersing agent as sample particles move through to the finest test sieve and pan both in meals without and with visual problems of agglomeration.

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References

ASABE, 2013. Method of determining and expressing fineness of feed materials by sieving. ANSI/ASAE S319.4 FEB2008 (R2012). Approved February 2008; reaffirmed February 2013, 8 pp.

Razzaghi, A., Larsen, M., Lund, P. & Weisbjerg, M.R., 2016. Effect of conventional and extrusion pelleting on in situ ruminal degradability of starch, protein, and fibre in cattle. Livest. Sci. 185, 97-105.

Retch brochure, 2013. Sieving for perfect quality control. Retch GmbH, Haan, Germany, 20 pp.

Stark, C.R. & Chewning, C.G., 2012. The effect of sieve agitators and dispersing agent on the method of determining and expressing fineness of feed materials by sieving. Anim. Prod. Sci. 52, 69-72.

Feeding rye grain to growing dairy bulls

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Introduction

In Northern Europe, diets for growing cattle are mainly based on grass silage and barley (*Hordeum vulgare*), oats (*Avena sativa*) or wheat (*Triticum aestivum*) supplementation. In some cases also rye (*Secale cereale*) could be a cost-effective concentrate supplement. Rye is highly valued particularly in Eastern and Northern Europe in producing traditional dark bread. Especially in Poland, rye is used also for animal feeding, mainly for pigs and cattle (Pieszka et al., 2015). Nevertheless, there is scepticism concerning high rye levels in the diet mainly due to high concentrations of alkylresorcinols compared with other grains (e.g. Ziegler et al., 2015). Surprisingly, there are only a few old reports in the scientific literature where effects on rye inclusion on intake, gain and carcass characteristics of growing and finishing cattle has been examined. Spiece (1986) reviewed some old beef cattle research reports from the 1970's in his dissertation concerning feeding rye to lactating dairy cattle and concluded that results from rye studies are conflicting. Antoniou (1980) stated that possible feed intake and growth depression effects due to rye inclusion are less evident in older than in young animals. In dairy cows, Pieszka et al. (2015) observed that feeding with concentrates containing 250 or 400 g rye/kg dry matter (DM) did not reduce feed intake or milk yield during the first 100 days of lactation compared to a control group fed no rye. Because there are very few studies using rye in growing cattle, the objective of the present experiment was to study the effects of partial replacement of barley grain by rye grain on feed intake, gain and carcass characteristics of growing and finishing bulls.

Materials and methods

A feeding experiment was carried out in the experimental cattle unit of the Natural Resources Institute Finland (Luke) in Ruukki. The experiment was conducted using 80 dairy bulls (48 Holstein and 32 Nordic Red) with an initial live weight (LW) of 320 (±34.9) kg. At the start of the experiment, the bulls were on average 250 (±10.4) days old. During the experiment, the bulls were housed in an uninsulated barn in pens (10.0 × 5.0 m; 5 bulls in each pen), providing 10.0 m^2/bull. A GrowSafe feed intake system (model 4000E; GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes so that each pen contained two GrowSafe feeder nodes.

At the beginning of the feeding experiment, both Holstein and Nordic Red bulls were randomly allotted to pens (three Holstein and two Nordic Red bulls per pen) which were then randomly allotted to four feeding treatments (four pens and 20 bulls per treatment). The bulls were fed a total mixed rations *ad libitum* (proportionate refusals of 5%). Rations were mixed by use of a mixer wagon (Trioliet, BW Oldenzaal, the Netherlands) once a day.

The experimental diets included grass silage (500 g/kg DM) and concentrates (500 g/kg DM). Four different concentrate mixtures (C1, C2, C3 and C4) included rye grain 0, 150, 300 and 450 g/kg DM, respectively. Ingredients, chemical compositions and nutritional values of the concentrate mixtures are in Table 1. The C1 was a typical Finnish commercial concentrate

mixture for growing cattle including mainly barley, wheat bran and oats. In the three other concentrate mixtures barley was partly replaced by rye. All four concentrate mixtures were isoenergetic and isonitrogenous. The metabolisable energy (ME) content of concentrates was calculated based on concentrations of digestible crude fibre, crude protein (CP), crude fat and nitrogen-free extract described by Luke (2018).

Table 1 Ingredients, chemical composition and feeding values of the four concentrate feeds (C1, C2, C3 and C4) and grass silages (GS1 = primary growth, GS2 = regrowth) used in the feeding experiment

	C1	C2	C3	C4	GS1	GS2
Ingredients, g/kg dry matter (DM)						
Barley	491	356	210	54	-	-
Rye	-	150	300	450	-	-
Wheat bran	144	144	144	144	-	-
Oats	130	119	120	130	-	-
Rapeseed meal	70	69	67	65	-	-
Molassed sugar-beet pulp	66	66	66	66	-	-
Wheat feed meal	65	65	65	65	-	-
Calcium carbonate	16	16	16	16	-	-
Vegetable oil mix	8	5	2	-	-	-
Sodium chloride	7	7	7	7	-	-
Magnesium oxide	2	2	2	2	-	-
Vitamin, mineral and trace element premix	1	1	1	1	-	-
Chemical composition and feeding values						
Number of feed samples	4	4	4	4	4	4
DM, g/kg	879	875	878	878	228	260
Organic matter, g/kg DM	939	939	940	944	948	948
Crude protein, g/kg DM	142	148	142	140	142	134
Ether extracts, g/kg dry DM	37	36	31	30	40	42
Neutral detergent fibre, g/kg DM	253	260	258	275	569	550
Starch, g/kg DM	369	373	391	404	9	9
Metabolisable energy, MJ/kg DM	12.4	12.4	12.4	12.4	11.0	11.1
Metabolisable protein, g/kg DM	96	96	96	96	82	82
Protein balance in the rumen, g/kg DM	5	5	5	5	20	12
Digestible organic matter in DM, g/kg DM	-	-	-	-	687	694
Silage DM intake index	-	-	-	-	103	102

Fermentation quality of grass silage (primary growth and second growth on average): pH 3.78; volatile fatty acids 14 g/kg DM; lactic + formic acid 52 g/kg DM; water soluble carbohydrates 64 g/kg DM; ammonium-N 44 g/kg N; soluble N 464 g/kg N.

Grass silage was produced at the experimental farm of Luke in Ruukki (64°44'N, 25°15'E) from a timothy (*Phleum pratense*) sward (on 20 June and 26 July 2016, primary growth and regrowth, respectively). The primary growth was fed during the first half (116 days) and regrowth during the second half (127 days) of the feeding experiment. The swards were cut by a mower conditioner (Elho 280 Hydro Balance), harvested using a precision-chop forage harvester (Lely Storm 130P) approximately 24 hours after cutting, treated with a formic acid-based additive (AIV ÄSSÄ, Eastman Chemical Company) applied at a rate of 5.8 kg/t of fresh forage and stored in bunker silos.

During the feeding experiment, silage sub-samples were taken twice a week, pooled over periods of approximately four weeks and stored at –20ºC prior to analyses. Thawed samples were analysed for DM, ash, CP, neutral detergent fibre (NDFom) exclusive of residual ash, crude fat (analysed as ether extract), starch, silage fermentation quality [pH, water-soluble carbohydrates (WSC), lactic and formic acids, volatile fatty acids, soluble and ammonia N content of total N], and digestible organic matter (DOM) in DM (D-value) as described by Pesonen et al. (2013). Concentrate sub-samples were collected weekly, pooled over periods of eight weeks and analysed for DM, ash, CP, NDFom, crude fat and starch. The ME concentration of the silages was calculated as 0.016 × D-value. The ME concentration of concentrates was calculated based on concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by Luke (2018). Amino acids absorbed from the small intestine (AAT) and protein balance in the rumen (PBV) were calculated according to Luke (2018). The relative intake potential of silage DM (SDMI index) was calculated as described by Huhtanen et al. (2007).

The bulls were weighed on two consecutive days at the beginning of the experiment and thereafter approximately once every 28 days. Before slaughter, the bulls were weighed on two consecutive days. The target for the average carcass weight was 350–360 kg. The bulls were selected for slaughter based on the LW recorded each 28 d and slaughtered in the Atria Ltd. commercial slaughterhouse in Kauhajoki, Finland in two batches which differed by 45 days. All four feeding treatments were represented in both batches. After slaughter, carcasses were weighed hot. Cold carcass weight was estimated as 0.98 of hot carcass weight. Carcasses were classified for conformation and fat using the EUROP classification (EC, 2006).

Results are shown as least squares means. Data were subjected to analysis of variance using the SAS GLM procedure. The statistical model used was:

$y_{ijkl} = \mu + \delta_j + \alpha_i + \theta_{ijl} + \beta_{xijk} + e_{ijkl}$

where, μ is the intercept and e_{ijkl} is the residual error term associated with k^{th} animal. α_i, is the effect of i^{th} diet (C1, C2, C3, C4), while δ_j is the effect of the slaughtering batch (j=1, 2) and θ_{ijl} is the effect of pen.

Effect of pen was used as an error term because treatments were allocated to animals penned together. Initial LW was used as a covariate (β_{xijk}) in the model for intake, gain and feed conversion parameters. When dressing proportion, carcass conformation and carcass fat score were tested, carcass weight was used as a covariate. Effect of the rye inclusion was further divided into linear and quadratic effects using orthogonal polynomial contrasts.

Results and discussion

According to feed analyses, the grass silage used was good both nutritionally and in terms of preservation (Table 1). It was restrictively fermented with high residual WSC concentration and relatively low lactic acid concentration.

The feeding experiment lasted on average 234 days (Table 2). Two bulls (one C1 and one C3 bull) were excluded from the study due to pneumonia and two bulls (both C3) due to accidents. There was no reason to suppose that the diets had caused these problems. The other 76 bulls remained healthy throughout the study.

The average daily DM, ME and CP intakes of the bulls were 10.9 kg/d, 127 MJ/d and 1522 g/d, respectively, during the total experimental period (Table 2). There were no differences in

DM, ME or nutrient intakes between the feeding treatments. However, CP intake tended to decrease linearly (P=0.06) with increasing rye proportion in the diet. Consistent with the present experiment, Schneider et al. (1990) observed that replacement of barley by rye in the concentrate for bulls did not reduce feed intake. In dairy cows, Pieszka et al. (2015) found no reduction in feed intake when feeding with concentrates containing rye at 250 or 400 g/kg DM compared to a control group fed without rye. On the contrary, Sharma et al. (1981) reported that DM intake was reduced when lactating dairy cows were fed 250, 500 or 750 g/kg DM rye in their grain mixture compared to barley.

Table 2 Intake, growth performance and carcass characteristics of the bulls fed different rye proportion in the diet. C1, C2, C3 and C4 diets included rye 0, 75, 150 and 225 g/kg dry matter (DM), respectively

	Diets					*P*-values	
	C1	C2	C3	C4	SEM	L	Q
Number of bulls	19	20	17	20			
Duration of the experiment, d	239	233	231	233	3.6	0.22	0.20
Intake							
DM, kg/d	11.1	10.9	10.9	10.5	0.29	0.15	0.59
DM, g/kg metabolic live weight	104	103	105	99	2.8	0.31	0.28
Metabolisable energy (ME), MJ/d	130	128	128	123	3.4	0.15	0.60
Crude protein , g/d	1552	1559	1520	1457	40.0	0.06	0.36
Metabolisable protein, g/d	985	973	970	933	25.5	0.15	0.60
Neutral detergent fibre, g/d	4470	4454	4474	4351	118.9	0.51	0.63
Starch, g/d	2092	2102	2204	2164	56.2	0.19	0.64
Live weight gain (LWG), g/d	1544	1549	1511	1545	35.6	0.77	0.65
Carcass gain, g/d	835	835	825	828	23.0	0.82	0.95
Feed conversion rate							
kg DM/kg LWG	7.2	7.0	7.2	6.8	0.25	0.54	0.40
MJ/kg LWG	84	83	85	80	3.0	0.58	0.41
kg DM/kg carcass gain	13.3	13.1	13.2	12.7	0.53	0.69	0.66
MJ/kg carcass gain	156	153	155	148	6.2	0.69	0.66
Live weight, kg							
Initial	321	321	315	322	8.7	0.98	0.69
Final	690	682	664	682	7.8	0.19	0.09
Slaughter age, d	490	481	482	483	3.6	0.20	0.14
Carcass characteristics							
Carcass weight, kg	360	355	348	354	5.1	0.27	0.28
Dressing proportion, g/kg	521	520	524	519	3.7	0.89	0.51
Conformation, EUROP	5.2	5.0	5.1	5.0	0.10	0.27	0.85
Fat score, EUROP	2.3	2.2	2.3	2.1	0.12	0.34	0.77

S.E.M., standard error of the mean; L, linear effect of rye supplementation; Q, quadratic effect of rye supplementation; Conformation: (1=poorest, 15=excellent); Fat score: (1=leanest, 5=fattest).

The average LW gain (LWG) and carcass gain of the bulls were 1543 and 832 g/d, respectively, and replacing barley by rye had no effects on growth performance (Table 2). Furthermore, there were no differences in feed or ME conversion rates among treatments.

Because all concentrate mixtures were isoenergetic and there were no differences in feed intake among treatments, there was also no difference in energy intakes. Therefore, it is logical that there were no differences either in gain parameters among treatments. Based on a meta-analysis of growing cattle feeding experiments, Huuskonen & Huhtanen (2015) concluded that energy intake was clearly the most important variable affecting LWG of growing cattle, whereas the results showed only marginal effects of protein supply on gain.

Earlier scientific information on the effects of rye on growth performance of growing and finishing cattle is very limited. Spiece (1986) reviewed this issue and concluded that feeding recommendations indicate that rye should not exceed 50% of grain in the ration for cattle. However, Spiece (1986) also concluded that some studies indicate that rye may be included by up to 80% in the grain ration for growing steers without reduction in intake or growth. Sharma et al. (1981) used sixty Holstein calves to evaluate the nutritive value of rye grain in calf starter diets. Calves were assigned randomly to one of five starters containing 0, 300, 600 and 800 g/kg DM dry rolled or 800 g/kg DM roasted rye in an 18-week growth trial. Average daily gain and feed intake were similar during the first six weeks, but calves receiving 600 g/kg DM dry rolled rye consumed less feed and grew slower than the barley control and those receiving 800 g/kg DM roasted rye during the following 12 weeks (Sharma et al., 1981). Nevertheless, the calves fed 800 g/kg DM dry rolled rye did not differ from the barley control. Ratios of feed to gain were not different among the five treatments (Sharma et al., 1981).

Average slaughter age, carcass weight and dressing proportion were 484 d, 354 kg and 521 g/kg, respectively, and no treatment differences were observed (Table 2). Recent datasets from Finnish slaughterhouses indicate that average slaughter age, carcass weight, conformation score and fat score for dairy bulls are 590 d, 330 kg, 4.5 and 2.4, respectively (Huuskonen, 2014). Hence, the bulls in the present experiment were younger and heavier and conformed better than the average dairy bull of the Finnish beef cattle population. Previous scientific results related to effects of rye inclusion on carcass characteristics on finishing cattle are very limited. However, also Schneider et al. (1990) reported no differences in dressing proportion, carcass conformation or carcass fat score of finishing bull when they received rye, barley, wheat or maize grain.

Conclusions

Results indicate that the addition of 150–450 g/kg DM of rye grain to concentrate mixtures for growing and finishing dairy bulls does not influence DM intake or gain compared to a barley based concentrate. No differences in carcass characteristics of the bulls were observed among the feeding treatments. Therefore, it can be concluded that rye grain is a suitable energy supplement to a good quality silage for growing and finishing dairy bulls. The rational for use of rye will depend on its price in relation to alternative concentrate feeds.

References

Antoniou, T., 1980. Identification, isolation, modification and partial characterization of an antinutritional factor in rye grain. Ph.D. Thesis. University of Manitoba, Winnipeg, Canada.

EC, 2006. Council Regulation (EC) No 1183/2006 of 24 July 2006 concerning the Community scale for the classification of carcasses of adult bovine animals. Official J. EU L 214, 1-6.

Huhtanen, P., Rinne, M. & Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows; a revision of the relative silage dry matter intake index. Animal 1, 758-770.

Huuskonen, A., 2014. A comparison of Nordic Red, Holstein-Friesian and Finnish native cattle bulls for beef production and carcass traits. Agric. Food Sci. 23, 159-164.

Huuskonen, A. & Huhtanen, P., 2015. The development of a model to predict BW gain of growing cattle fed grass silage-based diets. Animal 9, 1329-1340.

Luke, 2018. Feed Tables and Nutrient Requirements. [cited 10.4.2018]. Available at: http://www.luke.fi/feedtables

Pesonen, M., Honkavaara, M., Kämäräinen, H., Tolonen, T., Jaakkola, M., Virtanen, V. & Huuskonen, A., 2013. Effects of concentrate level and rapeseed meal supplementation on performance, carcass characteristics, meat quality and valuable cuts of Hereford and Charolais bulls offered grass silage-barley-based rations. Agric. Food Sci. 22, 151-167.

Pieszka, M., Kamyczek, M., Rudzki, B., Łopuzańska-Rusek, M. & Pieszka, M., 2015. Evaluation of the usefulness of hybrid rye in feeding Polish Holstein-Friesian dairy cows in early lactation. Ann. Anim. Sci. 15, 929-943.

Schneider, M., Baldeweg, P. & Flachowsky, G., 1990. Untersuchungen zum Einsatz von Mais, Roggen, Gerste bzw. Weizen als Konzentrat in der Mastrinderfütterung. Tierzucht 44, 178-179.

Sharma, H.R., Ingalls, J.R., McKirdy, J.A. & Sanford, L.M., 1981. Evaluation of rye grain in the diets of young Holstein calves and lactating dairy cows. J. Dairy Sci, 69, 441-448.

Spiece, K.L., 1986. The effect of feeding processed rye grain to lactating dairy cattle and the effect of rye bran and flour fractions on ruminal flow and gastrointestinal function. PhD Thesis. University of Manitoba, Winnipeg, Canada.

Ziegler, J.U., Steingass, C.B., Longin, C.F.H., Würschum, T., Carle, R. & Schweiggert, R.M., 2015. Alkylresorcinol composition allows the differentiation of Triticum spp. having different degrees of ploidy. J. Cereal Sci. 65, 244.251.

Effect of replacing barley grain with oat grain in the diet of dairy cows on methane production *in vitro*

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Introduction

Methane (CH_4) is a greenhouse gas that has a considerable impact on global warming and consequently contributes to climate change (IPCC, 1990). Enteric fermentation is the most important anthropogenic source of CH_4 emissions accounting for 22.7% of total anthropogenic CH_4 emissions and 15.9% of total CH_4 emissions (Baumgard *et al*., 2015). Methane emissions from enteric fermentation also represent an energy loss for the animal (Johnson and Johnson, 1995). Dietary strategies to decrease methane emissions have been widely investigated. Increasing diet digestibility increases enteric CH_4 emissions (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013). Addition of fat in the diet of ruminants has a suppressing effect on enteric CH_4 emissions (Czerkawski *et al*., 1966; Grainger and Beauchemin, 2011; Ramin and Huhtanen, 2013). Oats and barley differ in composition of storage compounds in the grains. The crude fat and neutral detergent fibre content is higher in oats compared to barley, whereas barley has a higher content of starch (LUKE, 2017). Preliminary results from *in vitro* gas production experiments have shown that using oats instead of barley in silage-based diets decreases CH_4 emissions by approximately 20% (Ramin and Huhtanen, unpublished data).
The aim of this study was therefore to investigate the potential of reducing enteric CH_4 emissions from dairy cows by replacing barley grain with oat grain on a grass silage based diet in an *in vitro* gas production experiment.

Materials and methods

The experimental feed material consisted of eight varieties of oats (*Avena sativa*) and eight varieties of barley (*Hordeum vulgare*). All varieties of oats and barley were with hull. The grains were ground through a 1-mm screen using a laboratory mill (Retsch, SM2000, Rheinische, Haan, Germany) and used in a mixture with silage, which was from an early-cut harvested from primary growth of a third-year lay dominated by timothy (*Phleum pratense*) with some red clover (*Trifolium pretense*). The animals donating rumen fluid for the *in vitro* gas production experiment were two dairy cows of the Nordic Red breed in late lactation, fed a total mixed ration (grass silage/concentrate ratio 600/400 g/kg on a DM basis) *ad libitum* and fitted with rumen cannulae. The *in vitro* gas production experiment consisted of three *in vitro* runs conducted during three subsequent days. Each run included 36 samples from 16 diets with two replicates and four blank samples containing only buffered rumen fluid. Feed samples and buffered rumen fluid (60 ml) were mixed in glass bottles (250 ml, DURAN®, Schott AG, Mainz, Germany) and incubated in a water bath. The incubated grain/silage ratio was 1:1 on a dry matter basis, each diet containing 0.5 grams of barley or oats and 0.5 grams of silage. The *in vitro* gas production experiment was conducted using a fully automated gas

production technique described by Cone *et al*. (1996) and Ramin and Huhtanen (2012), where total gas volume was automatically recorded by the system and corrected for prevailing air pressure. To predict CH_4 production *in vivo,* gas sampling was performed at 2, 4, 8, 24, 32 and 48 h of incubation. Each bottle was subject to pH measurements and sampled for volatile fatty acid analysis at 48 hours of incubation. I*n vitro* digestibility was determined as true dry matter digestibility (TDMD). Sampling for digestibility analysis was performed at 48 hours of incubation by transferring feed sample residues from each bottle into a nylon bag with a pore size of 11 µm. True DMD was determined by boiling the bags in a NDF solution for one hour.

Predicted *in vivo* CH_4 production was estimated using a set of models and calculations described by Ramin and Huhtanen (2012). All statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Data for predicted *in vivo* CH_4 production (ml/g DM), TDMD (%), total VFA production (mmol) and molar proportions of VFA (mmol/mol) were subjected to analysis of variance using the MIXED procedure in SAS. The results were considered as statistically significant when $P < 0.05$. To investigate possible relationships between predicted *in vivo* CH_4 production and grain composition, a simple regression analysis was performed using the PROC REG procedure in SAS software.

Results and discussion

Predicted *in vivo* CH_4 production (ml/g DM) decreased by 2.8 ml/g DM (8.5%) when oats was used as a substrate compared with when barley was used as a substrate ($P < 0.001$) (Table 1). To the writer's knowledge, no *in vitro* research has been published comparing predicted *in vivo* CH_4 production between oat based and barley based diets. It is difficult, if not impossible, to create an *in vitro* environment exactly similar to *in vivo* conditions. However, prediction of CH_4 production using this method was accurate in the study of Ramin and Huhtanen (2012) as the method has been evaluated with *in vivo* studies in which CH_4 was measured using the respiration chamber technique (Danielsson *et al*., 2017).

Total VFA production (mmol) was higher in the barley compared to the oat based diets ($P < 0.01$), which is consistent with *in vivo* results of Vanhatalo *et al*. (2006). Grain species had no effect on VFA molar proportions ($P > 0.05$), except for valerate and caproate, which were slightly lower or higher, respectively, when oats was used as a substrate ($P < 0.05$) (Table 1). Contrary to these findings, Vanhatalo *et al*. (2006) found a slightly higher proportion of butyrate in barley diets compared to oat diets when grain inclusion was 40% on a DM basis. The pH at 48 hours of incubation was lower in the barley compared to the oat based diets (Table 1). Higher pH in the oat-based diets was probably caused by the decrease in total VFA production observed in the oat based diets.

True DM digestibility was higher ($P < 0.001$) when barley was used as a substrate (85.5%) as compared with when oats was used as a substrate (79.5%; Table 1). Since the DM proportion of grain was only 50% in the feed mixture, the result falls well in line with values in national feed tables, reporting 10-12 %-units higher digestibility for barley compared to oats (LUKE, 2017).

In the regression analysis, true DM digestibility was the best predictor of CH_4 production (Figure 1). Among grain composition parameters, indigestible neutral detergent fiber (iNDF) was the best predictor of CH_4 production. The mean response in CH_4 production to increased iNDF concentration was -0.026 ml/g DM per 1 g iNDF /kg DM. Grain NDF concentration

was only slightly less associated to CH_4 production than iNDF concentration, and regression coefficients of iNDF and NDF did not differ markedly. Increased diet digestibility was positively correlated to CH_4 production, which was also reported by Blaxter and Clapperton (1965) and Benchaar *et al*. (2001) when CH_4 production was expressed as total CH_4 production per day and relative to GE intake. In this study, CH_4 production was expressed relative to DMI, which was also the case in a study by Ramin and Huhtanen (2013), where similar effects of digestibility on CH_4 production were observed when prediction equations were used to estimate CH_4 production. The lower digestibility of oats compared to barley together with the strong correlation between diet digestibility and CH_4 production in this study, suggest that one part of the lower CH_4 production of oats may be due to the lower digestibility of oats compared to barley.

Using crude fat content as a predictor instead of iNDF or NDF increased the prediction error slightly. However, the mean response in CH_4 production to crude fat (-0.113 ml/g DM per 1 g/kg DM crude fat) was greater than that of iNDF or NDF (Figure 2). The negative effect of increased dietary fat concentration on CH_4 production has also been shown in other studies (Czerkawski *et al*., 1966; Beauchemin *et al*., 2009; Grainger and Beauchemin, 2011). Higher fat content in oats compared to barley and the relatively strong correlation observed between fat content and CH_4 production implies that part of the CH_4 mitigating effect of oats is due to its higher content of fat.

Table 1 The effects of grain species on predicted *in vivo* methane production, true dry matter digestibility, pH, total VFA production and VFA molar proportions

Item	Barley	Oats	SEM	n	P-value
CH_4 (ml/g DM)	32.8	30.0	0.42	86	<0.001
TDMD (%)	85.5	79.5	0.19	94	<0.001
pH	6.11	6.26	0.005	96	<0.001
Total VFA production (mmol)	3.68	3.29	0.046	96	<0.001
VFA molar proportion (mmol/mol)					
Acetate	636	637	1.4	96	0.45
Propionate	183	182	0.9	96	0.48
Butyrate	129	129	0.7	96	0.63
Isobutyrate	13.4	13.3	0.18	96	0.60
Isovalerate	11.6	11.3	0.22	96	0.26
Valerate	21.2	20.4	0.19	96	<0.05
Caproate	6.7	7.0	0.07	96	0.001

TDMD = true dry matter digestibility, VFA = volatile fatty acid.

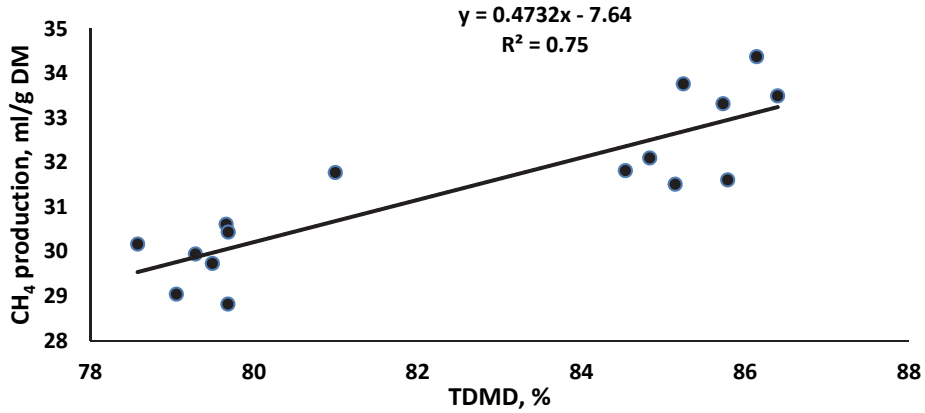

Figure 1 Relationship between predicted *in vivo* methane production and true dry matter digestibility (TDMD) of the different varieties of oats and barley.

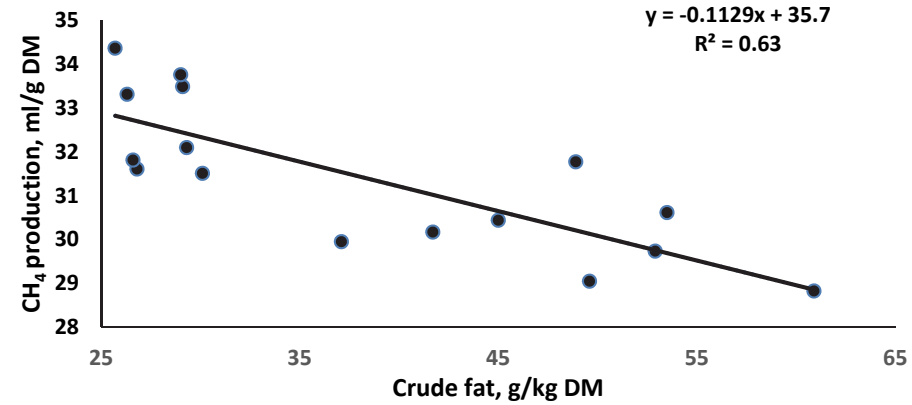

Figure 2 Relationship between predicted *in vivo* methane production and content of crude fat in the different varieties of oats and barley.

Conclusions

Replacing barley grain with oat grain in a grass silage based diet decreased predicted *in vivo* methane production by 8.5%. In addition, digestibility, fermentation and total VFA production *in vitro* decreased, whereas molar proportions of acetate, propionate and butyrate and the minor VFAs isobutyrate and iso-valerate remained unchanged. Feed digestibility and crude fat content correlated well with predicted *in vivo* methane production.

References

Baumgard, L., Baumgard, L., Gaughan, J., Prasad, C. S. & Sejian, V., 2015. Climate change impact on livestock: adaptation and mitigation. New Delhi: Springer.

Beauchemin, K. A., McGinn, S. M., Benchaar, C. & Holtshausen, L., 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92, 2118-2127.

Benchaar, C., Pomar, C. & Chiquette J., 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. Can. J. Anim. Sci.81, 563-574.

Blaxter, K. L. & Clapperton, J. L., 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19, 511-522.
Blaxter, K. L. & Clapperton, J. L., 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19, 511-522.
Cone, J., vanGelder, A., Visscher, G. & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113-128.
Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W., 1966. The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20, 349-362.
Danielsson, R., Ramin, M. Bertilsson, J, Lund. & Huhtanen, P., 2017. Evaluation of an in vitro system for predicting methane production in vivo. J. Dairy Sci. 100, 8881-8894.
Grainger, C. & Beauchemin, K. A., 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166–167, 308-320.
IPCC 1990. ClimateChange: The IPCC Scientific Assessment. IPCC, Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, Great Britain, New York, NY, USA and Melbourne, Australia.
Johnson, K. A. & Johnson, D. E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483-2492.
LUKE 2017. Rehutaulukot ja ruokintasuositukset. https://portal.mtt.fi/portal/page/portal/Rehutaulukot/Rehutaulukot/marehtijat. Jokionen: LUKE Luonnonvarakeskus. Cited 28.6.2017.
Ramin, M. & Huhtanen, P., 2012. Development of an *in vitro* method for determination of methane production kinetics using a fully automated *in vitro* gas system—A modelling approach. Anim. Feed Sci. Technol. 174, 190-200.
Ramin, M. & Huhtanen, P., 2013. Development of equations for predicting methane emissions from ruminants. J. Dairy Sci. 96, 2476-2493.
SAS, 2014. SAS 9.4. SAS institute Inc. Cary. NC. US.
Vanhatalo, A., Gäddnäs, T. & Heikkilä, T., 2006. Microbial protein synthesis, digestion and lactation responses of cows to grass or grass-red clover silage diet supplemented with barley or oats. Agric. Food Sci. 15, 252-267.

Cereals or sugar beet pulp - effects on feed consumption, milk yield, human edibles and domestic feed origin

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Introduction

The common practice of feeding human edible cereal grains (CG) to ruminants has been questioned (Eisler *et al*., 2014). The argument is that animal production can become more sustainable if ruminants primarily consume products that are not suitable for human consumption, such as forages and by-products. In diets based on by-products, CG can be replaced with sugar beet pulp (SBP) to provide dairy cattle with easily fermented carbohydrates. Under Swedish conditions, however, this strategy leads to a lower level of national self-sufficiency. Presently, domestic production of sugar beets is not sufficient to replace home grown barley in diets to dairy cows at today's levels. Therefore, imported SBP must be used. Previous studies have shown that grains can be replaced by by-products without affecting milk yield of cows in mid lactation (Ertl *et al*., 2015 & 2016; Karlsson *et al*., 2018). These studies have compared diets with much higher forage proportion than commonly used in high yielding Swedish dairy herds. The aim of the present study was, therefore, to investigate the effects of feeding dairy cows diets based on CG or SBP as total mixed rations (TMR) containing 50% concentrate on feed intake, milk production and use of human edibles as well as feeds of domestic origin.

Materials and methods

A feeding trial was conducted between May and June 2017 at the 'Lantmännen Lantbruk' research farm 'Nötcenter Viken' in Falköping, Sweden and was approved by the Ethical Committee for Animal Research in Gothenburg. Forty-eight dairy cows were used in a 2 x 2 Latin square design during two 21 day periods with registration and sampling during the last 5 days in each period. The cows were in mid lactation (125 ± 20; mean ± SD, days in milk at start of the study), both multiparous (n = 30) and primiparous (n=18), and of the breeds Holstein (n = 20), Swedish Red (n = 15), and crosses (n = 13). They were blocked by breed, parity and days in milk before randomly assigned to two treatment groups. The two treatments were TMR with concentrates based on mainly CG or SBP (Table 1). All cows received TMR *ad libitum* and feed intake was recorded individually (CRFI, BioControl Norway As, Rakkestad, Norway). Both feed mixes contained 50% silage on dry matter (DM) basis consisting of ¼ first cut and ¾ third cut perennial lays of mainly timothy, tall fescue, red clover and white clover. The silage blend contained 32% DM, 11.1 MJ metabolizable energy (ME)/kg DM, 165 g/kg DM of crude protein (CP) and 514 g/kg DM of neutral detergent fibre (NDF). The cows were housed in a loose house barn and milked twice daily. Milk sampling was conducted at four consecutive milkings during the five days of each period. Milk samples were analysed for fat, protein and lactose by Fourier transform infrared spectroscopy (CombiScope FTIR 300 HP, Delta Instruments B. V., Drachten, the Netherlands). Feed DM were determined according to Åkerlind *et al*. (2011) and analyses of ash, CP, NDF and starch were performed as described by Bertilsson and Murphy (2003).

Ether extract content in the concentrates was determined according to the European Commission Directive (EC No. 152/2009).

Table 1 Ingredients in the concentrate part of the total mixed ration (50% silage, 50% concentrate) and ingredients proportion of human-edibles according to Wilkinson (2011) and proportion of ingredients of Swedish origin

Ingredients (% DM)	Cereal grain based	Sugar beet pulp based	Human-edible proportion	Swedish origin proportion
Barley	38.8	0	0.8	1.0
Sugar beet pulp[1]	21.6	56.6	0.2	0.0
Rapeseed meal[2]	15.4	7.0	0.2	0.85
Wheat bran	15.0	12.0	0.2	1.0
Wheat feed flour	0	10.0	0.2	1.0
Molasses	2.0	2.2	0.2	0/1.0[3]
Distiller grain's[4]	2.0	7.0	0.2	1.0
Feed fat[5]	1.0	2.5	0.8	0.13
Limestone	1.4	0.7	0	1.0
Salt	1.2	1.1	0	0.0
Minerals and vitamins	0.2	0.2	0	0.07
Synthetic lysin[6]	0.45	0.50	0	0.0
Synthetic metionin[7]	0.14	0.19	0	0.0

[1]Dried with no inclusion of molasses (Nordic Sugar AB, Eslöv, Sweden). [2]Solvent-extracted and heat-moisture treated rapeseed meal with low levels of glucosinolates and eruic acid (ExPro, AAK Sweden AB, Karlshamn, Sweden). [3]Molasses included in sugar beet pulp mix came from Sweden while that in cereal grain feed mix were imported from elsewhere in Europe. [4]Fiber and yeast cells from ethanol manufacturing based on mainly wheat but also barley and triticale (Agrow Feed 90, Lantmännen Agroetanol, Norrköping, Sweden). [5]Fatty acids (99% fat; 45% C16:0, 37% C18:1, AkoFeed Cattle, AAK Sweden AB, Karlshamn, Sweden). [6]Rumen protected synthetic amino acid (LysiPEARL, Kemin Industries, Inc., Iowa, USA). [7]Rumen protected synthetic amino acid (MetaSmart Dry, Adisseo, Antony, France).

Table 2 Chemical composition (means ± SD) of concentrates used in the diets

	Cereal grain based	Sugar beet pulp based
Dry matter (g/kg)	870 ± 1	877 ± 4
Organic matter (g/kg DM)	930 ± 2	937 ± 3
Crude protein (g/kg DM)	161 ± 2	151 ± 6
Ether extracts (g/kg DM)	46 ± 3	21 ± 1
Neutral detergent fibre (g/kg DM)	251 ± 14	362 ± 10
Starch (g/kg DM)	264 ± 2	52 ± 5
Metabolizable energy (MJ/kg DM)[1]	12.5	12.3
Human-edible feed ingredients (g/kg DM)	437	210
Feed of Swedish origin (g/kg DM)	705	386

[1]Metabolizable energy content in the concentrates was estimated with tabulated values according to the Swedish Board of Agriculture (SJVFS 2011:40, Saknr M 39, 2011) and based on the original concentrate recipes.

Data was analysed with PROC MIXED in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using a change-over model with effect of treatment order, period, and treatment included. Effect of breed and parity as well as interactions were tested and removed if non-significant. Effects were considered as statistically significant when $P < 0.05$.

Results and discussion

Dry matter intake (DMI) was higher for cows fed the CG diet (Table 3). The cows had a higher intake of NDF when fed SBP diet (9.1 kg NDF per day) due to a higher NDF content in that diet. Maximum intake of NDF is around 1.5% of body weight for cattle (Gustavsson, 1989; Nycander, 1989) which would correspond to approximately 9.9 kg NDF for cows in the present study. Thus, it might have been the high NDF intake with SBP diet that limited DMI of this diet. One more reason for lower DMI with SBP diet could have been the high water holding capacity of SBP which could have increased rumen fill (Voelker & Allen, 2003). This in turn stimulates stretch receptors in the rumen wall, signalling satiety and reducing DMI. Intake of CP, ether extracts, starch, and energy were higher for cows fed the CG diet as a result of both a higher DMI and a higher content of those components in the CG mix. When designing concentrates, balancing CP and ME levels is important. After analysing the concentrates for chemical composition it was revealed that the fat content in the SBP concentrate turned out lower (Table 2) than expected (58 g ether extracts/kg DM) causing a lower energy content in the SBP diet. Energy content and energy intake in Table 2 and 3 had not been corrected for the lower content of fat found. Moreover, the differences between concentrates were diluted by the having 50% silage in the TMR.

Table 3 Treatment effects on daily intake, digestibility, feed efficiency and intake of human-edible feed ingredients and feeds of Swedish origin for the two total mixed rations (least square means with standard error of the means (SEM) and P-value)

	Cereal grain based (CG)	Sugar beet pulp based (SBP)	SEM	P-value
Dry matter intake (kg/d)	22.4	20.8	0.38	<0.01
Organic matter intake (kg/d)	20.7	19.3	0.35	<0.01
Crude protein intake (kg/d)	3.65	3.29	0.06	<0.01
Ether extracts intake (kg/d)	0.51	0.22	0.01	<0.01
Neutral detergent fibre intake (kg/d)	8.55	9.09	0.16	<0.01
Starch intake (kg/d)	2.96	0.54	0.04	<0.01
Metabolizable energy intake (MJ/d)[1]	275	254	4.65	<0.01
Organic matter digestibility (%)	71.9	71.0	0.40	0.06
Human-edible feed ingredients intake (kg/d)	4.89	2.18	0.07	<0.01
Feed of Swedish origin intake (kg/d)	19.1	14.5	0.30	<0.01

[1] Metabolizable energy content in the concentrates was estimated with tabulated values according to the Swedish Board of Agriculture (SJVFS 2011:40, Saknr M 39, 2011) and based on the original concentrate recipes. [2]Calculated as dry matter intake (kg)/kg energy corrected milk.

Milk yield and energy corrected milk (ECM) yields were higher for cows fed the CG diet (Table 4). When fed the CG diet, cows also had higher yields of fat, protein and lactose in the milk. The higher production was probably a result of the higher nutrient intake when fed CG diet compared to the SBP diet. Milk protein content was higher when cows were fed CG diet. One possible explanation could be that feeding higher levels of starch increases the utilization of rumen degradable protein and lowers ammonia concentrations in the rumen (Oba & Allen, 2003). Less available energy from soluble carbohydrates can cause a decrease in microbial protein synthesis in the rumen that in turn leads to less amino acids absorbed in the small intestine for cows fed the SBP diet. The CG concentrate contained 15% solvent extracted and heat treated rapeseed meal (ExPro), compared to 7% in the SBP concentrate. Rapeseed meal has a high content of rumen undegradable protein (Evans *et al*., 2016) which probably

contributed to an improved protein supply in cows fed the CG diet as compared to the SBP diet.

The proportion of human-edible feed was lower in the SBP diet than in the CG diet (Table 2). Typical Swedish feed rations contain 21-45% human-edible feeds (Swensson *et al*., 2017). Other studies comparing diets low in human-edible feed ingredients (Ertl *et al*., 2015, Ertl *et al*., 2016, Karlsson *et al*., 2018) conclude that the edible feed conversion ratio (human-edible output in milk per potential human-edible feed input) are much higher in diets low in human-edibles. However, those studies have fed between 60-80% forage, compared to the present study with 50% forage. Diets low in human-edibles are usually high in fibre due to a high inclusion of both forage and by-products, which could result in lower milk production.

Table 4 Treatment effects on daily yield of milk, ECM, milk components and milk composition for the two different total mixed rations (least square means with standard error of the means (SEM) and P-value)

	Cereal grain based (CG)	Sugar beet pulp based (SBP)	SEM	P-value
Milk yield (kg/d)	33.3	32.6	0.43	<0.01
Energy corrected milk yield (kg/d)[1]	33.5	32.8	0.45	0.005
Fat yield (kg/d)	1.38	1.35	0.02	0.14
Protein yield (kg/d)	1.12	1.08	0.02	<0.01
Lactose yield (kg/d)	1.49	1.45	0.02	<0.01
Fat content (%)	4.15	4.16	0.07	0.82
Protein content (%)	3.35	3.32	0.04	0.01
Lactose content (%)	4.44	4.45	0.02	0.49

[1]Energy-corrected milk, calculated according to Sjaunja *et al.* (1990).

The CG concentrate contained 71% ingredients of Swedish origin while the SBP concentrate contained 39% ingredients of Swedish origin. The CG concentrate was based on Swedish grain and SBP concentrate on imported sugar beet pulp. Today, most SBP used in cattle concentrates in Sweden is imported from other European countries since the Swedish production of SBP is only about 74 550 tonnes DM per year (Flygsjö *et al*., 2008; Statistics Sweden, 2017). As a comparison, the total production of barley in Sweden is around 1.3 million tonnes DM per year (Statistics Sweden, 2017). Relying on import make countries more vulnerable to the impact of events outside their borders, such as country-to-country conflicts or transport problems (Eckersten *et al*., 2015). By providing a higher level of self-sufficiency in terms of feed, Sweden would also secure self-sufficiency of food to a greater extent. Dairy cows can produce milk on only forage and grain, but inclusion of protein concentrate increases production (Spörndly, 2017). Domestic grain is a valuable feed source for dairy cattle but to reach higher production, the concentrate needs to be complemented with some protein rich ingredients where there is a range of products of Swedish origin to choose from (Table 1). Neither level of domestic self-sufficiency nor human-edible feed estimates take land-use efficiency into account, which is also an important factor when assessing sustainability aspects of food production (Wilkinson, 2011; Ertl *et al*., 2015 & 2016; Van Zanten *et al*., 2016).

Conclusions

In this trial, cows fed a TMR with concentrate based on SBP resulted in a lower intake of potential human-edible products while the CG based diet had a higher content of ingredients

of Swedish origin. The diet with concentrate based on CG had higher DMI and higher yield of ECM compared to cows fed concentrate based on SBP.

References

Bertilsson, J. & Murphy, M., 2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass Forage Sci. 58, 309-322.

EC No 152/2009. Commission regulation laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union L54/1:1-130.

Eckersten, H., Djurle, A., Albihn, A., Andersson, L., Båge, R., de Toro, A., Gärdenäs, A., Hultgren, J., Kvarnheden, A., Lewan, E., Nkurunziza, L., Rosén, K., Spörndly, R., Vågsholm, I., von Rosen, D., Yuen, J. & Magnusson, U., 2015. Framtida risker och hot mot svensk spannmåls- respektive mjölkproduktion – en analys av forskningsbehov för att bedöma risker. The Swedish University of Agricultural Science, Framtidens lantbruk - djur växter och markanvändning. Uppsala, Sweden. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-e-3154 (accessed 22 November 2017).

Eisler, M. C., Lee, M. R. F., Tarlton, J. F., Martin, G. B., Beddington, J., Dungait, J. A. J., Greathead, H., Liu, J., Mathew, S., Miller, H., Misselbrook, T., Murray, P., Vinod, V. K., Van Saun, R., & Winter, M., 2014. Steps to sustainable livestock. Nature 507, 32-34.

Ertl, P., Zebeli, Q., Zollitsch, W. & Knaus, W., 2015. Feeding of by-products completely replaced cereals and pulses in dairy cows and enhanced edible feed conversion ratio. J. Dairy Sci. 98, 1225-1233.

Ertl, P., Zebeli, Q., Zollitsch, W. & Knaus, W., 2016. Feeding of wheat bran and sugar beet pulp as sole supplements in high-forage diets emphasizes the potential of dairy cattle for human food supply. J. Dairy Sci. 99, 1228-1236.

Evans, E., Callum, C. & Dyck, B., 2016. Review: New findings regarding the feeding value of canola meal for dairy cows. J. Adv. Dairy Res. 4, 151.

Flygsjö, A., Cederberg, C. & Strid, I., 2008. LCA-databas för konventionella fodermedel – miljöpåverkan i samband med produktion. Version 1.1. SIK, SIK-rapport 772, Gothenburg, Sweden.

Gustavsson, A. H., 1989. Från forskning till test i rådgivningen. Biologisk foderstyrning. Cellväggar vägvisare till optimal grovfodergiva? SHSs/SLUs Utfodringskonferens, Kolmården, Sweden.

Karlsson, J., Spörndly, R., Lindberg, M. & Holtenius, K., 2018. Replacing human-edible feed ingredients with by-products increases net food production efficiency in dairy cows. J. Dairy Sci. (in press).

Nycander, L., 1989. Skattning av foderintag hos mjölkkor med hjälp av Neutral Detergent Fiber (NDF) och osmält neutral detergent fiber (ADF). The Swedish University of Agricultural Science, Examensarbete 11, Uppsala, Sweden.

Oba, M. & Allen, M. S., 2003. Effects of Diet Fermentability on Efficiency of Microbial Nitrogen Production in Lactating Dairy Cows. J. Dairy Sci. 86, 195-207.

Sjaunja, L.O, Baevre, L., Junkkarinen, L., Pedersen, J. & Setälä, J., 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Performance Recording of Animals: 27th Biennial Session of the International Committee of Animal Recording, Paris, France.

Statistics Sweden, 2017. Agricultural statistics 2017. Statistics Sweden, Agricultural Statistics Unit, Örebro, Sweden. https://www2.jordbruksverket.se/download/18.695b9c5715ce6e19dbbeb7c3/1498809539729/JS_2017.pdf (accessed 11 April 2018).

Spörndly, 2017. Bara vall och spannmål – lönar det sig? Vallkonferens 2017, Uppsala, Sweden.

Swensson, C., Lindmark-Månsson, H., Smedman, A., Henriksson, M. & Modin Edman, A. K., 2017. Protein efficiency in intensive dairy production: a Swedish example. J. Sci. Food Agric. 97, 4890-4897.

Van Zanten, H. H. E, Mollenhorst, H., Klootwijk, C. W., van Middelaar, C. E. & de Boer, I. J. M., 2016. Global food supply: land use efficiency of livestock systems. Int. J. Life Cycle Assess. 21, 747-758.

Voelker, J. A. & Allen, M. S., 2003. Pelleted beet pulp substituted for high-moisture corn: 1. Effects on feed intake chewing behavior, and milk production of lactating dairy cows. J. Dairy Sci. 86, 3542-3552.

Wilkinson, J. M., 2011. Re-defining efficiency of feed use by livestock. Animal 5, 1014-1022.

Åkerlind, M., Weisbjerg, M., Eriksson, T., Udén, P., Ólafsson, B. L., Harstad, O. & Volden, H., 2011. Feed analyses and digestion methods. In: Volden, H. (e.d) NorFor – the Nordic feed evaluationsystem. EAAP Publ. No. 130. Wageningen Academ. Publ. Wageningen, The Netherlands. 9

Effect of tetracycline and penicillin on ruminal methane production and total gas production from dairy cow feces

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Introduction

In a recent study by Hammer et al. (2016) it was observed that more methane is produced from feces from cows treated with broad spectrum antibiotics (tetracycline), compared to feces from untreated cows. This suggests that antibiotics treatment changes the microflora in the gastrointestinal tract. There are currently no studies on how methane production in the rumen is affected when a cow is treated with antibiotics. The aim of the present study was to investigate effect of antibiotic treatments on feed utilization and methane production in healthy lactating dairy cows.

Material and methods

Twenty-four healthy lactating Swedish Red dairy cows were used. The cows were fed a total mixed ratio (TMR) (grass silage/concentrate ratio 600/400 g/kg on a DM basis) ad libitum. Cows were blocked according to udder health, parity and stage of lactation and allocated to three treatment groups and one control group, with six cows per group. Sampling occurred at a control week with a first sampling occasion (baseline period), the treatment period when antibiotics were given (Period 1) was superseded by a follow-up period after 4 weeks (Period 2) to allow detection of responses to the treatments. The 5-d treatments were: bensylpenicillinprokain, Penovet® vet. (Boehringer Ingelheim Vetmedical) 20 mg/kg BW intra-muscularly once daily; a 5-day intra mammary treatment of 600 mg Bensylpenicillinprokain, Carepen® vet. (Boehringer Ingelheim Vetmedical) into the right front udder quarter and a 4-day treatment of tetracycline, Engemycin® vet. (Intervet) 10 mg/kg BW given intra-muscularly once daily. Mass flux of methane in exhaust gases was measured by a portable open-circuit head chamber system (GreenFeed system, C-Lock Inc., Rapid City, SD) as described by Huhtanen et al. (2015). To evaluate the effect of treatment on digestion, total gas production from fecal samples from both treated and untreated cows was measured in glass syringes (Menke and Steingass, 1988). In vitro total gas production was measured until Day 98 after the last day of antibiotics treatment. The MIXED procedure of SAS (9.3, SAS Inst., Inc., Cary, NC) was used, for analyzing of methane fluxes the cows own control period was used as a covariate in the model. Cow was used as a random effect. Least square means were calculated using the LSMEANS/Slice=time. The data on gas production from feces was analyzed by repeated measurement for the effect of treatment within week. Least square means were calculated using the LSMEANS/PDIFF and statistical differences between treatments were determined following the Tukey adjustment. All differences were declared significant at $P<0.05$.

Results and discussion

There were no statistically detectable effects of treatment on individual methane production (g/day) or production in g/kg DM intake. In vitro fecal gas production was inhibited by all three antibiotic treatments (P<0.001). Over the 98-d measurement period, the control samples emitted a total of 13.9 (s.e ± 0.61) mL gas/g DM, Carepen vet. 11.4 (s.e ± 0.75) mL/g DM, Penovet vet. 10.6 (s.e ± 0.86) mL/g DM and tetracycline 6.62 (s.e ± 0.86) mL/g DM (Figure 1). Opposite to the findings of Hammer et al. (2016), the antibiotics had no detectable effect on gas fluxes in vivo from cows but essentially decreased gas production in vitro. Further analysis of fermentation patterns, digestibility and microbial community analysis might confirm current results.

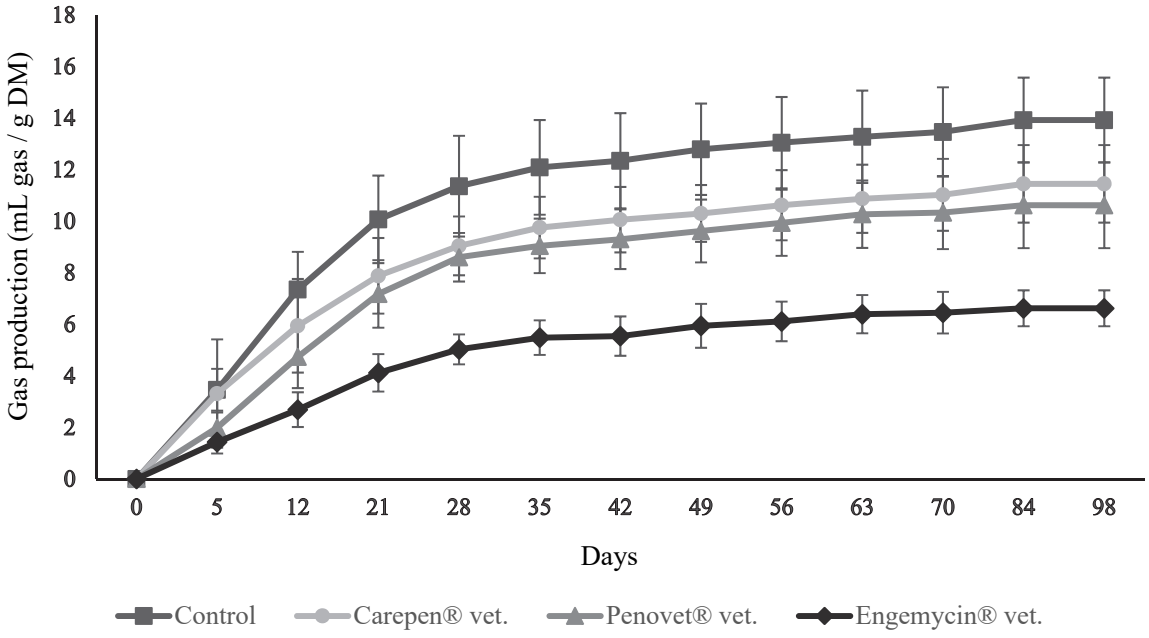

Figure 1 In vitro gas production (mL/g DM) from fecal samples of cows treated with three different antibiotics and from a control group.

References

Hammer, T.J., Fierer, N., Hardwick, B., Simojoki, A., Slade, E., Taponen, J., Viljanen, H. & Roslin, T., 2016. Treating cattle with antibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles. Proc. R. Soc. B 283, 7 pp, DOI: 10.1098/rspb.2016.0150

Huhtanen, P., Cabezas-Garcia, E.H., Utsumi, S. & Zimmerman, S., 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J. Dairy Sci. 98, 3394-3409.

Menke, H.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Develop. 28, 7-55.

The new Gas Endeavour system from Bioprocess Control AB for *in vitro* assessment of animal feeds

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Introduction

In vitro rumen incubation analyses have already been used for years to evaluate the nutritive qualities of feeds, originally employing end-point measurements focusing on feedstuff digestion. The relation between accumulation of fermentation gases and metabolisable energy content of the feed was established in the 1970s. Since then, measurement techniques based on *in vitro* gas production have been further developed for feed evaluation experiments (Cone et al., 1996; Getachew et al., 1998; Getachew et al., 2008; Murray et al., 2014). Much of these early reports rely on a manometric gas measurement principle. Not many publications on liquid displacement systems based on a volumetric gas measuring principle are available due to the limitations of instrumentation setup.

This work describes a new volumetric gas measuring technique specially developed for monitoring production of ultra-low gas volumes, with various applications in batch fermentation tests. The technique has been successfully applied and validated for quantifying biochemical methane potential from various biodegradable organic matters. An automated measuring system based on this volumetric measuring technique can offer continuous monitoring of gas production from *in vitro* digestibility tests with high throughput and significant reduction of labour and time intensity.

Background

There are many protocols available on how to perform *in vitro* digestibility tests. Some of them are adapted for utilisation of the gas measurement technique, but they differ in experimental set up and are generally modified and adapted to the specific researcher´s purpose. Because of this, it is often difficult to evaluate results from different studies and values can vary substantially. Thus, there is a need for a test standard and general procedure, but also for a measurement quality standard of the gas measurement technique for *in vitro* digestibility tests.

One issue that is not fully addressed in the current protocols, is the equipment and experimental set up that is used for these kinds of tests. Many times, these are developed in-house and specific for each laboratory. A solution to minimise differences is the use of a complete lab platform such as the Gas Endeavour (Figure 1 and 2). The Gas Endeavour is specially designed for low gas volume and flow analysis and includes everything needed to perform *in vitro* digestibility tests; i.e. temperature controlled continuously mixed test vessels, optional vessels for carbon dioxide removal when methane analysis is performed, and a robust and reliable gas measuring system with a resolution of approximately 2 ml or 9 ml. In a study where three different ways of measuring the biochemical methane potential of cellulose were tested, the Gas Endeavour´s predecessor, AMPTS (Automatic Methane Potential Test System), provided the highest accuracy and repeatability (Esteves et al., 2011). Examples of studies where the AMPTS has been used are: investigation of methane potential

from algae farming on available sludge streams from a waste water treatment plant (Rusten & Sahu, 2011), evaluation of different pre-treatments of sugarcane bagasse (Badshah et al., 2012) and evaluation of the effects from different chemical and biological additives on a substrate mixture (Strömberg et al., 2011).

In this study the system is presented, and the results of a long term *in vitro* ruminant fermentation are discussed and compared with the classical VOS procedure. These tests were performed with the predecessor of the new Gas Endeavour.

System

The Gas Endeavour has been used to perform several methane potential tests for biogas production on various types of substrates, as well as a number of *in vitro* digestibility studies with ruminant feeds. As can be seen in the below figures, the Gas Endeavour is available in various configurations. In Fig. 1 and 2, the unit on the left-hand side is the gas detection unit where measurements take place and data is stored. The unit in the middle is a CO_2 removal unit and consists of 15 small vessels with 3M NaOH. The unit on the right-hand side is the incubator with test vessels.

Figure 1 The Gas Endeavour with 250 ml bottles in a thermostatic shaking water bath.

Figure 2 The Gas Endeavour with 500 ml bottles and mechanical agitation in a thermostatic water bath.

The system is available with a 2- or 9-ml measurement resolution (Fig. 3 and 4), and has either 500-ml bottles with mechanical mixing and a regular thermostatic water bath, or 250-ml bottles in a shaking thermostatic water bath. The system can also be set up in a way to measure both total gas and methane simultaneously, by using two flow cells per bottle with a

CO_2 removal unit in between (Fig. 5). The detection unit, which can be seen on the left-hand side of Fig. 1 and 2, is a gas measurement unit where gas is collected in a flow cell by water displacement. When a pre-defined gas volume has been accumulated, the cell opens and releases the gas which is registered in the embedded CPU. Every opening corresponds to roughly 2 ml (or 9 ml, depending on the resolution) of gas and for each opening the ambient temperature and pressure are registered for calculations of normalised values (0°C, 1 atmosphere and zero moisture content).

Figure 3 Flow Cell Unit with 2 ml measurement resolution.

Figure 4 Flow Cell Unit with 9 ml measurement resolution.

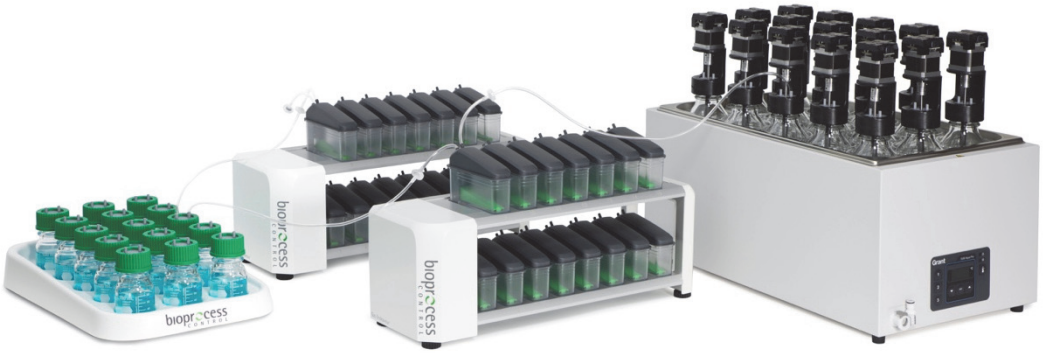

Figure 5 The Gas Endeavour can be set up in a way to monitor both total gas and methane production simultaneously.

Among other applications, the Gas Endeavour can be used to conduct ruminant fermentation trials, feed additive studies, monogastric nutrition trials, biodegradability and compostability tests, greenhouse gas emission studies, silage studies, specific anammox activity tests, biochemical oxygen demand (BOD) analyses, aerobic and anaerobic respiration tests, and determining the dynamic profile of the target analysis.

A long-term *in vitro* feed digestibility test was performed with the predecessor of the Gas Endeavour, where the accumulated gas volume was correlated to the organic matter digestibility of six standard samples and a straw sample.

Materials and methods

The incubation intended to imitate the standard VOS 96-h procedure, with a set of six calibration samples that are normally included in each run. The incubation involved both gas measurement and gravimetric determination of organic matter disappearance in the incubation vessels. It was performed in conjunction to the lab's weekly routine for rumen *in vitro* organic matter digestibility (IVDOM) determinations of forage samples according to the 96-h VOS procedure (Lindgren 1979; Åkerlind et al., 2011). Proportions of rumen fluid, buffer and sample were similar to the VOS procedure with 10 ml rumen fluid, 290 ml VOS buffer ((Lindgren, 1979), containing per litre: 8.50 g $NaHCO_3$, 5.80 g K_2HPO_4, 0.50 g $(NH_4)_2HPO_4$, 1.00 g NaCl, 0.50 g $MgSO_4$•7 H_2O, 0.01 g $FeSO_4$•7 H_2O and 0.10 g $CaCl_2$) and 4 g of air-dry sample.

A set of six calibration samples with IVDOM values of 686 to 901 g/kg OM, that are included in each IVDOM batch at the lab, were incubated in duplicate and so was a barley straw sample with IVDOM value of 505 g/kg OM. A single blank bottle with 290 ml VOS buffer and 10 ml rumen fluid was also included.

The rumen fluid was from a maintenance fed non-lactating cow and collected in the morning. Handling of rumen fluid and buffer was similar to the lab's IVDOM procedures with straining of rumen fluid through a 1-mm screen, mixing with buffer and dispensing into incubation bottles without previous CO_2 flushing. Incubation was conducted over 96 hours.

After termination, each bottle was split into three glass filter tubes with porosity P1 (100-160 μm) and rinsed according to the VOS procedure with hot water and acetone. The samples were then dried overnight at 103°C and ashed for 3 h at 500°C according to the standard procedures for VOS to get a measure of remaining organic matter amount and hence organic matter digestibility in vitro.

Results and discussion

The *in vitro* digestibility test was performed at the Department of Animal Nutrition and Management, SLU, Uppsala, with the predecessor of the new Gas Endeavour. The accumulated gas volume was monitored over time, and remaining organic matter amount was measured after the incubation period. Table 1 shows average organic matter digestibilities, obtained with the VOS method for many replicates and for the actual batch (shown as mean from two water baths). Presented is also organic matter digestibility (OMD) obtained with the Gas Endeavour, together with the final gas amount. Average results of accumulated gas volume over time are presented in Figure 6 with standard deviation of duplicates.

Table 1 Vessel contents, organic matter digestibility in % (VOS) at lab as average of numerous replicates and as measured with actual VOS batch. OMD from the Gas Endeavour was determined gravimetrically, similar to the VOS procedure

Bottle	Sample	Sample (gram)	Strained rumen fluid (ml)	VOS buffer (ml)	VOS long term average	VOS in actual batch	OMD in Gas Endeavour	Final gas (ml)
1	Grass S1	4.00	10	290	73.4	72.8	75.0	379
2	Grass S1	4.00	10	290	73.4	72.8	75.2	379
3	Grass S2	4.00	10	290	78.8	77.2	79.6	380
4	Grass S2	4.00	10	290	78.8	77.2	79.5	409
5	Grass S3	4.00	10	290	68.6	68.3	70.3	309
6	Grass S3	4.00	10	290	68.6	68.3	70.9	336
7	Grass S4	4.00	10	290	81.2	80.6	82.0	291
8	Grass S4	4.00	10	290	81.2	80.6	80.2	275
9	Grass S5	4.00	10	290	84.0	80.7	84.2	388
10	Grass S5	4.00	10	290	84.0	80.7	82.9	352
11	Grass S6	4.00	10	290	90.1	88.0	88.2	491
12	Grass S6	4.00	10	290	90.1	88.0	89.5	470
13	Straw	4.00	10	290	-	50.5	57.1	171
14	Straw	4.00	10	290	-	50.5	57.4	172

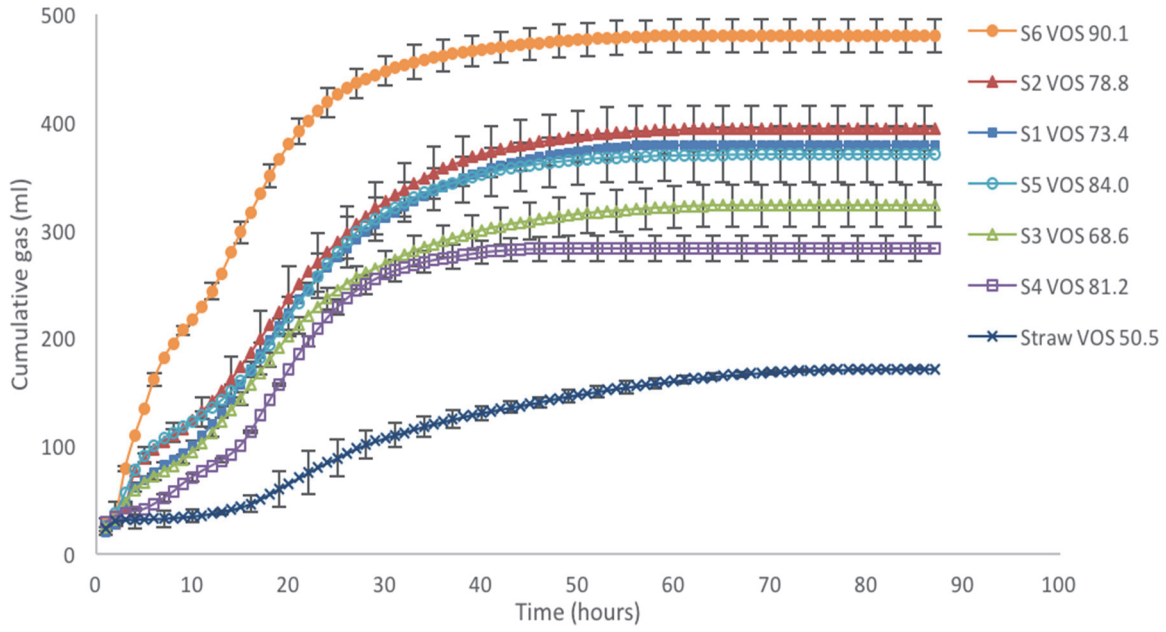

Figure 6 Accumulated gas volume over time, with final pH value.

There was a negligible amount of remaining organic matter in the blank (15 mg compared to 366-1565 mg in the samples) and the accumulated gas volume was 24 ml. The blank was not considered in this compilation. The Gas Endeavour resulted in a slightly higher remaining organic matter amount whereby the relative error compared to the VOS analysis was *circa* 3%. A clear difference in accumulated gas volume and gas production kinetics can be seen between the various grasses and straw. Figure 7 shows how the correlation between endpoint

gravimetric OMD and gas volume changes for each hour. As can be seen with these samples, it would not pay off to measure gas for more than approx. 30 h. After that, the correlation decreases, which could be caused by microbial recycling and a more unpredictable gas production. Overall, however, the results from the two methods were well correlated, with a correlation of approximately 0,88 for a standard *in vitro* rumen fermentation of 24 hours.

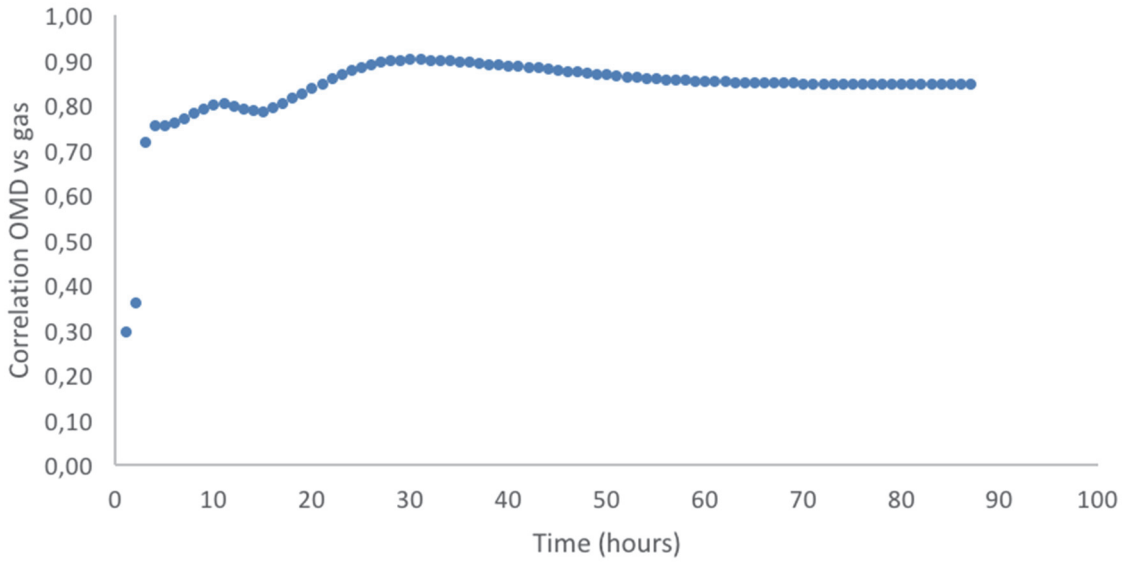

Figure 7 Correlation between cumulative gas volume per hour and endpoint organic matter digestibility.

Conclusions

In this work, the new Gas Endeavour of Bioprocess Control was presented. Result of a long term *in vitro* feed digestibility test, performed with the predecessor of the Gas Endeavour, are presented. A clear correlation between gas production and OMD was found, with decreasing correlation after 30 hours of incubation.
The newly developed Gas Endeavour offers the additional possibilities to study gas composition in real-time. It has a more accurate measurement of low flow of highly water soluble gases, while maintaining the proved measurement principle of its predecessor.

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References

Badshah, M., Liu, J. & Mattiasson, B., 2012. Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments. Biores. Technol. 114, 262-269.

Cone, J. W., van Gelder, A. H., Visscher, G. J. W., & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Tech. 61, 113–128.

Esteves, S., Devlin, D., Dinsdale, R. & Guwy, A., 2011. Performance of various methodologies for assessing batch anaerobic biodegradability. Proc. IWA Symp. Anaerobic Digest. Solid Waste and Energy Crop, Vienna, August 28-September 01.

Getachew, G., Blümmel, M., Makkar, H. P. S., & Becker, K., 1998. In vitro gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Tech. 72, 261–281.

Getachew, G., DePeters, E. J., & Robinson, P. H., 2008. Gas production provides effective method for assessing ruminant feeds. Calif. Agr. 58, 54–58.

Lindgren, E., 1979. The nutritional value of roughages estimated in vivo and by laboratory methods. Rapport 45, Uppsala, Sweden: Departm. Animal Nutr. Managem., Swedish University of Agricult. Sci.. 1-66 (in Swedish).

Murray, J. M. D., McMullin, P., Handel, I., & Hastie, P. M., 2014. Comparison of intestinal contents from different regions of the equine gastrointestinal tract as inocula for use in an in vitro gas production technique. Anim. Feed Sci. Tech. 187, 98–103.

Rusten, B. & Sahu, A.K., 2011. Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy, Water Sci. Technol. 64, 1195-1201.

Strömberg, S., Nistor, M. & Liu, J., 2011. Evaluation of substrate characteristics using the Automatic Methane Potential Test System (AMPTS). Proc. IBBK Progr. Biogas II, Stuttgart-Hohenheim, March 30-April 01.

Åkerlind M., Weisbjerg M., Eriksson T., Thøgersen R., Udén P., Ólafsson B. L., Harstad O. M. & Volden H., 2011. Feed analyses and digestion methods. In: Volden, H. (ed) NorFor – The Nordic Feed Evaluation System. EAAP publication No. 130. Wageningen Acad. Publ., Wageningen, the Netherlands. pp 41-54.

Preservatives can improve aerobic stability of potato by-products

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Introduction

There is a requirement for a more sustainable production in the food industry, where many by-products become bio-waste along the process chain. Food waste does not represent only economic and ethical problem but it also diminishes natural resources, which are limited in the world. According to Stenmarck *et al*. (2016), about 88 million tonnes of food waste is generated in Europe, which includes both edible food and inedible parts associated with food, comprising the entire food chain. This waste represents about 20% of the total food produced and the sectors contributing the most are households and processing. When it comes only to the fruit and vegetable industry, waste is even greater and, according to FAO (Wadhwa & Bakshi, 2013), it can reach nearly 50%, with losses occurring during agricultural production, processing, distribution and by consumers.

These vegetable by-products could potentially be used as animal feeds, which in general would strengthen the sustainability of both food and feed systems. An increasing demand for alternative feed resources in ruminant production is due to a growing requirement for a more sustainable food production from livestock.

Vegetable residues can be composted and used as soil amendment or used in biogas production, but they result in only a limited added value to the end product. A higher added value option would be to use these by-products, as animal feeds, but the challenge is how to preserve and store such residues, as they are typically wet and prone to fast deterioration. Preservatives can be added to materials to preserve and/or enhance quality, such as increasing aerobic stability (Rinne *et al*., 2017; Franco *et al*., 2018a), which allows e.g. more efficient logistics. Use of vegetable by-products as animal feeds should be efficient in terms of nutrient utilization, level of production reached and provision of safe feeds to secure animal health, occupational health of animal caretakers and finally the well-being of consumers using the animal products.

Europe has the highest level of potato consumption in the world (almost 90 kg per capita per year; FAO, 2008) resulting in a large amount of potato by-products generated and wasted. Potato by-products represent a great potential for use of bio-waste as an animal feed, if aspects related to feed safety, feed value and aerobic stability can be solved. The objective of the current study was to evaluate the effect of preservatives on aerobic stability of potato by-products.

Materials and methods

Potato by-products were collected from two Finnish companies. Samples were collected from the production line, kept refrigerated overnight and aerobic stability tests were started the next day.

Four types of potato by-products were used:

- Whole discarded peeled potatoes (WDP)
- Chopped discarded peeled potatoes (CDP)

- End waste, which included peels, discarded potatoes, pieces and soil from washing (EWP_1)
- End waste with peels (EWP_2).

Whole potatoes were ground before the trial and other fractions were used as such. A 4 × 4 treatment design was used, with four types of potato by-products and four preservative treatments as follows:

- Control without preservative (C)
- Formic acid based preservative at 5 l/ton (FA; AIV 2 Plus Na, Eastman Chemical Company, Oulu, Finland)
- Formic and propionic acid based preservative at 5 l/ton (FAPA, AIV Ässä Na, Eastman Chemical Company, Oulu, Finland)
- Salt based preservative 4 l/ton (Salt, Safesil, Salinity Agro, Västra Frölunda, Sweden).

Details of the preservatives are in Table 1.

One and half kg of raw material was weighed into a plastic container and the preservative was carefully mixed with it and, from each batch, one kg was used for aerobic stability measurement. Two stability methods were used: rise in temperature and visual inspection of deterioration.

Table 1 Description of the preservatives used in the experiment

Abbr.	Company	Composition	Name	Amount used
FA	Eastman Chemical Company	76% Formic acid 5.5% Sodium formate	AIV 2 Plus Na	5 l/t
FAPA	Eastman Chemical Company	58% Formic acid 20% Propionic acid 2.5% Potassium sorbate 5.2% Sodium formate	AIV Ässä Na	5 l/t
Salt	Salinity Agro	20% Sodium benzoate 10% Potassium sorbate 5% Sodium nitrite	Safesil	4 l/t

Aerobic stability of the by-products was carried out according to Luke standard method, where thermocouple wires in polystyrene boxes were connected to a data logger. Measurements started directly after adding the additives. Temperature from each of the boxes was automatically recorded at 10-minute intervals. Aerobic stability was defined as time taken to increase temperature of the raw material by 2°C above ambient temperature. There were three replicates per treatment, which were weighed before and after the aerobic incubation in order to measure aerobic losses. Replicates were prepared for each treatment and the preservatives were mixed separately for each replicate. However, total amount of some potato fractions (for instance CDP) was so small that preservative treatments could not be evaluated with this method, since it requires a great relatively large amount of sample. For CDP, only control and FAPA treatments were applied. EWP_2 was so liquid that it was not possible to perform temperature measurement and losses.

Aerobic stability of all potato by-products were measured by visual appearance of moulds and yeasts by placing a 3-cm layer (about 150 grams) of the potato mass in a plastic container which was covered with a slightly perforated plastic film and kept at +20ºC. Aerobic stability was visually evaluated by observing the growth of moulds and yeasts on the surface of the potato mass once a day using the scores: 0 = no spoilage; 1 = slight spoilage; 2 = moderate

spoilage; and 3 = severe spoilage. There were three replicates per treatment. Samples were discarded when they reached Score 3. Aerobic stability through visual inspection of spoilage was defined as hours until the sample reached Score 3.

All potato by-products were sampled and stored in a -20°C freezer prior to analysis according to standard laboratory methods. Samples were analysed for dry matter (DM) by freeze drying. A drying period of 4 days was used in a Christ gamma 2-20 with controller LMC-2, (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), ash (method 942.05), crude protein (CP; method 968.06), neutral detergent fibre (NDF) according to AOAC (1990) and starch (Salo and Salmi, 1968). Fresh samples were also analysed for enterobacteria, lactic acid bacteria, yeasts and moulds just before starting the aerobic stability tests.

Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. The sums of square was further partitioned into contrasts, where fractions and preservative effects were tested as well as preservatives within fractions. Replicates were taken as a random effect into the model.

Results and discussion

The potato by-products had lower DM and higher NDF (Table 2) than the reference value for whole potatoes in the Finnish feed tables (Luke, 2018). This was logical as water was used in the process, and the proportion of peels was higher than in whole potatoes. In addition, the concentrations of CP of the raw materials were higher than the reference value. In terms of microbiological quality, EWP_1 had the highest contamination of enterobacteria, lactic acid bacteria and yeasts, which can be explained by the composition and conditions under which this sample was taken. All waste produced along the production chain was collected and transported outside of the processing plant and placed in a big container, which contained rotten potatoes, peels, chopped pieces with black spots and also soil. The best hygienic quality was found for the WDP fraction, which represented peeled and clean potatoes ready to be further processed, but was discarded due to black spots or physical damage. Potato by-products presented a higher pH than ideal for proper preservation by itself, requiring preservative treatments to extend stability.

We were not able to detect differences in temperature rise during a 3-week period, because the temperature did not increase 2 degrees above room temperature even when samples were already deteriorating based on visual inspection. It indicates that this methodology was not suitable to measure aerobic stability of this type of raw material, probably due to the high requirement of heat to increase the temperature of a wet by-product. The specific heat of water requires 4,184 joules (1,000 calories) to raise the temperature of 1 kg of water by 1°C. On average, the moisture content of potato by-products was 850 g/kg, which would require 3,560 joules to increase the temperature of 1 kg of raw material by 1°C. The loss of aerobic stability was estimated based on 2°C increasing temperature above the ambient, which means that double amount of joules (7,120) would be required. As a comparison parameter, it would take only 5,000 joules to raise 2°C in temperature of, for instance, a total mixed ration with 400 g/kg DM concentration.

Seppälä *et al*. (2012) reported incapacity to measure aerobic stability of total mixed rations due to high DM concentrations of the raw materials, which probably was caused by a rapid dissipation of heat to the air preventing a measurable heat increase. These examples indicate

that alternative techniques are needed to evaluate aerobic stability of raw materials that do not show increase in heat. Franco *et al*. (2018b) reported a high correlation ($R^2 = 0.979$) between increase in temperature and CO_2 produced by aerobic bacteria showing that CO_2 can be used as an indicator of deterioration and as an alternative method to evaluate aerobic stability. Also, visual inspection has been successfully used (Rinne et al., 2017; Franco *et al*. 2018b), and that method was used in the current experiment as shown further below.

Table 2 Chemical composition and microbial quality of potato by-products before onset of aerobic stability test including comparison to a reference value

	WDP	CDP	EWP_1	EWP_2	Potato (Luke, 2018)
Dry matter (DM), g/kg	148	147	80	163	220
In DM, g/kg					
Ash	53	51	61	71	55
Crude protein	118	113	128	114	95
NDF	119	133	299	68	70
Starch	608	679	461	590	620
Enterobacteria, CFU/g	3.2×10^4	6.0×10^4	9.2×10^4	3.3×10^4	
LAB, CFU/g	4.6×10^6	4.2×10^7	5.2×10^7	2.7×10^7	
Yeasts, CFU/g	1.7×10^4	2.5×10^5	2.5×10^6	2.5×10^4	
Moulds, CFU/g	$<1.0 \times 10^2$	1.6×10^3	$<1.0 \times 10^2$	2.5×10^2	
pH	5.59	4.63	5.01	5.72	

WDP: whole discarded potatoes; CDP: chopped discarded potatoes; EWP_1: end waste, which includes peel, discarded potatoes, pieces and soil from washing; and EWP_2: end waste with peels. NDF: neutral detergent fibre; CFU: colony-forming unit; LAB: lactic acid bacteria.

There was no effect of preservative on weight losses during the 3-week period for WDP and EWP_1 (Figure 1), but preservatives increased losses of CDP. Losses were not affected by type of preservative in comparisons between FA and FAPA as well as FA and FAPA preservatives against Salt. Losses were maybe misleading, since some boxes allowed greater losses by leakage of liquid.

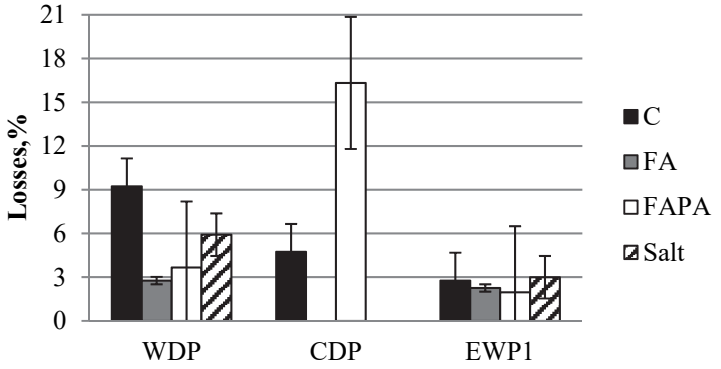

Figure 1 Losses (% of fresh weight) of different fractions of potato by-products treated with preservatives. WDP: whole discarded potatoes; CDP: chopped discarded potatoes; EWP_1: end waste, which includes peel, discarded potatoes, pieces and soil from washing. C: Control; FA: formic acid based preservative; FAPA: formic and propionic acid based preservative; Salt: salt based preservative. WDP: C vs Preservative = 0.23; CDP: C vs Preservative <0.01; EWP_1: C vs Preservative = 0.79; FA vs FAPA = 0.69; FA and FAPA vs Salt = 0.15. SEM = 2.0.

Preservatives had no effect on losses across all potato by-products (Figure 2; left). The WDP tended (P = 0.07) to have higher losses than EWP_1 (Figure 2; right).

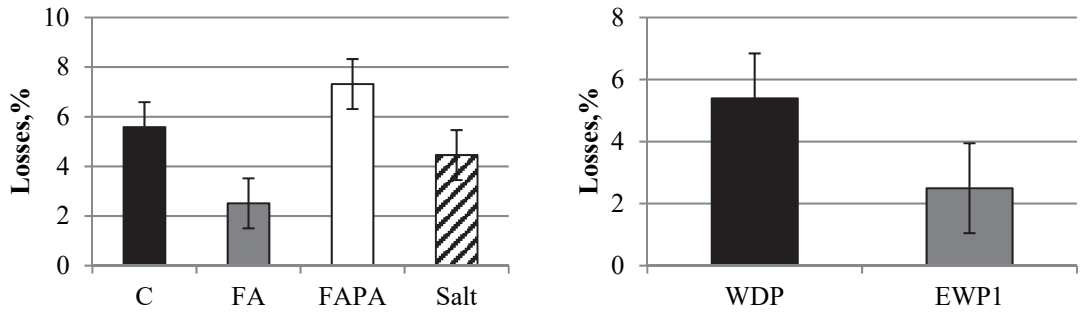

Figure 2 Losses (% of fresh weight) of potato by-products treated with preservatives (left) and different fractions (right). WDP: whole discarded potatoes; EWP_1: end waste, which includes peel, discarded potatoes, pieces and soil from washing. C: Control; FA: formic acid based preservative; FAPA: formic and propionic acid based preservative; Salt: salt based preservative. Left: C vs Preservative = 0.29; Right: WDP vs EWP_1 = 0.07. SEM = 2.0.

Visual inspection of moulds and yeasts gave useful information to evaluate efficacy of the preservatives and differences among by-products (Figure 3). There was an interaction between fraction and preservative, particularly, because of the Salt treatment, which resulted in longer aerobic stability for WDP and EWP_2, but shorter for CDP and EWP_1. Salt proved to be efficient depending of the type of raw material, while both FA and FAPA treatments presented the same pattern for all by-product fractions. These results are in line with those of Rinne *et al*. (2017) and Franco *et al*. (2018a) who found improvements on aerobic stability of carrot by-products preserved with formic acid based preservatives. FAPA resulted in a longer preservation period than FA, which can be explained by the presence of propionic acid (Table 1) in FAPA.

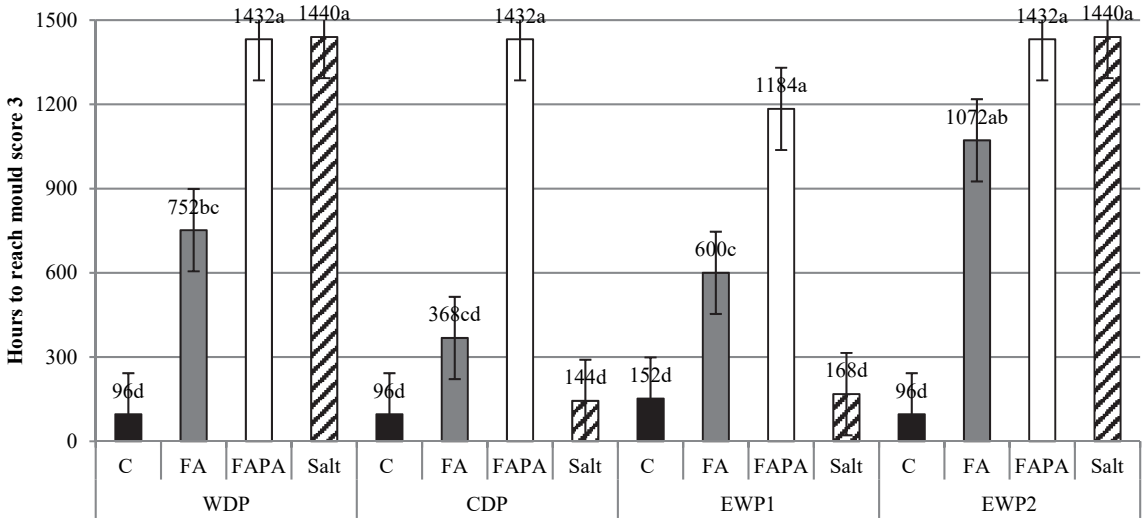

Figure 3 Hours to reach spoilage score 3 of different potato by-product fractions treated with preservatives. WDP: whole discarded potatoes; CDP: chopped discarded potatoes; EWP_1: end waste, which includes peel, discarded potatoes, pieces and soil from washing; EWP_2: end waste with peels. C: Control; FA: formic acid based preservative; FAPA: formic and propionic acid based preservative; Salt: salt based preservative. Fraction <0.01; Preservative <0.01; Fraction*Preservative <0.01; C vs Preservative <0.01; FA vs FAPA <0.01; FA and FAPA vs Salt <0.01. Means without same letter differ significantly (P<0.05). SEM = 78.6.

Conclusions

Increase in temperature was not a suitable method to evaluate aerobic stability of potato by-products, but visual inspection proved to be efficient to detect differences between by-products and preservatives. Preservatives extended the preservation period of the potato by-products, with formic and propionic based preservative being superior to the others. However, weight losses ranging from 2.5 % for formic acid to 7.3 % for formic and propionic acid based preservative, during the aerobic phase could not be avoided by preservatives.

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References

AOAC, 1990. Official methods of analysis. Arlington, VA: Association of Official Analytical Chemists, Inc., 1298 p. ISBN 0-935584-42-0.

FAO, 2008. International Year of the Potato. FAOSTAT. Available at: http://www.fao.org/potato-2008

Franco, M., Jalava, T., Järvenpää, E., Kahala, M. & Rinne, M., 2018a. Carrot by-product fermentation quality and aerobic stability could be modified with silage additives. XVIII Intern. Silage Conf., 24-26 July 2018, Bonn, Germany (in press). https://www.isc2018.de/

Franco, M., Stefanski, T., Jalava, T., Kuoppala, K., Huuskonen, A. & Rinne, M., 2018b. Aerobic stability of crimped wheat grain manipulated by additive treatments detected using different methods. XVIII Intern. Silage Conf., 24-26 July 2018, Bonn, Germany (in press). https://www.isc2018.de/

Luke, 2018. Feed Tables and Nutrient Requirements. Available at: www.luke.fi/feedtables

Rinne, M., Jalava, T., Siljander-Rasi, H., Kuoppala, K., Blasco, L., Kahala, M. & Järvenpää, E., 2017. Improving the usability of carrot by-products as animal feeds by ensiling. Proc. 8th Nordic Feed Sci. Conf., 13-14 June 2017, Uppsala, Sweden. Available at: http://www.slu.se/globalassets/ew/org/inst/huv/nfsc/nfsc-2017-proceedings.pdf

Salo, M.-L. & Salmi. M., 1968. Determination of starch by the amyloglucosidase method. J. Sci Agr. Soc. Finland 40, 38-45.

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M., 2012. The aerobic stability of total mixed ration can be managed by silage additive. XVI International Silage Conference, 2-4 July 2012, Hämeenlinna, Finland.

Stenmarck, Å., Jensen, C., Quested, T. & Moates, G., 2016. Estimates of European food waste levels. European Commission (FP7), Coordination and Support Action. Stockholm, Sweden. Available at: https://www.eu-fusions.org/phocadownload/Publications/Estimates%20of%20European%20food%20waste%20levels.pdf

Wadhwa, M. & Bakshi, M.P.S., 2013. Utilization of fruits and vegetables wastes as livestock feed and as substrates for generation of other value-added products. FAO Publication 2013/04. H.P. Makkar Technical Editor. Available at: http://www.fao.org/docrep/018/i3273e/i3273e.pdf

Effect of compound composition on water stability of pellets

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Introduction

Concentrate feedstuffs are often industrially processed into compound mixtures and subsequently pelletized to prevent segregation of ingredients and to ease on-farm allocation. For cattle, the conventional way to pelletize compound concentrates is by grinding premixed materials prior to entering the pellet mill, where rollers press the compound meal through a steel die.

Rumen fermentation and escape of nutrients from the rumen in compound concentrates would likely be affected by how easily pellets disintegrate. Pellets produced by conventional means typically have high sinking velocity in water and low stability. However, factors affecting stability in water of concentrate pellets for cattle are not well described.

The aim of this study was to investigate if pellet stability in water of compound concentrate is affected by nutrient and ingredient composition.

Materials and methods

In total, 32 samples of pelletized compound concentrates were obtained from two commercial feed mills in Denmark owned by Danish Cooperative Farm Supply (DLG, Vesterbrogade 4A, DK-1620 Copenhagen V, Denmark). Both compound concentrates for dairy cows (n = 23) and for fattening bulls (n = 9) were sampled.

Ingredients were milled using a hammer mill with a 3.5 mm screen before mixing. The mixed compound concentrates were pelletized using a conventional pellet press with steam addition to achieve a temperature of 81°C (pellet press with 3.8 × 80 mm die).

Bulk density of the cereals and cereal mixtures was determined in triplicate by measuring weight of each concentrate in a hard plastic container with a defined volume. Water stability index (WSI) of experimental concentrate pellets was tested at Fôrtek, Norway, using the procedure of Baeverfjord et al. (2006) where the amount of DM retained in net baskets after soaking for 120 min in 25°C deionised water.

Data on ingredient composition and calculated nutrient content were collected from production recipes. Ingredients were grouped for correlation analysis: grains = wheat, barley, maize, rye, triticale, and oats; oilseed by-products = soybean meal, soypass, rapeseed meal, rape seed cakes, sunflower meal, and linseed meal; fibre rich feeds = dried sugar beet pulp, soybean hulls, dried grass pellets, and dried citrus pulp; molasses = sugar beet and sugar cane molasses.

Pearson correlation analysis was performed among WSI, nutrient composition, and ingredient composition of compound concentrates using the CORR procedure of SAS. Strong correlation was declared if $P \leq 0.0001$ and moderate correlation when $0.0001 < P \leq 0.01$.

Results and discussion

Water stability indices are in Table 1 for the 32 compound concentrates and varied from 2 to 40% and averaged 14.2% (SD = 8.6%). Considering the subdivision of concentrates into type for cows and calves, WSI was lowest for the cow type compound concentrates as compared to the calf type. Composition of the 32 compound concentrates covered wide ranges for both nutrients and ingredient groups.

Table 1 Water stability index (WSI, %), bulk density (g/L), nutrient (g/kg), and ingredient composition (g/kg) of 32 commercial compound concentrates tested

Variable	Mean	SD	Minimum	Maximum
All, n = 32				
WSI	14.2	8.6	2.1	40.0
Bulk density	623	32	572	686
Ash	62	24	42	153
Crude protein	238	82	140	380
Crude fat	56	22	32	110
Starch	180	130	31	386
NDF	248	39	200	340
Grains	215	203	0.0	575
Oilseed by-products	444	317	22	990
Fibre rich feeds	130	135	0	489
Minerals	21	28	0	123
Molasses	9.3	5.6	0	20
Molasses + fats	21	15	5.0	65
Cows, n = 23				
WSI	10.5	5.2	2.1	19.8
Bulk density	619	31	572	677
Ash	56	7.1	42	70
Crude protein	258	79	160	380
Crude fat	63	22	40	110
Starch	136	93	37	335
NDF	260	39	210	340
Grains	142	137	0	452
Oilseed by-products	508	326	22	990
Fibre rich feeds	149	153	0	489
Minerals	10	7.4	0	30
Molasses	9.3	6.2	0	20
Molasses + fats	24	17	5.0	65
Calves, n = 9				
WSI	23.6	8.7	12.8	40.0
Bulk density	635	34	603	686
Ash	75	41	52	153
Crude protein	181	64	140	300
Crude fat	37	4.9	32	45
Starch	302	144	31	390
NDF	218	14	200	243
Grains	417	223	0	575
Oilseed by-products	269	224	100	707
Fibre rich fees	88	52	0	191
Minerals	45	41	20	123
Molasses	9.5	3.7	0	15
Molasses + fats	15	4.0	5.0	20

NDF = neutral detergent fibre.

In the current data, WSI was weakly correlated to bulk density (Table 2). However, when considering only the cow type, WSI was moderately positive correlated to bulk density, indicating that denser pellets disintegrate more slowly.

The WSI of compound concentrate pellets was not correlated to ash content, moderately correlated to crude protein and NDF content, and strongly correlated to crude fat and starch content (Table 2). Considering the subdivision of concentrates into type for cows or calves, similar correlations among WSI and nutrient contents were observed within both types, though, correlations were weaker reflecting lower number of observations in the subgroups.

Table 2 Pearson correlation analysis of WSI against bulk density (BD), nutrient and ingredient composition in 32 commercial compound concentrates

		Nutrients[1]					Ingredient groups[2]				
	BD	Ash	CP	Crude fat	Starch	NDF	Grains	Oilseed byprod.	Fibre rich	Molas-ses	Molas-ses+fat
All, n = 32											
r	0.36	0.01	-0.59	-0.67	0.72	-0.46	0.74	-0.51	0.05	0.23	-0.29
P	0.04	0.96	<0.01	<0.0001	<0.0001	<0.01	<0.0001	<0.01	0.77	0.20	0.11
Cows, n = 23											
r	0.65	-0.28	-0.49	-0.60	0.68	-0.25	0.68	-0.41	0.24	0.33	-0.19
P	0.01	0.19	0.02	<0.01	<0.01	0.25	<0.01	0.05	0.27	0.12	0.38
Calves, n = 9											
r	-0.11	-0.48	-0.49	-0.71	0.46	-0.16	0.48	-0.50	0.92	0.35	-0.01
P	0.79	0.19	0.18	0.03	0.22	0.68	0.19	0.17	<0.01	0.36	0.98

[1]CP = crude protein; NDF = neutral detergent fibre; [2]See materials and methods section for definition of ingredient groups

The observed correlations between WSI and ingredient groups likely reflected correlations with nutrients, e.g. similar correlations were observed for starch and grains, and for crude protein and oilseed by-products. Indeed, there could have been differences between each type of ingredient within a defined ingredient group, but there were too few samples to obtain robust correlations analyses.

The observed strong correlation between WSI of pellets and starch/grain content in the compound was likely caused by gelatinisation of starch during the pelletizing process where steam was added.

For the calf type concentrates, a moderate positive correlation was observed between WSI and fibre rich ingredients. This was surprising as no correlations was observed across all concentrates or within the cow type group. Thus, it is likely that this was caused by the low number of samples.

Conclusions

It appears that stability of compound concentrate pellets in water is positively correlated to starch content and negatively correlated to fat content. Protein and NDF contents seem to have negatively affected water stability of the pellets. The effect of nutrient composition on water stability of pellets strongly reflected ingredient group composition.

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References

Baeverfjord, G., Refstie, S., Krogedal, P., Åsgård, T., 2006. Low feed pellet water stability and fluctuating water salinity cause separation and accumulation of dietary oil in the stomach of rainbow trout (Oncorhynchus mykiss). Aquacult. 261, 1335-1345.

Grass silage for biorefinery – separation efficiency and aerobic stability of silage and solid fraction

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Introduction

A green biorefinery concept involves processing of green biomass into a range of products (Mandl, 2010). Grasses have an excellent potential for biomass production under boreal conditions and they provide versatile properties as raw material for green biorefinery. Ensiling allows green biomass to be processed all year round. The green biorefinery approach generally starts with mechanical separation of liquid and solid fractions. These fractions can then be further processed into other products (McEniry *et al*., 2011). Solid fractions from green biorefinery can be used e.g. as feed for ruminants (Kautto *et al*., 2018), to produce insulation boards (Grass, 2004) or hydrolysed into simple sugars for further processes (Niemi *et al.,* 2017). Liquid fractions can be used as feeds for pigs and cows (Rinne *et al*., 2018). Another possible application of the liquid fraction may be raw material for extraction of lactic acid and amino acids (Ecker *et al*., 2012).

Raw material quality and processing technology significantly influence the yield and composition of the liquid and solid fractions. Franco *et al*. (2018) suggested that there is high correlation between liquid yield and composition end the silage quality. Silage quality can be then used to predict the biorefinery potential of grass silages. Furthermore, the production of grass silage can be modify to meet the regiment of specific green biorefinery.

A variety of technological solutions can be used for liquid-solid separation and choice of method has a great impact on liquid yield and composition. A further challenge is the preservation of solid and liquid fractions after extraction, due to a rapid deterioration. Aerobic stability reflects stability of a feed over time after exposure to the air. Short aerobic stability may influence dry matter (DM) intake, quantity of feed refusals and will affect production costs by increasing feed losses. Preservatives can be added to preserve and/or enhance quality and increase aerobic stability (Seppälä *et al*., 2016). The aim of this experiment was to compare three liquid-solid separation methods of grass silage on liquid yield, composition and retained compounds in liquid. Effect of preservatives on aerobic stability of silage, solid fraction and solid fraction with water added and used as such or in a total mixed ration (TMR) was also assessed using two indicators - increase in temperature and visual inspection.

Materials and methods

The grass silage was produced in Jokioinen, Finland, from a first cut (21st and 22nd of June 2017) of mixed timothy and meadow fescue lay with the following composition: DM 193 g/kg, crude protein (CP) 132 g/kg DM and organic matter digestibility 749 g/kg OM. The grass was precision chopped and ensiled in a clamp silo with a formic acid based additive with a target application rate of 5 l/ton (AIV 2 Plus, Eastman Chemical Company, Oulu, Finland).

The silage was separated into liquid and solid fractions using three pressing methods as follows:

- Farm scale twin screw press (FTS; Haarslev Industries A/S, Søndersø, Denmark). Liquid-solid separation was made by feeding the press from a TMR wagon equipped with a scale. The liquid was pumped into 1000-l containers and the amount of liquid was estimated based on volume. This allowed mass balances (liquid and solid yields from the original silage) to be calculated. In 8 batches with one week intervals, a total of 34410 kg of silage was processed. Each batch was processed over two days. Before starting the measurements, the FTS was running for 15 min to fill the press and reach optimal performance. The solid fraction was fed to dairy cows in a feeding trial (Kautto *et al*., 2018)
- Laboratory scale twin screw press (LTS; Angel Juicer Ltd., Busan, South Korea). Batches of 300 g were pressed. Before starting the measurements, the LTS was filled with 150 g of silage in order to reach optimal performance. Liquid was quantitatively collected and weighed.
- Laboratory scale pneumatic press (LPP, Luke in-house built equipment, Jokioinen, Finland). Liquid-solid separation was made placing 150 g of sample into a mesh bag and applying pressure between two piston plates during 2 minutes at 6 bars (× 100 kPa) pressure. The mesh bags were wetted and pressed before the actual samples were processed. Liquids were quantitatively collected and weighed. There were 3 replicates per sample and a mean value was used for statistical analyses.

Silage from batches 4 and 7 from the FTS were used for LTS and LPP. The average composition of liquid and solid fractions and the original silages are in Table 1. Retained proportion of DM, CP and ash in liquid were calculated for each liquid-solid separation method.

Samples for chemical analysis were taken along the experiment and analysed as described by Seppälä *et al*. (2016) at the laboratory of Luke in Jokioinen, Finland.

Table 1 Chemical composition of original silages, and solid and liquid fractions.

	FTS			LTS			LPP	
	Silage	Solid	Liquid	Silage	Solid	Liquid	Solid	Liquid
Dry matter, g/kg	204	430	63	214	497	85	310	70
In dry matter, g/kg								
Ash	71	42	197	70	43	183	55	229
Crude protein	142	107	279	144	99	262	118	271
Neutral detergent fibre	609	727	-	609	Nd*	-	Nd*	-
Ammonia-N, g/kg N	30	16	3	30	Nd*	Nd*	Nd*	Nd*
Organic matter digestibility	724	695	-	724	Nd*	-	Nd*	-

FTS: farm scale twin screw press; LTS: laboratory scale twin screw press; LPP: laboratory scale pneumatic press. *Not determined.

Further, an aerobic stability experiment was arranged using a 3 × 2 × 3 factorial design, with three types of raw material (silage, solid fraction or solid fraction with added water to reach the DM content of the silage), two forms of raw material (as such or as part of TMR) and three preservative treatments, including a control without preservative, a formic and propionic acid based preservative (FAPA, AIV Ässä Na, Eastman Chemical Company, Oulu, Finland) and a propionic acid based preservative (PA, Eastman Stabilizer Crimp, Eastman Chemical Company, Oulu, Finland) both at a rate of 3 l/t of fresh matter. Diets recipes are in Table 2.

Aerobic stability was evaluated according to the Luke standard temperature method, where thermocouple wires in polystyrene boxes were connected to a data logger. Temperatures from each of the treatments were automatically recorded at 10-minute intervals. Aerobic stability was defined as the time taken to increase temperature 2°C above the ambient temperature (Seppälä et al., 2016).

Aerobic stability by visual inspection of silage and solid fractions of FTS was also evaluated. Samples were placed (3 cm layer) in a plastic container which was covered with a slightly perforated plastic film and kept at +20ºC. Visual inspection was conducted once a day and scored: 0 = no spoilage; 1 = slight spoilage; 2 = moderate spoilage; and 3 = severe spoilage. Aerobic stability by visual inspection of spoilage was defined as hours to reach Score 3.

Table 2 Composition of the diets used to measure aerobic stability through increase in temperature.

	As such			In total mixed ration		
	Silage	Solid	Solid+water	Silage	Solid	Solid+water
Water, g/kg			723			566
In g/kg dry matter						
Grass silage	1000			502		
Solid fraction		1000	277		500	218
Barley				160	160	69
Oats				160	160	69
Rapeseed meal				160	160	69
Minerals				18	20	9

Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. Effect of liquid-solid separation method on liquid yield, composition and retained compounds in liquid was evaluated using a Tukey test. The factorial scheme to evaluate the aerobic stability was partitioned into contrasts, where type of raw material, used form and preservative were included as fixed effects in the model. Effect of preservatives on aerobic stability measured through visual inspection was tested taking replicates as a random effect into the model.

Results and discussion

The grass silage (Table 1) used in this experiment was representative for a typical grass silage used in Northern Europe (Huhtanen *et al*., 2006).

Separation of grass silage into fractions resulted in solid fractions with higher DM and NDF concentrations and lower CP and ash than the original silage (Table 1). Also Wachendorf *et al*. (2009) reported that separation resulted in a solid fraction with lower ash concentration and higher fibre than the original silage. In the present study, the concentrations of CP and ash in the solid fraction decreased by on average 33% (SD = 11.0) and 50% (SD = 13.2) relatively to the original grass silage, respectively. Similarly, McEniry *et al*. (2012) reported a 55% decrease of CP and ash in the solid fraction compared to the fresh pre-ensiled material. McEniry & O`Kiely (2013) reported decreasing of CP concentration of 66% on average in solid fraction compared to the fresh material pre-ensiling. The larger reduction of CP in McEniry & O`Kiely (2013), compared to present study, may be explained by their material pretreatment with a detergent (sodium dodecyl sulphate) in deionized water at 60°C to enhance the separation process.

Separation method affected liquid yield and liquid DM concentration (Table 3) with a lower liquid yield for LPP and higher liquid DM for LTS (P<0.05). There was an effect of separation method on DM, CP and ash retained in liquid (P<0.05). The LPP treatment resulted in lower DM, CP and ash retained in the liquid (P<0.05). Higher DM retained in liquid was obtained for LTS, while CP and ash retained in liquid were not different between FTS and LTS.

Table 3 Effect of pressing methods on liquid yield, composition and retained compounds in liquid

	FTS	LTS	LPP	SEM
Liquid yield	0.576[a]	0.601[a]	0.345[b]	0.0218
Liquid dry matter (DM), g/kg	71[b]	84[a]	69[b]	1.4
In liquid DM, g/kg				
Crude protein (CP)	270[a]	263[a]	271[a]	1.2
Ash	189[a]	178[a]	218[a]	11.7
Amount retained in liquid as proportion of original silage				
DM	0.193[b]	0.237[a]	0.112[c]	0.0056
CP	0.361[a]	0.422[a]	0.209[b]	0.0112
Ash	0.535[a]	0.606[a]	0.351[b]	0.0308

FTS: farm scale twin screw press; LTS: laboratory scale twin screw press; LPP: laboratory scale pneumatic press. SEM: standard error of the mean. Means within the same row without same superscript differ (P<0.05).

The effects of preservatives and different combinations of raw material in the diet (Table 2) on aerobic stability measured through increase in temperature are in Figure 1.

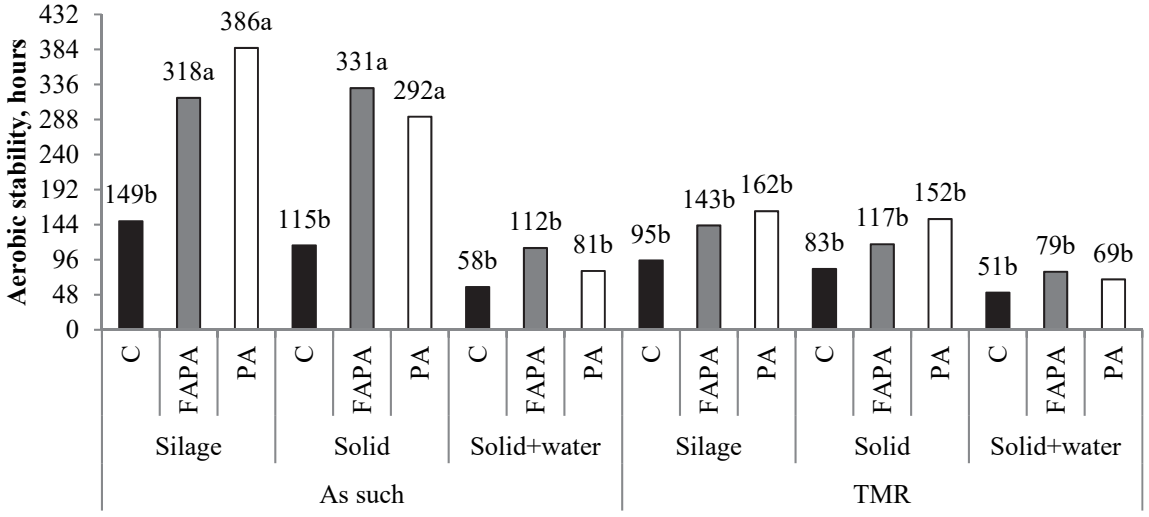

Figure 1. Effect of preservatives on aerobic stability of grass silage, solid fraction and solid fraction + water used as such or in TMR assessed through increasing in temperature. Preservative <0.001; Silage vs Solid used as such =0.060; Silage vs Solid in TMR =0.417; Silage as such vs Silage in TMR <0.001; Solid as such vs Solid in TMR <0.001; Silage vs Solid+water as such <0.001; Silage vs Solid+water in TMR =0.001; As such vs TMR <0.001. C: control without preservative; FAPA: formic and propionic acid based preservative; PA: propionic acid based preservative. Means without same letter differ (P<0.05).

Silage and solid fraction treated with preservatives had the highest aerobic stability compared to remaining treatments in this study (Figure 1). Similarly as reported by Seppälä *et al*. (2016), silage had longer stability than TMR. It should be noted that in both cases, the silages was of high hygienic quality and preserved with a formic acid based additive. Seppälä *et al*.

(2016) indicated that high yeast count in brewer's grain is likely to cause low aerobic stability of TMR. Also in the current study, the concentrate components may have had lower hygienic quality. Addition of water to the solid fraction (to achieve the same DM as in silage) reduced aerobic stability. It may be speculated that high DM of the solid fraction may have restricted microbial growth. Also Seppälä *et al*. (2016) suggested that high DM of silage may restrict growth of spoiling microbes. But on the other hand, high DM silage are more prone to heating, probably due to a higher air ingress and lower concentrations of fermentation end-products, which may be quite efficient in preventing aerobic spoilage. There was no difference between silage and solid fraction used as such or in a TMR on aerobic stability measured through increasing in temperature. Both preservatives improved aerobic stability of the diets.

Figure 2 presents the effect of preservatives on aerobic stability of grass silage and solid fraction assessed by visual inspection. There was an interaction between raw material and preservative. Preservatives provided higher aerobic stability than control and effect of preservative was stronger in silage than in the solid fraction.

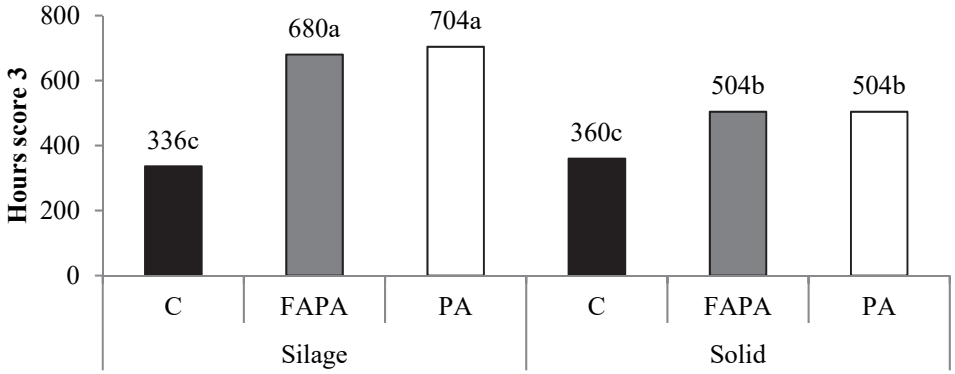

Figure 2. Effect of preservatives on aerobic stability of grass silage and solid fraction of grass silage assessed through visual inspection. Silage vs Solid <0.001; Preservative in silage <0.001; Preservative in solid <0.001; Preservative <0.001; Raw material*Preservative <0.001; FAPA vs PA = 0.458. C: control without preservative; FAPA: formic and propionic acid based preservative; PA: propionic acid based preservative. Means without same letter differ (P<0.05).

Both methods (increasing in temperature and visual inspection) indicated that preservatives were effective in increasing aerobic stability of grass silage and solid fraction from a biorefinery process. However, visual inspection resulted in longer aerobic stability than that evaluated through increase in temperature, which indicates the need of a lower threshold for visual inspection, so that both methods can match. There was a high correlation between methods (R^2 = 0.798), indicating that both techniques are suitable to measure aerobic stability of silage and solid fraction.

Conclusions

Twin screw presses, farm and laboratory scale, resulted in higher liquid yield and greater amount of retained compounds in liquid fraction as compared to a pneumatic press. Preservatives extended aerobic stability of silage, solid fraction and solid fraction added with water used as such or in a TMR.

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References

Ecker, J., Schaffenberger, M., Koschuh, W., Mandl, M., Böchzelt, H.G., Schnitzer, H., Harasek, M. & Steinmüller, H., 2012. Green Biorefinery upper Austria-pilot plant operation. Sep. Purif. Technol. 96, 237-247.

Franco, M., Winquist, E. & Rinne, M., 2018. Grass silage for biorefinery – A meta-analysis of silage factors affecting liquid-solid separation. XVIII Intern. Silage Conf., 24-26 July 2018, Bonn, Germany (in press). www.isc2018.de

Grass, S., 2004. Utilisation of grass for production of fibres, protein and energy. In: Proc. OECD Workshop on Biomass and Agriculture: sustainability, markets and policies. Paris, France: OECD Publ. Service. p. 169-177.

Huhtanen, P., Nousiainen, J. & Rinne, M., 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15, 293-323.

Kautto, O., Franco, M., Stefanski, T., Mäntysaari, P., Kuoppala K. & Rinne, M., 2018. Grass silage for biorefinery - Dairy cow responses to diets based on solid fraction of grass silage. Proc. 9th Nordic Feed Sci. Conf. (in press).

Mandl, M.G., 2010. Status of green biorefining in Europe. Biofuel Bioprod. Bior. 4, 268-274.

McEniry, J., King, C. & O'Kiely, P., 2011. The grass is greener - alternative applications for Irish grass. T-Research 6, 26-27. Available at: http://www.teagasc.ie/publications/2011/1002/TResearch_201106.pdf

McEniry, J., Finnan, J., King, C. & O'Kiely, P., 2012. The effect of ensiling and fractionation on the suitability for combustion of three grassland species harvested at sequential growth stages. Grass Forage Sci. 67, 559-568.

McEniry, J. & O´Kiely, P., 2013. The estimated nutritive value of three common grassland species at three primary growth harvest dates following ensiling and fractionation of press-cake. Agric. Feed Sci. 22, 194-200.

Niemi, P., Pihlajaniemi, V., Rinne, M. & Siika-aho, M., 2017. Production of sugars from grass silage after steam explosion or soaking in aqueous ammonia. Ind. Crop. Prod. 98, 93-99.

Rinne, M., Keto, L., Siljander-Rasi, H., Stefanski, T. & Winquist, E., 2018. Grass silage for biorefinery – Palatability of silage juice for growing pigs and dairy cows. XVIII Intern. Silage Conf., 24-26 July 2018, Bonn, Germany (in pess), www.isc2018.de.

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M., 2016. Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass Forage Sci. 71, 458-471.

Wachendorf, M., Richter, F., Fricke, T., Grab, R. & Neff, R., 2009. Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. I. Effects of hydrothermal conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds, and nutrient balances. Grass Forage Sci. 64, 132-143.

Milk production, feed sorting behaviour and social interactions in the feeding area in cows fed TMR or compact TMR

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Introduction

Swedish dairy cows are to an increasing extent fed concentrates and roughage mixed together, in a total mixed ration (TMR). Total mixed rations may be beneficial especially for organic herds, as it facilitates the use of homegrown protein feeds, which often are rich in starch and thereby can cause rumen disturbances when fed separately to the roughage. When the diet is mixed, the risk of rumen acidosis is decreased due to the more even intake of fibre and starch. However, it has been shown that animals tend to sort the diet if it is possible, leading to differences in composition over time, commonly resulting in higher fibre concentration the longer the feed has been available to the animals (Khan *et al.*, 2014). In other words, low ranked cows, that may be pushed away from the feed and get to eat last, are offered a less nutrient dense feed compared with high ranked cows. Since sorting of the diet will result in a variable diet composition over the day, it may result in problems like sub-acute rumen acidosis (Shaver, 2002).

This sorting behaviour has been suggested to decrease when providing the cows with a so-called compact TMR (CTMR; Kristensen, 2015), where the concentrate ingredients are soaked before adding forage and mixing the diet for a longer time. Soaking of the concentrates makes small, starchy particles stick better in the mix and a prolonged mixing time decreases the proportion of long particles. A more finely chopped feed results in fewer particles that are rejected (Leonardi & Armentano, 2003) and the addition of water to a TMR can decrease sorting behaviour (Fish & DeVries, 2012). However, water addition has in some cases been shown to increase rather than decrease sorting (Felton & DeVries, 2010). Shorter particle size of forage-based diets has been shown to increase feed intake and milk production, as well as protein concentration of the milk (Nasrollahi *et al.*, 2015). Other effects may be reduced eating and ruminating times (Storm & Kristensen, 2010) or shortened total eating time (Nasrollahi *et al.*, 2014) which will give the cow more time to rest.

The aim of the study was to evaluate the effects of a CTMR, achieved by decreased dry matter (DM) content and increased mixing of the diet, on milk production and dairy cow behaviour at the feed bunk. It was hypothesized that feeding a CTMR diet would result in equal or increased milk production and less sorting behaviours and fewer aggressive interactions at the feed bunk compared with a traditional TMR diet.

Materials and methods

Two dietary treatments were tested in 40 mid-lactation dairy cows in a change-over experiment with randomized block-design. The cows were blocked according to parity and stage of lactation and, within block, they were randomly allocated to one of two groups of 20 animals each. The experimental periods consisted of two weeks for adaptation and one week for measurements and the experiment lasted six weeks in total. The cows were housed in a free-stall barn in two separate units. The feed bunk was shared between the units, but divided in length so that cows did not reach the feed supplied on the opposite side of the feed bunk. In

addition to the experimental animals, non-experimental cows were housed in the barn to achieve a realistic traffic around the feed table. In total, 59-61 cows were housed in each unit, having access to 32 feeding spaces with headlocks per unit during the measurement period.

Feed was available *ad libitum* and was mixed and distributed twice daily with the aim of approximately 10% feed residues. The same second harvest grass/clover silage (precision chopped before ensiling) and concentrate (pelleted and crushed) was used for the diets with the difference that the forage in the CTMR was mixed for 60 minutes in a mixer with knives (SiloKing,Tittmoning, Germany). Then concentrate and approximately 30% water was added during an additional mixing for 10 minutes in a mixer without knives (DeLaval, Tumba, Sweden). The TMR consisted of forage from the bunker silo and concentrates mixed without knives for 10 minutes. The increased mixing time and addition of water in the CTMR was aiming to decrease particle size and lower the DM content of the feed. The silage:concentrate ratio was 60:40 on DM basis according to organic standards for milk production (KRAV, 2017). Average DM content of the silage was 42% and the diet contained (/kg DM) 12.5 MJ metabolizable energy (ME), 187 g crude protein (CP) and 367 g neutral detergent fibre (NDF). Particle size was evaluated using the Penn State particle separator (Lammers *et al*., 1996) with two sieves and a bottom pan. The cows were milked twice a day at 6 AM and 5 PM in an automatic milking rotary (DeLaval, Tumba, Sweden). Milk yield was automatically recorded at each milking and test milking was performed at morning and evening milking two consecutive days during the measurement periods. Milk samples were analysed with a Delta Combiscope (Combiscope FTIR 300, Delta instruments, the Netherlands) for fat, protein and lactose content. Energy corrected milk yield (ECM) was calculated according to the formula determined by Sjaunja *et al.* (1990).

Behavioural studies were performed during the measurement periods. Continuous observations to study sorting behaviour and aggressive interactions between cows at 0 to 1 h after feeding and 2 to 3 h post feeding.

Data on feed sorting behaviour and aggressive interactions were analysed with mixed models including period, treatment and hour as fixed effects. Data on milk yield were analysed with PROC MIXED (SAS 9.4), a mixed model including block, period and treatment as fixed effects and cow as random. Interactions between the fixed effects were tested but found non-significant and excluded from the models. Effects were considered significant when $P<0.05$.

Results and discussion

Mean distribution of particle sizes in fresh weight of the feeds at feeding were in percentage: 6, 64 and 30 *vs*. 31, 34 and 34 of >19 mm, 8 to19 mm and <8 mm in CTMR and TMR, respectively (data not shown). Feed sorting behaviours were more frequent in the group fed TMR compared with CTMR, which supported our hypothesis. Cows showed more digging and eating from underneath in this group, while eating from the side and throwing feed did not differ between treatments (Table 1). Digging and eating from underneath are typical sorting behaviours in cattle (Leonardi & Armentano, 2003). Hypothetically, the decrease in sorting behaviour could have resulted in less difference between the feed residuals and the newly mixed diet, and the results regarding aggressive interactions (Table 2) indicated that there were fewer aggressive interactions in the CTMR treatment compared to the TMR treatment. It could be speculated that the cows were less prone to compete for new feed in the CTMR treatment. Total number of aggressive interactions was higher around feeding compared with two to three hours after feeding (P = 0.005; SEM = 1.25; data not shown).

Table 1 Feed sorting behaviours in lactating dairy cows fed a total mixed ration (TMR) or a compact TMR (CTMR) at a feed bunk. Presented as LSmeans and standard error of the mean (SEM)

	Treatment			
Number of sorting behaviours/h	TMR	CTMR	SEM	*P*-value
Total	42.6	16.9	4.74	0.002
Digging	23.4	6.8	3.13	0.003
Eating from underneath	10.1	1.0	1.13	< 0.001
Eating from the side	7.0	8.6	1.20	0.36
Throwing	2.1	0.5	0.67	0.11

Table 2 Number of aggressive interactions in lactating dairy cows fed a total mixed ration (TMR) or compact TMR (CTMR) at a feed bunk. Presented as LSmeans and standard error of the mean (SEM)

	Treatment			
Number of aggressive interactions/h	TMR	CTMR	SEM	*P*-value
Total	14.8	8.5	1.25	0.004
Lower head	3.9	2.0	0.58	0.041
Head butting	5.6	2.5	0.60	0.003
Push	1.5	1.5	0.65	1.0
Squeeze	3.4	2.3	0.65	0.25

There were no differences in milk yield or composition between treatments (Table 3). Thus, the hypothesis that feeding CTMR would result in equal or increased milk production compared to feeding TMR was supported.

Table 3 Daily milk yield and composition from cows fed total mixed ration (TMR) or compact TMR (CTMR). Presented as LSmeans and standard error of the mean (SEM)

	TMR	**CTMR**	**SEM**	***P*-value**
Milk yield, kg	33.4	32.9	0.78	0.10
ECM[1], kg	31.8	31.4	0.82	0.47
Fat (%)	3.66	3.69	0.08	0.72
Protein (%)	3.39	3.36	0.04	0.56
Lactose (%)	4.50	4.44	0.04	0.24

[1]ECM, energy corrected milk

Conclusions

Increased mixing time and addition of water to a diet with 60% grass silage resulted in fewer aggressive interactions among dairy cows. This may have been the result of a more homogenous diet, also supported by a decrease in sorting behavior. However, there were no differences in milk yield or milk composition between the two dietary treatments.

References

Felton, C.A., & DeVries, T.J., 2010. Effect of water addition to a total mixed ration on feed temperature, feed intake, sorting behaviour, and milk production of dairy cows. J. Dairy Sci. 93, 2651-2660.

Fish, J.A. & DeVries, T.J., 2012. Short communication: Varying dietary dry matter concentration through water addition: effect on nutrient intake and sorting of dairy cows in late lactation. J. Dairy Sci. 95, 850-855.

Khan, M.A., Bach, A., Castells, L., Weary, D.M. & von Keyserlingk M.A., 2014. Effects of particle size and moisture levels in mixed rations on the feeding behaviour of dairy heifers. Animal 8, 1722-1727.

KRAV, 2017. Standards for Organic Production. Uppsala, Sweden.

Kristensen, N.B., 2015. Djurhälso- & utfodringskonferensen. Växa Sverige, Sollentuna, 25-26 aug.

Lammers, B.P., Buckmaster, D.R. & Heinrichs, A.J., 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79, 922-928.

Leonardi, C. & Armentano L.E., 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86, 557-564.

Nasrollahi, S.M., Ghorbani, G.R., Khorvash, M. & Yang, W.Z., 2014. Effects of grain source and marginal change in Lucerne hay particle size on feed sorting, eating behaviour, chewing activity, and milk production in mid-lactation Holstein dairy cows. J. Anim. Phys. Anim. Nutr. 98, 1110-1116.

Nasrollahi, S.M., Imani, M. & Zebeli, Q., 2015. A meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. J. Dairy Sci. 98, 8926-8939.

Shaver, R.D., 2002. Rumen acidosis in dairy cattle: bunk management considerations, in:

Sjaunja, L.-O., Baevre, L., Junkkarinen, L., Pedersen, J. & Setälä, J., 1990. A Nordic proposal for an energy corrected milk (ECM) formula. 26th session of the Int. Comm. Record. Prod. Milk Anim. (ICRPMA).

Storm, A.C. & Kristensen, N.B., 2010. Effects of particle size and dry matter content of a total mixed ration on intraruminal equilibration and net portal flux of volatile fatty acids in lactating dairy cows. J. Dairy Sci. 93, 4223-4238.

Effect of Maxammon grain treatment on performance of finishing beef cattle

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Introduction

Feed is a major cost and an important contributor to the environmental impact of beef production. An improvement in feed efficiency should result in higher margins and lower environmental impact per unit of production. Diets that are predominantly composed of cereal grains have a relatively higher risk of acidosis than diets based on forage and might lead to clinical disorders and impaired production. Harbro Limited's *Maxammon* process was developed to preserve cereal crops harvested with high moisture content and involves treatment with urea and enzyme to produce ammonia, which increases pH to approximately 9.0 and simultaneously increases the crude protein content.

A study was carried out on a commercial beef finisher unit to compare the production performance and incidence of diarrhoea in cattle finished on a *Maxammon* treated barley diet with a propionate treated barley (*Prograin*) diet, which was routinely used on the farm in the past.

Materials and methods

The study was carried out on a 600 acre beef breeder and finisher unit with approximately 900 cattle in Aberdeenshire, North East Scotland. Barley was harvested from the farm and formed the basis of the finishing ration fed to store cattle that comprised a mix of on-farm bred cattle and cattle purchased from local sales. In this study, 217 continental crossbreed steers (predominantly Limousin and Charolais) with an average weight of 481.5 kg (SD = 37.9 kg) were housed in a shed and allocated to 4 pens on the basis of weight and breed to ensure equal weights and breed representation in each group. After allocation to groups on 27/07/2017, three transition diets were fed to all animals. The first animals were sold after 83 days and the last animals to be sold were removed after 125 days on feed. Average duration of the feeding period was 114 days (SD = 10 days).

The diets, including the transition rations, are in Table 1 and were formulated to ensure that the two treatment groups received similar amounts of protein, meaning that the *Maxammon* diets received a lower allocation of pot ale syrup. All animals received the same allocation of minerals, Yea-Sacc (Alltech) and RumiTech (essential oils – Agolin). Group 1 and Group 2 (62 and 31 animals respectively) were fed a diet based on *Maxammon* treated barley harvested on-farm (15 kg urea and 5 kg *Maxammon*/tonne of barley treated). Groups 3 and 4 (both with 62 animals) were fed a diet based on barley harvested on-farm and preserved with propionic acid (*Prograin*). *Prograin* inclusion rates varied according to moisture content of the cereal, which ranged from 17.81 to 20.98% in this study. Hence, *Prograin* inclusion ranged according to the recommendations of the supplier from 6.5 to 7.5 litres/tonne. All dietary components were analysed by NIR.

Animals were weighed when housed, on the 8th week of the feeding period and when sent for slaughter. On 6 occasions, approximately every three weeks through the feeding period, 10 faecal pats were randomly selected (5 faecal pats for the small pen with 31 animals), generating a total of 95 samples from pens of cattle fed with *Maxammon* and 120 from the

animals fed on *Prograin*. Faeces was scored for diarrhoea from 1 to 5, 1 being very dry forming a pile of more than 50 mm high, and 5 being moist to liquid with blood or mucus. Samples were randomly selected: the researcher walked along two standard transects across each pen and selected the first 10 (or 5) faecal pats encountered on that transect.

Data were analysed using *R* (R Core Team, 2016). Effect of treatment on continuous response variables was tested using analysis of variance (with pen as the experimental unit) and categorical variables were tested by Chi square test of proportions. A threshold level of 1.2 kg/day for average daily liveweight gain was arbitrarily selected in consultation with the farmer and other local farmers on the basis that a value below this would be considered to be an unsatisfactory level of performance.

Table 1 Rations and nutritional composition of the diets fed to cattle on the trial

Component (kg/head as fed)	**Maxammon**				**Prograin**			
	27-31 Jul	1-5 Aug	6-14 Aug	Final diet	27-31 Jul	1-3 Aug	4-7 Aug	Final diet
Pot ale syrup	1.75	-	-	-	3.25	2.89	2.53	3.45
Molasses	-	0.9	0.87	0.96	-	-	-	-
Straw	1.83	2.19	0.86	0.58	1.81	1.08	0.72	0.50
Prograin Barley	-	-	-	-	5.06	6.14	7.59	11.33
Maxammon Barley	6.37	7.7	9.58	13.14	-	-	-	-
Grampian Beefmax Minerals + Rumitech +Yea-Sacc	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Total as fed (kg)	10.08	10.92	11.44	14.81	10.25	10.24	10.97	15.41
DMI (kg)	7.8	9.0	9.4	12.2	7.1	7.2	7.9	11.1
Nutrient (% of DM)								
Dry matter	77.2	82.7	82.4	82.4	69.7	70.4	71.9	71.9
CP	13.4	10.9	12.3	12.8	13.2	13.3	13	13.3
NDF	29.1	30.1	22.1	16.3	30.4	25.8	23.7	21.6
Oil	2.3	2.4	2.6	1.7	2	2.2	2.4	2.5
Starch	33.5	34.8	41.5	43.1	23.3	27.9	31.4	33.4
Sugar	1.7	3.9	3.8	3.5	2.8	3	3.1	3.2
Na	0.26	0.24	0.22	0.18	0.28	0.26	0.24	0.18
K	0.89	0.89	0.76	0.71	1.04	0.92	0.81	0.77
Ca	0.57	0.54	0.48	0.39	0.63	0.60	0.53	0.41
Mg	0.15	0.12	0.13	0.12	0.21	0.21	0.19	0.19
Cl	0.50	0.54	0.48	0.40	0.52	0.49	0.43	0.33
P	0.45	0.27	0.30	0.31	0.66	0.65	0.61	0.60
S	0.22	0.19	0.18	0.18	0.28	0.26	0.24	0.23

Results and discussion

Table 2 shows selected results from the study. Feed conversion ratio was higher in the *Maxammon* groups than in *Prograin* fed animals ($p < 0.05$). Proportion of animals with a daily liveweight gain lower than 1.2 kg/day was higher in *Prograin* fed animals ($p < 0.05$). Proportion of animals with diarrhoea (faecal score of 3 or 4 or 5) was higher in the *Prograin* fed group ($p < 0.05$).

Table 2 Average daily gain, liveweight, proportion of animals below 1.2 kg daily gain and proportion of animals with diarrhoea (score ≥ 3/5 and ≥4/5). The on-feed average daily liveweight gain (ADLWG) and final weight (LW) data were obtained for individual animals and presented here as mean values of the means of two pens subjected to each treatment. The mean feed conversion ratio (FCR) was derived from individual level live weights and pen level feed intakes and are presented as the pen means for each treatment. The *p* values presented here for ADLWG, LW and FCR are derived from *t* tests applied to pen means with Diet as the explanatory variable

Variable	***Prograin***	***Maxammon***	***p* value**
Performance measures	(n = 122 animals)	(n = 92 animals)	
Mean on-feed average daily liveweight gain (ADLWG) (kg/d ± sd)	1.65 ± 0.42	1.82 ± 0.29	0.25
Mean final weight (LW) (kg) ± sd	649 ± 57	665 ± 57	0.22
Mean feed conversion ratio (FCR) (kg DM feed/kg liveweight gain)	8.02 ± 2.0	7.13 ± 1.3	0.042
Count ADLWG ≤ 1.2 kg/d	14/122 (12%)	2/92 (2%)	0.01244
Faecal observations	(n=120 samples)	(n = 95 samples)	
Count with faeces ≥3	44/120 (37%)	17/95(18%)	0.00398
Count with faeces ≥4	21/120 (18%)	3/95(3%)	0.00195

Conclusions

The animals fed the diet based on *Maxammon* treated barley showed more efficient production performance than the animals on the *Prograin* diet. The *Maxammon* diet also led to a lower incidence of diarrhoea than the *Prograin* group and a smaller proportion of animals failing to meet the target specifications.

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New co-products from bio-energy processing as a potential new feed source evaluated for feed registration: research progress and update

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Introduction

Recently, relatively new non-human consumed industry oil seeds (yellow and brown Carinata) have been developed for use in the bio-fuel/bio-energy processing industry to produce jet/airplane fuel in Canada. This processing results in large amounts of new co-products from Carinata (*Brassica Carinata*) seeds which could be used as feed sources. However, these co-products are relatively new and are not yet in the Feed List in the Canadian Food Inspection Agency (CFIA) as registered feed sources. In order to be registered, detailed feed quality and animal nutritional information must be provided including performance by various types of animals. The objectives of this program are therefore: (1) to evaluate these new co-products from bio-fuel/bio-energy processing industry to assist registration in the CFIA, and (2) develop a strategy to efficiently utilize these co-products by developing pelleted products (BPP) for dairy cows.

P1: Chemical, nutrient, and digestion profiles of newly developed Carinata seeds in comparison with Canola seeds

This project has been published recently by our team (Ban et al., 2018). In this study, we investigated physiochemical and nutritional characteristics of newly developed yellow and brown Carinata seeds in comparison with yellow and brown canola seeds. The results showed that Carinata seeds contained high levels of crude protein (CP), non-protein nitrogen, lower fibre and lignin, higher glucosinolates and lower metabolizable energy contents at a production level, greater rumen undegraded protein (RUP), and total metabolizable protein than canola seeds. Feed milk value (FMV) of Carinata seeds was higher than canola seeds.

P2: Nutritional and metabolic characteristics of co-products (Carinata meal) from bio-fuel/bio-energy processing in dairy cows in comparison with conventional Canola meal

The detailed results of this study were published by Ban et al. (2017). In this study, we evaluated the nutritive value of Carinata presscake and meal for dairy cows in comparison with canola meal. Results showed that, compared to canola meal, glucosinolates were much higher in Carinata presscake (168.5 μmol/g) and meal (115.2 μmol/g) (3.4 μmol/g) (and that CP and soluble CP content in Carinata presscake and meal were higher in Carinata presscake and meal. The higher soluble CP proportion resulted in high rumen degradation for Carinata. Total metabolizable protein value for Carinata presscake and meal were lower than for canola meal. It was concluded that, based on the nutrient profile, rumen degradation and intestinal digestion, Carinata co-products can be used as an alternative feed protein source if the high levels of glucosinolates content in the co-products can be reduced (Ban et al., 2017).

P3: Development of blend pelleted products based on new co-products (Carinata meal) from bio-fuel processing, pulse screenings and lignosulphonate compounds in dairy

cows: potential N to energy synchronization, rumen degradation kinetics and intestinal digestibility

This study has been reported recently by Guevara Oquendo et al. (2018a). In this study, we developed and tested eight different blend pelleted products based on combinations of co-products from bio-fuel (Carinata meal) and bio-oil (canola meal) processing, pea screenings and lignosulphonate (feed additive) at different levels. Results showed that the Carinata meal based pelleted products contained higher rumen undegraded protein (RUP), lower effective degradation of CP, higher effective degradation of neutral detergent fiber , lower indigestible neutral detergent fibre at 288 h, higher intestinally absorbable feed protein and higher total digestible protein than canola based pelleted products. Through blending of co-products, we could more efficiently utilize the new co-product and optimize nutrient supply to dairy cows.

P4: Metabolic characteristics and feed milk value of BPP based on combination of new co-products from bio-fuel processing, pulse screenings and lignosulphonate in dairy cattle

This study has been completed recently and the manuscript has been submitted for publication (Guevara Oquendo et al., 2018b, unpublished). In this study, we modelled nutrient supply to dairy cows from the eight different blending pellet products that we developed in P3. The results showed that the Carinata based pelleted products contained high levels of truly digestible nutrients and had a high feed milk value (4.8 kg milk/kg DM feed). The dairy production performance and metabolic trials will be carried out in a near future.

P5 (ongoing): Milk production performance of newly developed blend pellet products (BPP) based on TMR in lactating dairy cows

The project is ongoing which include the following three phases (Aya Ismael, 2018):

In the 1st phase, we are studying, on a molecular basis, pelleting induced molecular structural changes in relation to nutrient utilization and availability in pellet products based on combinations of co-products from bio-fuel/bio-oil processing, low-grade peas and lignosulphonate at different levels for ruminants. In the 2nd phase, we will develop a feeding strategy to use the best pellet products for dairy cows, and in the 3rd phase, we will investigate possible associations between molecular structural features with nutrient availability ("feed value") and milk production performance ("feeding value") in lactation dairy cows when fed blend pellet products based TMR.

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References

Ismael, A., 2018. Study association of molecular structure spectral features with nutrient profiles and availability and milk production performance of newly developed blend pellet

products (BPP) and BPP based on TMR in lactation dairy cows. M.Sc. Project Proposal. University of Saskatchewan, Canada.

Ban, Y., Khan, N.A. & Yu, P., 2017a. Nutritional and metabolic characteristics of brassica Carinata coproducts from biofuel processing in dairy cows. J. Agric. Food Chem. 65, 5994-6001.

Ban, Y., Pratesa, L.L. & Yu, P., 2017b. Investigating molecular structures of bio-fuel and bio-oil seeds as predictors to estimate protein bioavailability for ruminants by advanced non-destructive vibrational molecular spectroscopy. J. Agric. Food Chem. 65, 9147-9157.

Ban, Y., Pratesa, L.L. & Yu, P., 2018. Biodegradation characteristics and nutrient availability of newly developed Carinata seeds in comparison with canola seeds in dairy cattle. Anim. Feed Sci. Technol. (In press, DOI: 10.1016/j.anifeedsci.2018.03.014).

Guevara-Oquendo, V.H., Christensen, D.A., McKinnon, J.J., Rahman, Md.M. & Yu, P., 2018a. Potential nitrogen to energy synchronization, rumen degradation kinetics, and intestinal digestibility of blend pelleted products of new co-products from bio-fuel processing, pulse screenings and lignosulfonate compound in dairy cows. Anim. Feed Sci. Technol. 236, 196–207.

Guevara-Oquendo, V.H., Christensen, D.A., McKinnon, J.J., Refat, B., Rahman, Md.M. & Yu, P., 2018b. Metabolic characteristics, truly digested nutrient supply, and feed milk value of blend pelleted products based on combination of co-products from bio-fuel/bio-oil processing, pea screenings and lignosulfonate at different levels for dairy cattle. Animal Feed Sci. Technol. (submitted).

Dry matter losses from different silo structures

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Introduction

Major research resources have been spent to improve the ensiling process. The ultimate objective is to accomplish a secure preservation of the ensiled material with limited changes in nutrients and a high hygienic standard of the end-product. However, the problem of silage heating up after opening and during unloading is evident on many farms. This problem leads to rapid deterioration of feed quality, large losses and sometimes situations where a considerable amount of silage must be discarded. Heating does not always occur, but takes place in a seemingly haphazard way on different farms and in different years. Presence of air in the silo is a prerequisite for heating and yeast starts the process in most cases (Wilkinson & Davies, 2013), which always leads to losses of organic matter and the production of carbon dioxide and heat. A set of experiments, funded by the Swedish Farmers´ Foundation for Agricultural Research, have been carried out in laboratory scale silos to study the effect of air ingress during fermentation (Spörndly & Persson, 2015a) and during unloading silos (Spörndly & Nylund, 2016) as well as the effect of yeast prevalence on crops at commercial farms (Spörndly & Persson, 2015b). Temperature measurements have also been performed in full-scale bunker silos to monitor ongoing processes during the ensiling and unloading phases (Spörndly & Nylund, 2016). The present study reports dry matter (DM) losses measured by silo balances and chemical analyses from different types of silo structures at commercial farms.

Materials and methods

Losses during ensiling, storing and unloading of mainly grass-clover crops were studied on 12 farms. During the study, a total of 12 bunker silos, 6 tube silos, 3 tower silos and 60 round bales in duplicate were monitored (Table 1). In-going green crops (mostly grass/clover leys) for ensiling in large silos were weighed by an axel weighing kit (Dini Argeo s.r.l, Italy) with four load cells with a limit of 10000 kg each and an accuracy (d-value) of 5 kg. Unloaded silage was weighed by the farmers using on-farm mixer wagon scales. Both the axel weighing kit and the mixer wagon balances were calibrated using a pair of beam load cells (Flintec SB5, Flintab AB, Sweden) of with a limit of 1020 kg each and an accuracy (combined error) of 0.5 kg. This Flintab scale was also used for weighing round bales before and after storage. Samples from in-going crops were taken from each wagon before the crop was loaded into the bunker, tube or tower silos. For round bales, samples of in-going material was taken from every second bale. At unloading the big silos, samples were taken three times per week and for round bales, the same bales sampled in the beginning were sampled at opening. All samples were frozen at -18ºC and dry matter was determined on each individual sample by drying for16 h in 60ºC and weighed hot. For silage samples, a correction for volatiles was applied, using the regression: 0.99 x g DM/kg +10 (Åkerlind et al, 2011). All DM analyses were made on the individual samples and DM losses were calculated by recalculating dry weight to corresponding quantity of wet weight. Analysis of ash, crude protein (CP), pH and metabolizable energy for ruminants (ME) was performed on 8-20 pooled samples per silo, depending on silo size. Ash was analysed by incineration at 550°C

for 5 h, CP as Kjeldahl-N x 6.25 determined with a Kjeltec 1030 (Foss A/S, Denmark) and ME was estimated from in vitro organic matter digestibility (Lindgren, 1979). For statistical calculations, the Mixed procedure and Pearson correlation procedure of the SAS package were used (SAS 9.4, SAS institute Inc., Cary, NC, USA) with silo type and farm as fixed independent variables.

Results and discussion

Silo balance for all measured silos are in Table 1. The DM content in the bunker silos was in average 33.0%, in the tube silos 30.8%, in the tower silos 26.9% and in the round bales 53.9%. Silo balances in fresh weight (kg out of the silos minus kg into the silos) were mainly negative. However, in three cases with bunker silos, silo balance were positive.

Table 1 Silo balances for bunker silos, tube silos, tower silos and round bales.

Farm	Green crop weighed in. kg fresh	DM.%	Silo balance, out - in, kg fresh	DM loss as discarded, %	DM loss total, %	DM loss excl. discarded, %	Total, ME loss, %	Total CP loss, %
				Bunker silos				
1	328000	21.5	-16467	1.6	3.1	1.5	3.2	3.1
2	442220	36.4	-36727	2.8	18.1	15.3	12.6	18.1
1	369120	35.6	-8495	0.7	3.4	2.7	4.5	3.4
1	160870	39.6	4950	1.3	7.8	6.5	9.5	7.8
2	488900	35.6	-22215	3.6	16.0	12.4	14.9	16.0
2	403720	39.6	15672	1.5	14.7	13.2	16.7	14.7
3	272720	23.7	-23104	0.9	7.7	6.8	13.8	7.7
4	892659	31/36	-28857	0.8	6.4	5.6	5.7	6.4
5	422680	33.6	-25264	1.4	21.7	20.2	23.4	21.7
6	1235830	24.0	-147166	1.8	15.0	13.2	18.6	15.0
7	119220	33.4	-5938	9.8	29.2	19.4		
7	165060	39.7	5218	14.8	26.2	11.4	23.9	16.5
	Bunker silos - mean			**3.4**	**14.1**	**10.7**	**13.3**	**11.9**
	Bunker silos - SD			**4.4**	**8.7**	**6.1**	**7.1**	**6.4**
				Tube silos				
8	492020	30.4	-55326	0.1	18.4	18.3	15.0	18.4
9	328460	28.6	10378	0.8	-2.1	-2.9	-2.5	-2.1
9	154360	29.8	4470	0.7	4.5	3.7	4.2	4.5
10	169080	40.0	-4673	9.6	8.5	-1.0		8.5
7	143720	32.4	-23898	0.0	21.7	21.7	22	22.8
7	313930	23.6	-83858	0.0	18.1	18.1		
	Tube silos - mean			**1.9**	**11.5**	**9.6**	**9.7**	**10.4**
	Tube silos - SD			**3.8**	**9.4**	**10.9**	**10.9**	**10.2**
				Tower silos				
11	394220	18.1	-128382	0.2	20.9	20.7	22.7	20.9
7	259470	33.9	-73718	0.0	24.3	24.3	22.3	24.2
7	286600	28.8	-93283	0.0	24.9	24.9	25.4	24.4
	Tower silos - mean			**0.1**	**23.4**	**23.3**	**23.5**	**23.2**
	Tower silos- SD			**0.1**	**2.2**	**2.3**	**1.7**	**2.0**
				Round bales				
12	21201	46.7	-139	0.0	1.4	1.4	0.6	1.4
7	29370	61.2	-240	0.0	0.8	0.8		
	Round bales - mean			**0.0**	**1.1**	**1.1**	**0.6**	**1.4**
	Round bales - SD			**0**	**0.4**	**0.4**		

This was interpreted as rainwater entering into the silo mass as DM contents of the silages in these silos also were lower compared to the green crop. Average precipitation during the storage time of 8 months (July – February) was 450 mm resulting in 144000 kg water falling on a bunker silo of 8 x 40 m. It is reasonable to believe that a portion of this rainwater entered the silo, if the surfaces were not perfectly tight and without a gentle slope to allow water to run off. All bunker silos were constructed with three walls. It is probable that all bunker silos were subjected to variable amounts of water leakage, indicated by average wet weight silo balance of only -3.1% compared to -14.1% for the DM balances (Table 1). The horizontal tube silo has a construction where rainwater does not enter easily. Hence, the difference between the wet weight and DM silo balance for tube silos was smaller, -8.5% compared to -11.5%. The tower silos in this study were filled with lower DM content of the fresh crop than recommended. Average fresh weight balance of tower silos was -31.2% compared to the DM silo balance of -23.4%, which indicates a substantial amount of effluent loss (not measured in this study).

Four farms participated with two silos of the same type during two different years and one farm had all four silo structures (Table 1). Therefore, a statistical analysis including also the effect of farm was possible. However, the interaction between the two could not be included in the model due to the low number of observations with different silo types on the same farm. Both the effect of silo structures and farm proved to be highly significant ($p<0.0002$) and the contrast between the silo structures are presented in Table 2.

Table 2 Contrast significance levels for DM losses of four silo structures using silo type and farm as fixed variables, both significant at $p<0.0002$

Silo type	Bunker silo	Tube silo	Tower silo	Round bales
Bunker silos	-	0.0159	0.2624	<0.0001
Tube silos	0.0159	-	0.1030	0.0003
Tower silos	0.2624	0.1030	-	<0.001
Round bales	<0.0001	0.0003	<0.001	-

Results showed that the round bales had considerably lower DM losses than all the larger silo constructions. The tube silos proved to have lower losses than bunker silos but DM losses in tower silos were not different from bunker or tube silos.

The marked difference between the large silo types and the small round bale silos suggest an effect of lower seal integrity and longer unloading time of the large-scale silos. Spörndly & Persson (2015b) showed that low intensity air ingression during storage in laboratory silos resulted in a drastically higher temperature immediately after opening the silos. They also showed that the low intensity air ingression during storage hardly influenced silage quality or losses at opening of the silos. Therefore, it is possible that the great difference between large and small silo constructions arise during the long unloading time of large silos in contrast to round bale silos that are emptied the same day they are opened. Spörndly & Nylund (2016) supported this hypothesis in laboratory scale silos where the DM losses were 6% at opening but 29% after a 63-day unloading period. The average unloading time of the bunker silos in the present study was 95 days, varying from 34 to 186 days but the correlation between unloading time and loss was only 0.37 (Table 3).

Spörndly & Nylund (2016) presented detailed results of temperature development inside five bunker silos used in the present study. They registered maximum silo temperatures from 26.5 to 44.5°C with mean temperature of 23.7°C. The elevated temperature were maintained also during the winter period when the ambient temperature was far below 0°C. However, no

correlation between silo temperature and total DM losses in these five silos could be observed (Table 3).

Table 3 Silo size, filling speed, unloading time, silage maximum temperature, pH at start and end of unloading time, DM loss calculated using the ash content in fresh crop and silage, and invisible DM loss (loss excluding discarded silage) using all-in/all-out method

Farm	Silo size	Filling speed,	Unloading time,	Silage max. temp.,	pH at un-loading,		DM loss, calculated from ash content,	Invisible DM loss,
		ton/h	days	℃	Start	End	%	%
				Bunker silos				
1	30 x 6 x 3 m	2.4	73	28.5	3.9	4.2	1.1	1.5
2	43 x 8 x 3 m	17.8	118	26.5	4.3	4.1	-8.2	15.3
1	30 x 6 x 3 m	2.2	84	35	4.3	4.2	5.3	2.7
1	20 x 5 x 3 m	2.0	70		4.5	4.4	5.4	6.5
2	43 x 8 x 3 m	8.2	45	31.5	4.2	4.2	2.4	12.4
2	43 x 8 x 3 m	6.8	115		4.7	4.5	-2.7	13.2
3	30 x 7 x 3 m	6.5	34		3.9	3.9	2.8	6.8
4	42 x 12 x 3 m	2.9	126	44.5	4.2	4.3	10.1	5.6
5	24 x 9 x 2.4 m	2.6	69		4	4.1	-5	20.2
6	36 x 10 x 4 m	10	186		4.2	4.6	2..7	13.2
Mean			92		4.2	4.3	1.4	9.7
SD			45.0		0.3	0.2	5.4	6
				Tube silos				
8	87 x 3.3 m		41		4	3.9	6.1	18.3
9	60 x 3.3 m		94		4	3.9	-7.4	-2.9
9	30 x 3.3 m		66		4.2	4	-1.1	3.7
10	50 x 2.7 m		83		3.8	3.9	-11.5	-1
Mean			71		4	3.9	-3.5	4.5
SD			23.1		0.2	0.1	7.7	10.6
				Tower silos				
11	450 m^3		232		4.2	3.8	-9.5	20.7
				Round bales				
12	1.8 m^3		1		4.4	5.3	-10	1.4

The aerobic microbial development during unloading, started by yeast, seems likely to be the main reason to the high losses for large silo constructions. A literature review by Borreani et al (2018) compiling six farm scale silo studies also stated that it is during the feed out phase that the greatest losses occur. Chen & Weinberg (2009) showed that the rise in temperature was well correlated with increasing DM losses when laboratory silos were opened. Spörndly and Persson (2015b) also found this in studies with laboratory scale silos where green crops from seven farms were ensiled in laboratory silos. They showed that time from silo opening to start of yeast development and temperature rise was shorter if the silos were not completely airtight during the 90-d storage period. This also leading to a better survival of yeast present in the green crop. If this is the case, substrate for the yeast growth, resulting in heat production, should be mainly in the form of easily degradable carbohydrates and could also be lactic acid, if lactate assimilating yeasts are active. This was shown by Ranjit & Kung (2000) in laboratory silos, who saw a marked increase in silage pH and increased DM losses a few days after silos were opened and exposed to air. Silage pH in silos in the present study was measured throughout the unloading time. In Table 3, pH at the beginning and end of the unloading time is shown, but no change in pH was evident.

Aerobic microbial growth, mainly from easy degradable organic matter with end-products in the form of carbon dioxide and heat, should theoretically lead to an increased content of ash in the silage. This has been suggested as a simple indicator of DM losses in farm scale silos (Ashbell & Weinberg, 1992). They suggested that DM losses could be calculated as DM loss (%) = 1-($ash_{fresh\ crop}/ash_{silage}$). When applying this equation to the ash contents obtained in this study (Table 3), no such correlation existed between this method and the all-in/all-out method ($r<0.1$). In eight silos, the ash content was lower in the silage and in eight, it was higher.

A carefully packing during filling of bunker silos achieves a high density (kg/m^3 or kg DM/kg^3) and is a way to prevent air leaking deep into the silo mass, which limits aerobic deterioration, heating and DM losses, particularly after opening the silo (Johnson et al, 2002; Holmes & Muck, 2007). An attempt was made to measure the silage density in the present study. This was successful only at some farms and data is, therefore, not presented. However, an indication of how well the crop was compacted at filling of the bunker silos is the time the filling lasted. Dividing total mass filled into the silo with total filling time gives an estimate of kg is filled per hour. We assumed a tractor for packing was running in the silo during the filling time, except during the nights. Resulting filling speeds are presented in Table 3 and the correlation with total DM losses was 0.55. However, the 10 bunker silos included one clear outlier (Farm 5, Table 3). By excluding this farm the correlation became 0.85 ($p<0.004$), as illustrated in Figure 1.

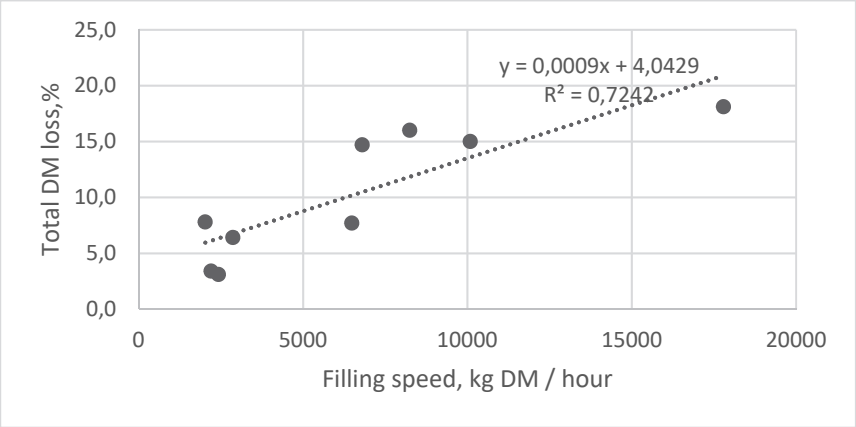

Figure 1 Total DM loss as a function of filling speed in 9 bunker silos. Hours are counted from start to end of filling, including night time hours.

Conclusions

The main difference between DM losses among silo types was a considerably lower loss in round bale silos (< 1% losses), compared to large bunker, tube and tower silos, which all averaged over 10% losses. The reason to the difference is suggested to be aerobic conditions during long unloading times of large silos. It was also suggested that the high seal integrity achievable with stretch film packages of round bales enables ensiling at high DM contents and also at moderate densities. Indirect methods for estimation of silage DM losses such as change in pre- and post-ensiling temperature did not prove useful.

A recommendation for managing bunker silos, drawn from this study, is to avoid filling the silo fast to enable a continuous compaction of the crop in thin layers. This can be done e.g. by filling two silos simultaneously instead of one after the other.

References

Ashbell, G. & Weinberg, Z.G. 1992. Top silage losses in horizontal silos. Can. J. Eng. 34, 171–175.

Borreani, G., Tabacco, E., Schmidt, R. & Muck, R., 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952-3979

Chen, Y. & Weinberg, Z., 2009. Changes during aerobic exposure or wheat silages. Animal Feed Sci. Technol. 154, 76-82.

Holmes, B. J., & Muck, R.E., 2007. Packing bunkers and piles to maximize forage preservation. In; Proc. 6th Int. Dairy Housing Conf. ASABE and Harvest and Storage.

Johnson, L. M., Harrison, J.H., Davidson, D., Mahanna, W.C., Shinners, K. & Linder, D. , 2002. Corn silage management: Effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. J. Dairy Sci. 85, 434–444.

Lindgren, E. 1979. The nutritional value of roughages determined in vivo and by laboratory methods. Swedish University of Agricultural Sciences. Departm. Animal Nutr. Managem. Uppsala. Report no. 45, 63 pp. (in Swedish).

Ranjit, N. K. & Kung Jr., L., 2000. The effect of Lactobacillus buchneri. L. plantarum. or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83, 526–535.

Spörndly, R. & Nylund, R. 2016. Temperature development and dry matter losses of grass silage in bunker silos. In: Udén et al (eds) Proceedings of the 7th Nordic Feed Sci. Conf., Uppsala, Sweden. Departm. Animal Nutr. Managem. Report 293. p. 41-46.

Spörndly, R., & Persson, A. 2015a. The effect on silage quality of air ingression during fermentation in experimental silos. In: Udén et al (eds) Proc. 6th Nordic Feed Sci. Conf., Uppsala. Sweden. Department of Animal Nutrition and Management. Report 291, p 60-65.

Spörndly, R. & Persson, A. 2015b. Yeast in fresh crop and silage from 15 Swedish farms and its impact on silage aerobic stability. . In: Udén et al (eds) Proc. 6th Nordic Feed Sci. Conf., Uppsala, Sweden. Departm. Animal Nutr. Managem. Report 291, p 66-70.

Wilkinson, J. M., & Davies, D.R. 2013. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1–19.

Åkerlind, M., Weisbjerg, M, Eriksson, T., Tørgersen, Udén, P., Harstad, O.M. & Volden, H., 2011. Feed analyses and digestion methods. In: Volden, H. (ed) NorFor – The Nordic Feed Evaluation System. EAAP Publication No 130. Wageningen Acad. Publ., the Netherlands.

Preservation of black soldier fly larvae (BSFL) by anaerobic fermentation

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Introduction

Considering that an indirect production of human food in form of meat and dairy products is less efficient than direct food production from agricultural lands (Foley et al., 2011) and that, it is unlikely that human consumption of animal-derived food will reduce in the future, it is necessary to find more sustainable solutions for feeding farm animals.

According to van Huis et al. (2013), insect rearing could be one way to enhance food and feed security considering that insect larvae can feed on waste biomass, including fruits and vegetable peels, food wastes, sewage, manure, slurry, etc. transforming it into high value food and feed resources (Čičková et al., 2015). One kg of insect larvae biomass can be produced from on average 2 kg of feed biomass (Makkar et al, 2014). Black soldier fly (BSF) larvae (Hermetia illucens) have been specifically pointed out as a candidate for replacing fish and soybean meals in animal feeds due to its favourable nutritional properties (Makkar et al., 2014).

The most common way of storage and processing of fresh larval mass is by drying. This process is, however, energy demanding and therefore, other alternatives have been considered. One of them is preservation in anaerobic condition by bacterial fermentation. Studies with fermented insect larvae are scare. Rangacharyulu et al. (2003) studied the inclusion of fermented silkworm pupae in diets for carps, as a substitute for fishmeal, and reported that under conditions of their study, ensiled pupae was nutritionally superior to untreated pupae or fishmeal. The objective of our study was to investigate the possibility to preserve and store black soldier fly larvae by anaerobic fermentation with regard to yields of protein meal and fat.

Materials and methods

Pre-pupae larvae of BSF, reared on food waste, were provided by the Department of Energy and Technology of SLU and ensiling was done at the Department of Animal Nutrition and Management of SLU. The larvae were euthanized by freezing at -18˚C for 20 min before being gently crushed by rollers. Glass tubes (100 ml) equipped with water-locks were used as silos. Treatments in triplicate included: (i) addition of NaCl at 15% of fresh larval weight, (ii) addition of sugar (a mixture of glucose, fructose, and sucrose) at 3.75% of fresh larval weight, (iii) Promyr NT570 (30-40% formic acid, <25% propionic acid and <20% sodium formate) at 18 ml per kg of fresh larval weight and (iv) control (no additive). Silos were stored at room temperature (20-22˚C) for 98 days and weighed at 0, 2, 7, 14, 21, 28, 50, and 98 d to determine weight losses. Weight losses were assumed to be the result of carbon dioxide production.

A representative sub-sample of euthanized larvae was dried at 60˚C until constant weight, ground in a coffee grinder and analysed for crude protein (CP; Kjelldahl method; Nordic Committee on Food Analysis, 1976), crude fibre (CF; Jennische & Larsson, 1990), fat (EE, according to O.J.E.C., 1998) and ash (by incineration at 550˚C during 3 h). Nitrogen free extract was calculated as NFE=100 - CP - CF - EE - ash. Chemical composition of ensiled

larvae was measured in a similar manner. Upon silo opening (98 days), silage pH was measured in the liquid phase of the silage by a pH meter. Mean weight of fresh larvae, calculated after weighing five groups of 50 larvae with a precision scale, was 198 mg (± 11). Data on chemical composition of larvae before and after ensiling are in Table 1. Fresh larval samples (30 g) were used for enumeration of lactic-acid bacteria (LAB), yeasts and moulds, clostridial spores and enterobacteria as described by Mogodiniyai Kasmaei et al. (2015).

Results and discussion

Chemical composition of the fresh BSF larvae compares well to data published in the literature with protein and fat contents over 30% on DM basis (Table 1). Packing density of larvae in the silos was 271 kg DM m^{-3}. Microbial analyses of fresh larvae revealed a high and diverse microbial composition with high counts of enterobacteria (5.94 log cfu/g), LAB (5.49 log cfu/g), clostridia (5.24 log cfu/g) and yeasts (5.04 log cfu/g).

Table 1. Chemical composition of black soldier fly larvae (BSF, Hermetia illucens) before and after 98 days of ensiling (n=3) with different additives (control, salt, sugar and acid)

	DM	CP	CF	EE	NFE	Ash
Before ensiling	35.8	39.4	5.2	35.7	10.3	9.4
Ensiled larvae						
Solid phase						
Control	n.a.	32.2	5.9	40.7	11.1	10.2
Salt	n.a.	28.7	4.1	25.8	10.9	30.4
Sugar	n.a.	30.9	6.1	41.4	12.2	9.4
Acid	n.a.	39.7	6.0	39.0	6.3	9.1
Liquid phase	**pH**					
Control	6.8	12.7	n.a.	-	n.a.	n.a.
Salt	5.3	6.7	n.a.	-	n.a.	n.a.
Sugar	5.7	13.8	n.a.	-	n.a.	n.a.
Acid	5.6	14.1	n.a.	-	n.a	n.a.

DM: dry matter % in fresh basis; CP: crude protein; CF: crude fibre; EE: ether extract; NFE: nitrogen free extract; ash (% DM basis) and n.a.: not analysed.

Chemical composition of the solid phase of the ensiled larvae resembled that of the pre-ensiled larvae with the exception that salt treated biomass had lower values for CP and EE due to the dilution effect of the added salt (15 % on fresh basis). The added salt also explains the high value of ash content in this treatment. The proportion of protein found in the liquid phase, again with the exception for the salt treatment, seems to be similar among treatments. No fat was detected in the liquid phase.

Fermentation in all the treatments was very intensive resulting in excessive volume changes within the tubes causing liquid effluents to being forced upwards into the gas locks. These effluents were collected and weighed. The highest average formation of effluent was observed in acid treated larvae (12.4 g), followed by control (6.9 g), whereas salt and sugar treatments formed the least (3.1 g and 3.3 g, respectively). Quality of fermentation is often reflected in fermentation losses (CO2). A good fermentation process carried out by lactic acid bacteria (LAB) normally results in low fermentation losses (3 to 4%) whereas fermentation losses in undesirable clostridia dominated processes can be considerably higher (McDonald

et al., 2001). The high losses in our study (Figure 1) suggests that fermentation was dominated by undesirable microfloras even in additive treated samples. Such losses are unacceptable in a conventional forage silage and are a result of a mal-fermentation. The high pH (Table 1) and odorous smell of the silages provide further evidence of undesirable fermentation of the ensiled larval biomass. Signs of undesirable fermentation were observed even in the salt treatment where DM losses were the lowest (10% DM loss). Further studies are required to investigate the possibilities to obtain a silage with an acceptable fermentation quality. It is likely that direct acidification with sulphuric acid (Jackson et al., 1984) would have been a more reliable method of preservation than by lactic acid fermentation. The latter was clearly not successful in terms of hygienic quality and losses.

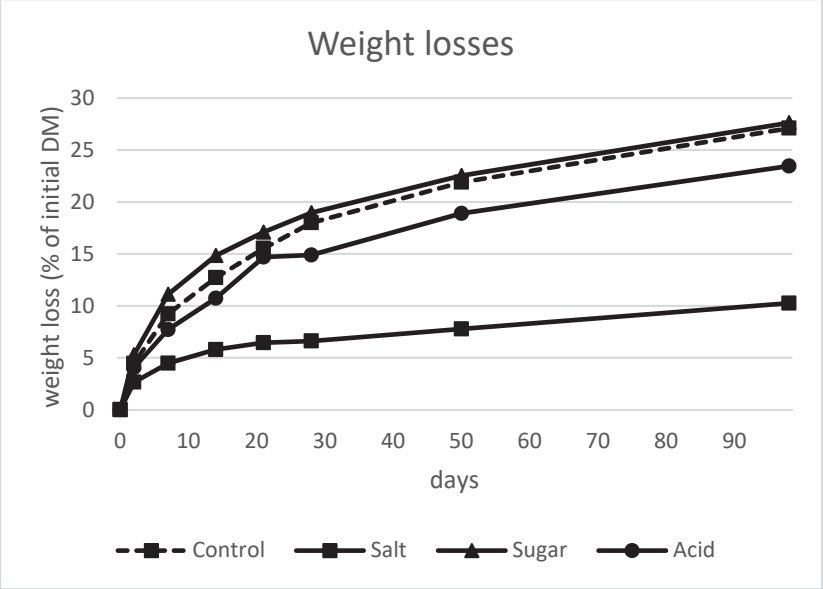

Figure 1. Dry matter (DM) losses in black soldier fly larval (*Hermetia illucens*) silages estimated from fresh weight losses during a 98 d conservation period. Salt=addition of NaCl at 15% of fresh larval weight, sugar=addition of sugar at 3.75% of fresh larval weight, acid=addition of organic acid (a mixture of formic acid, propionic acid and sodium formate) at 18 ml per kg of fresh larval weight.

Conclusions

High counts of enterobacteria, LAB, clostridia and yeasts were found in the fresh larvae. Chemical composition of the solid silage phase was similar to that of the pre-ensiled larvae when salt dilution was taken into account. The fermentation weight losses at 98 d were 10 to 28% and unacceptable. Hygienic quality was also poor as the silage pH was high accompanied by bad smell, probably as a result of clostridial fermentation.

References

Čičková, H., Newton, G.L., Lacy, R.C. & Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Management 35, 68-80.

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockstro, J., Sheehan, J., Siebert, S., Tilman, D. & Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature 478, 337-342.

Jennische, P. & Larsson, K., 1990. SLL rapport nr 60. Traditionella svenska analysmetoder för foder och växtmaterial. Uppsala, Sweden.

Makkar, H.P., Tran, G., Heuzé, V. & Ankers, P., 2014. State of the art on use of insects as animal feed. Anim. Feed Sci. and Technology. 197, 1–33.
McDonald, P., Henderson, A. R. & Heron, S. J. E., 1991. The Biochemistry of Silage. Chalcombe Publications, Marlow, Bucks, UK.
Mogodiniyai Kasmaei, K., Passoth, V., Spörndly, R. & Udén, P., 2015. A new sterilization and inoculation method in silage research. Grass and Forage Sci. 70, 668–673.
Rangacharyulu, P.V., Giri, S.S., Paul, B.N., Yashoda, K.P., Jagannatha Rao, R., Mahendrakar, N.S., Mohanty, S.N. & Mukhopadhyay, P.K., 2003. Utilization of fermented silworm pupae silage in feed for carps. Bioresource Technology 86, 29-32.
van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. & Vantomme, P., 2013. Edible insects: future prospects for food and feed security. Forestry Paper FAO 2013:171. Rome, Italy.
http://www.fao.org/docrep/018/i3253e/i3253e.pdf [2015-02-25].

Rumen *in vitro* total gas production of timothy, red clover and the mixed silage after extrusion

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Introduction

Forage from temporal leys is the major feed for Swedish dairy cows. Due to limited forage intake capacity, the energy demand for high yielding dairy cows cannot be supplied with forage only (Veerkamp 1998). Concentrate feeds, mostly grain in Sweden, are needed to supply the cow with enough energy and protein.

Extensive particle size reduction has been shown to increase forage intake and rumen passage rate considerably in sheep (Lárusson & Sveinbjörnsson, 2016). There are indications of particle size effects on intake also in dairy cows (Nasrollahi *et al.*, 2015). Processing beyond the commonly used precision chopping technique for harvesting silage may be required to achieve the full potential for affecting intake and digestion in cattle. Extruders represent an extensive processing technique that is used in biogas plants for fibrous material, including grass silage (Rodriguez *et al.*, 2017). It is a mechanical process where screws mix and shear the material causing particle size reduction and plant cells to break open. The result is depolymerisation and decrystallisation of fiber constituents which increase access for bacteria and enzymes and cause improved fermentation in biogas reactors (Hjorth *et al.*, 2011).

The objective of the pilot study presented here was to see if there were effects on gas production *in vitro* by extruding silage before incubation.

Materials and methods

Crops and extrusion

Timothy and red clover were cut manually on June 13, 2015, wilted to 35 – 37% DM, chopped at 20-30 mm length and ensiled in air-tight glass jars for 90 days. After glass jar emptying, silage was stored frozen at -20°C until extrusion.

A mixed ley dominated by timothy and red clover was also harvested in June 2017, wilted to 31% DM, precision chopped and ensiled in a bunker silo for ca 200 days before being extruded.

Extrusion was performed on a Bio-Extruder MSZ-B15e (Lehmann Maschinenbau, Germany) set at 50% rotation speed as the default treatment and with 20% rotation speed as an additional treatment for the mixed bunker silage. The extruded silage, as well as the control samples of the untreated material was then stored frozen in -20°C until in vitro incubation.

In vitro incubation

Two *in vitro* incubation batches were run. Incubations were performed in a Gas Endeavour system (Bioprocess Control, Lund, Sweden) with 15 bottles of 500 mL capacity. Each batch included duplicates of control samples and extruded silage of timothy, red clover and mixed bunker silage, respectively (totally 12 bottles). In addition, each batch included duplicates of the mixed silage extruded at a lower rotation speed (20%) and a single blank bottle.

Before incubation, approximately 4 g DM of forage sample was weighed into each bottle, whereafter bottles were placed in a waterbath at 38°C and gassed with CO_2. Rumen fluid was obtained from dry cows at maintenance feeding level, strained through a screen with approx. 1-mm openings and mixed with buffer (Goering & Van Soest, 1970) in proportions (1:4). The buffer:rumen fluid mixture was pre-incubated at 38°C for approx. 1 h, before incubation of samples was initiated by dispensing 300 mL of the mixture into the bottles. Gas volumes were recorded continuously, while the value from 1 h and, thereafter, from each 6th h up to 72 h were used in the analysis.

Data analysis

Data was analyzed by SAS 9.4 with a MIXED model that included the effect of batch, extrusion, crop, time and the interaction between them. Bottle within batch was the repeated subject. Results are presented as least square means with standard error of the mean and probability for treatment effect at time points.

Results and discussion

Extrusion resulted in larger gas production across all three silage types (Fig. 1), the numerical difference being 15 mL at 12 h and 30 mL (18%) at 72 h. This is similar to findings from biogas experiments, where these effects were ascribed a larger surface area from particle size reduction (Weiss and Bruckner, 2008). As reviewed by Rodriguez *et al.* (2017), methane production from grass in a 28 d biogas reactor fermentation increased by 62% after extrusion. When fermentation continued for 90 d, the difference levelled out so that methane production was 9% larger from extruded grass compared to the unextruded material.

When different extrusion intensities were compared (Fig. 2), gas volume ranged according to intensity. Rodriguez *et al.* (2017) also reported that methane production potential from ryegrass was proportional to extrusion intensity in terms of opening size of the extruder.

Possible positive i*n vivo* effects from particle size reduction chould be mediated by a) increased passage rate allowing an increased intake and b) increased fermentation rate balancing a reduced digestibility from increased passage rate. Methane production in biogas reactors is a process different from rumen fermentation with a conversion of volatile fatty acids to methane requiring considerably longer time than rumen fermentation. However, present results suggest an increased availability of extruded silage for rumen microbes that should be of relevance at typical rumen retention times.

Conclusions

The results from this pilot study suggests that rumen fermentability of precision chopped grass and clover silage is increased by extrusion of the silage and that the effect is proportional to intensity as defined by extruder rotation speed.

References

Goering, H.K. & Van Soest, P.J. 1970. Forage fiber analyses. (Apparatus, reagents, procedures, and some applications.). Agriculture Handbook 379, United States Department of Agriculture.

Hjorth, M., Gränitz, K., Adamsen, A.P. & Møller, H.B. 2011. Extrusion as a pretreatment to increase biogas production. Biores. Technol., 102:4989-4994.

Lárusson, T. & Sveinbjörnsson, J. 2016. The effect of pelleting hay upon feed intake, digestibility, growth rate and energy utilization of Icelandic lambs. 7th Nordic Feed Science Conference, Uppsala, 14-15 of June 2016. SLU/HUV Rapport 293, p 67-72.
Nasrollahi, S.M., Imani, M. & Zebeli, Q., 2015. A meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. J. Dairy Sci. 98, 8926–8939.
Veerkamp, R F. Selection for economic efficiency of dairy cattle using information on live weight and feed intake: A Review. J. Dairy Sci., 1998, 81, 1109-1119.

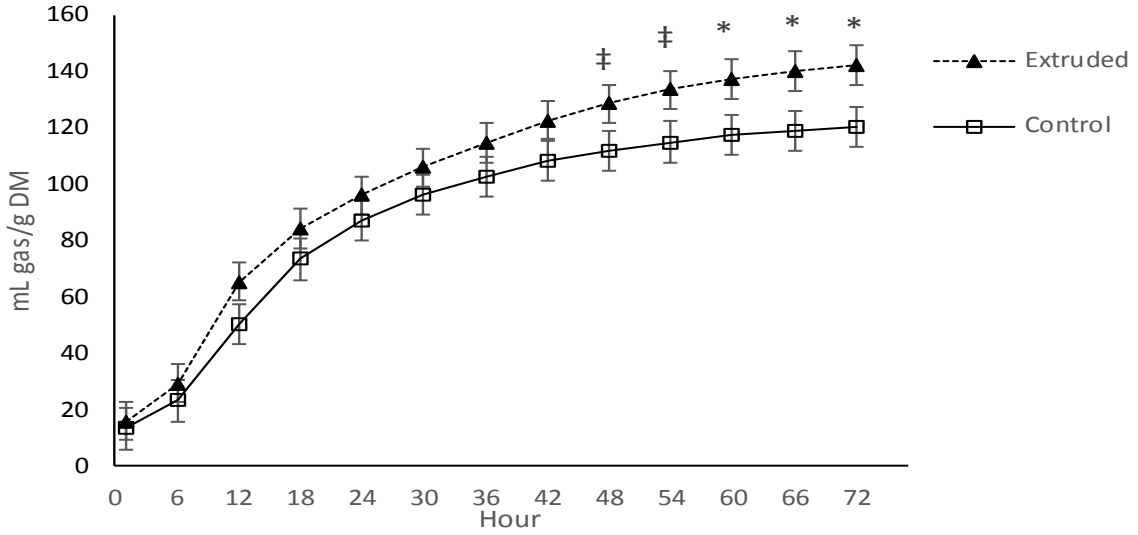

Figure 1 Overall effect of extrusion across three silage types (red clover, timothy and mixed grass clover bunker silages) on *in vitro* gas formation. Bars indicate standard error of the mean. ‡= $P \leq 0.10$; * = $P \leq 0.05$.

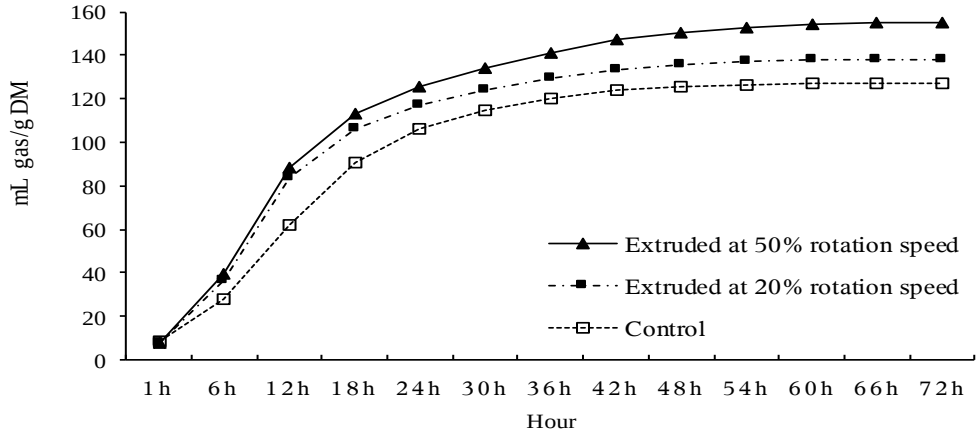

Figure 2 *In vitro* gas production from a mixed grass clover silage extruded at two intensities. Control is precision chopped at harvest.

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