Potential of fibrolytic enzymes in ensiling grass for a biorefinery process

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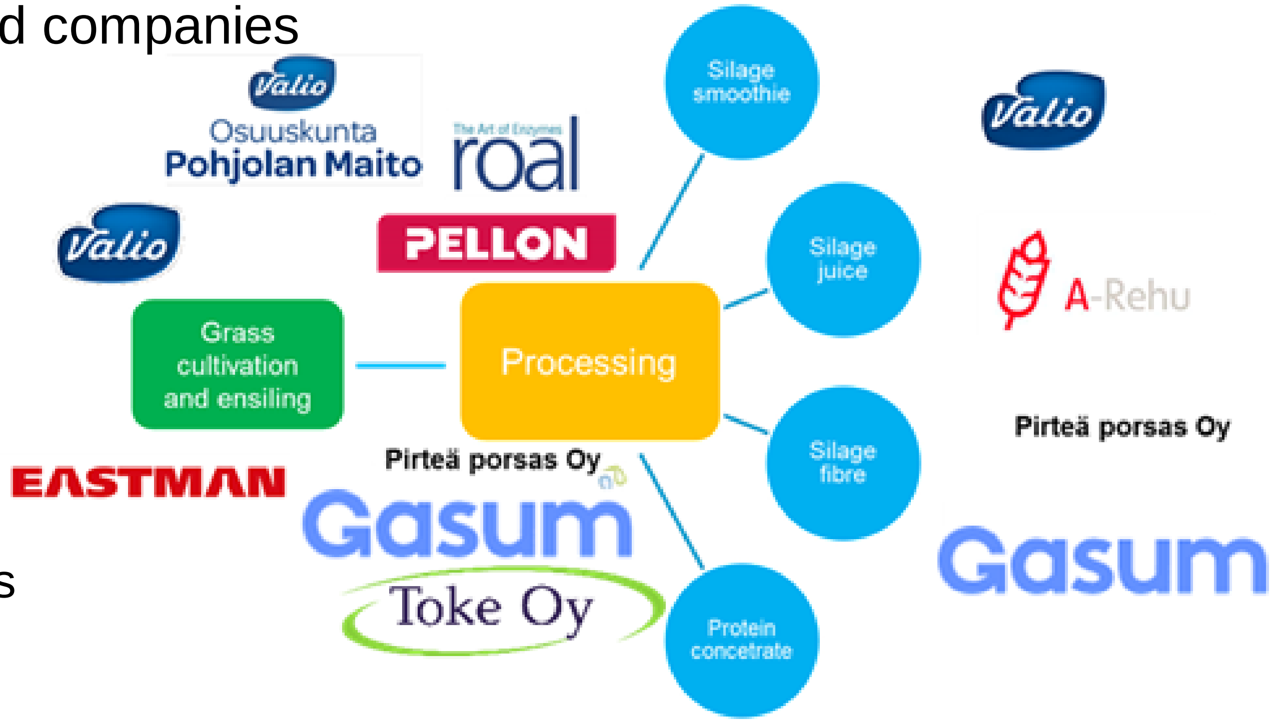
This trial is part of Innofeed -project
Biorefining ensiled grass into inventive feed products

- Developing and testing methods to process grass silage into novel feeds suitable for monogastrics
- Targets: improve protein self sufficiency, profitability and sustainability of agricultural production in Finland
- Project is carried out 2015-2018 by research organizations VTT and Luke
- Funded by TEKES and companies
  - A-Rehu
  - Gasum
  - Pohjolan Maito
  - Pellon
  - Pirteä Porsas
  - Roal
  - Eastman
  - Toholammin Kehitys
  - Valio



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Surplus grass biomass as raw material for green biorefineries

- Grass grows well in humid temperate areas with a capacity for high biomass production compared to annual crops
- Existing technology is available for its cultivation, harvesting and ensiling
- Due to its low lignin content, it is easier to process than wood or straw
- Offers a versatile raw material for feed and other purposes
- When preserved as silage, grass biomass can be refined all year round

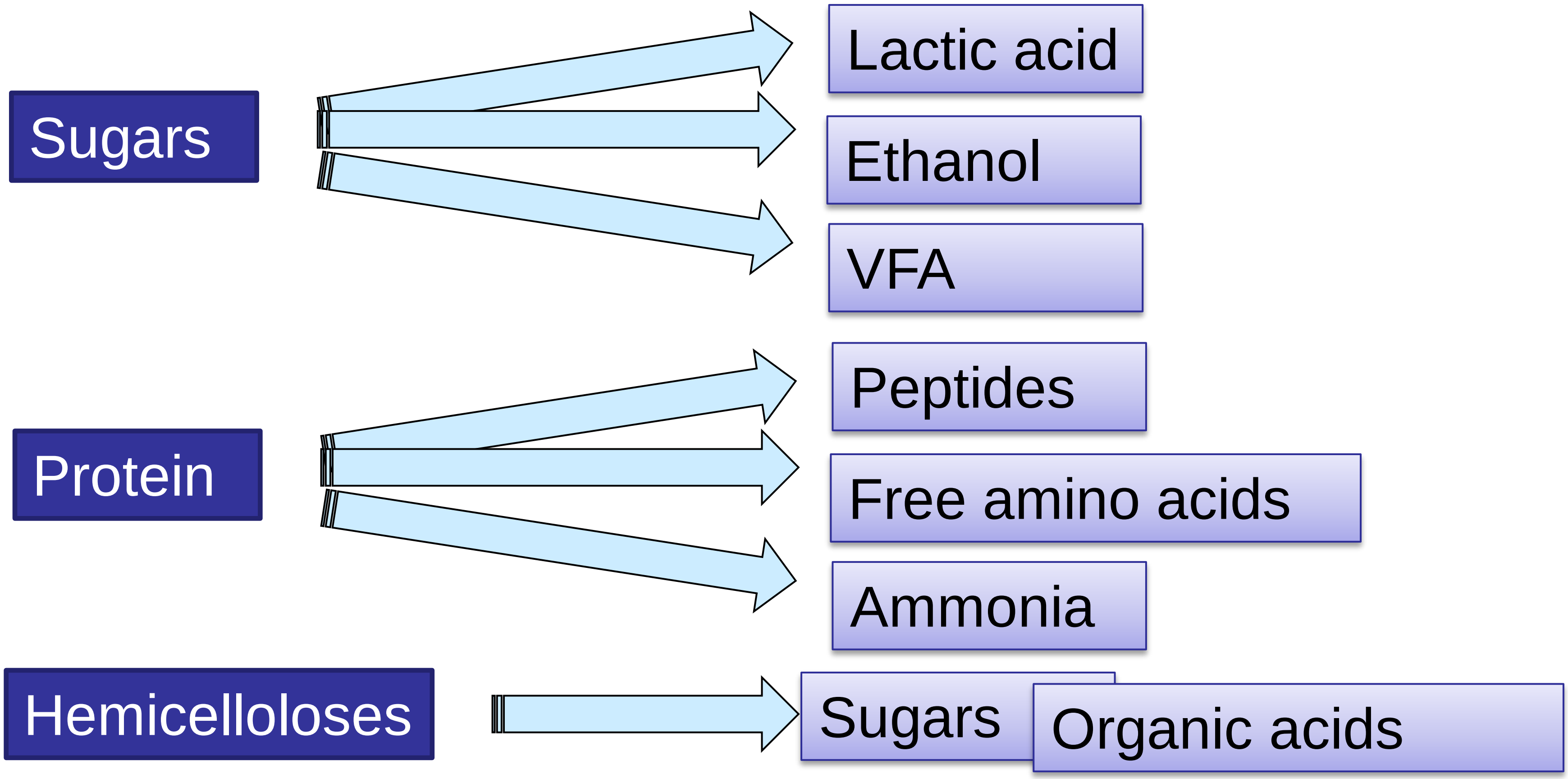
Grass potential in Finland

Potential to increase production from current level:

- Increase production level on current grass fields
- Increase fields under intensive grass production (e.g. from fallow areas, peat lands)
- traditional usage of grass is not increasing - surplus grass available

Photo: ©Luke Marketta Rinne

Some changes in chemical composition during ensiling



The extent of changes can be manipulated by prewilting, proper ensiling process and by silage additives

Separating silage juice from fibre has been suggested as the first step of silage processing

- In this study, we wanted to evaluate if fibrolytic enzyme application prior to ensiling could be used as a pretreatment for a biorefinery process to improve the press-juice yield as well as content of soluble nutrients in press-juice.

- The ultimate aim of the processing was to create suitable grass based feed for monogastrics

Photo: ©Luke /Niina Pitkänen

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Material and methods

Timothy meadow fescue swards

- Grown at Jokioinen for dairy cattle
  - First regrowth (**RG1**) grass was harvested on 4 August
  - Second regrowth (**RG2**) was harvested on 11 September
- Typical farm scale harvesting: mower conditioner, prewilting, precision chopper, a formic acid based additive was applied
- After harvesting grass was transported to laboratory for further treatments

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Enzyme treatment added in laboratory

- Flashzyme Plus (kindly provided by Roal Ltd., Rajamäki, Finland) with cellulase and hemicellulase activities was used

- The grass was divided into 4 batches, which received the following enzyme applications (mL enzyme solution per kg grass DM):
  - Low, 0.10
  - Medium, 0.50
  - High, 2.50
- In addition, a Control treatment without enzyme addition was prepared

Ensiling

Treated grass was ensiled in 12 L silos

- RG1: two replicate silos per treatment
- RG2: three replicate silos per treatment

The silos were stored in room temperature and opened after an ensiling period 471days for RG1 and 433 days for RG2.

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Juice extraction from silages

- Juice extraction was performed with an in-house mechanical compressor.
- Silage samples were packed into mesh bags, pressed for 2 min and the press-juice was weighed.

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Chemical composition of the grass prior to ensiling

	First regrowth (RG1)	Second regrowth (RG2)
Date of harvest in 2014	4 August	11 September
Dry matter (DM), g/kg	296	241
In DM, g/kg		
Ash	93	105
Crude protein	131	121
Water soluble carbohydrates	103	132
Neutral detergent fibre (NDF)	523	533
Indigestible NDF	77	64
In vitro OMD[1)]	0.729	0.742

Fermentation quality of the silages, RG1

	Enzyme level				SEM1)	Statistical significance2)		
	Control	Low	Medium	High		L	Q	C
Dry matter (DM), g/kg	284	278	274	272	0.2	↘ **	NS	NS
pH	4.44	4.34	4.25	4.13	0.024	↘ ***	NS	NS
In dry matter, g/kg								
Crude protein,	146	148	151	154	0.7	↗ ***	NS	NS
Neutral detergent fibre	516	508	470	413	4.2	↘ ***	**	NS
Water sol. carbohydrates	24	27	31	34	1.1	↗ **	NS	NS
Ethanol	30	28	42	45	2.9	↗ **	NS	NS
Lactic acid	44	53	60	80	3.3	↗ ***	NS	NS
Acetic acid	19	21	21	27	0.5	↗ ***	*	*
Ammonium N, g/kg total N	55	54	49	43	2.1	↘ *	NS	NS

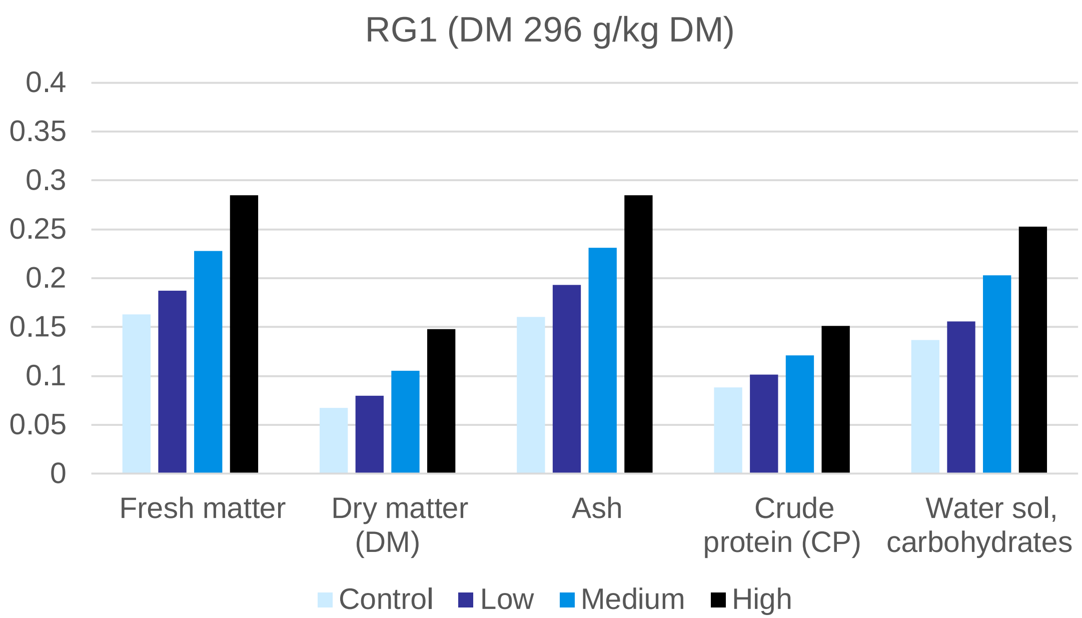
formic acid content of RG1 was 0.047 g/kg fresh matter, good application level

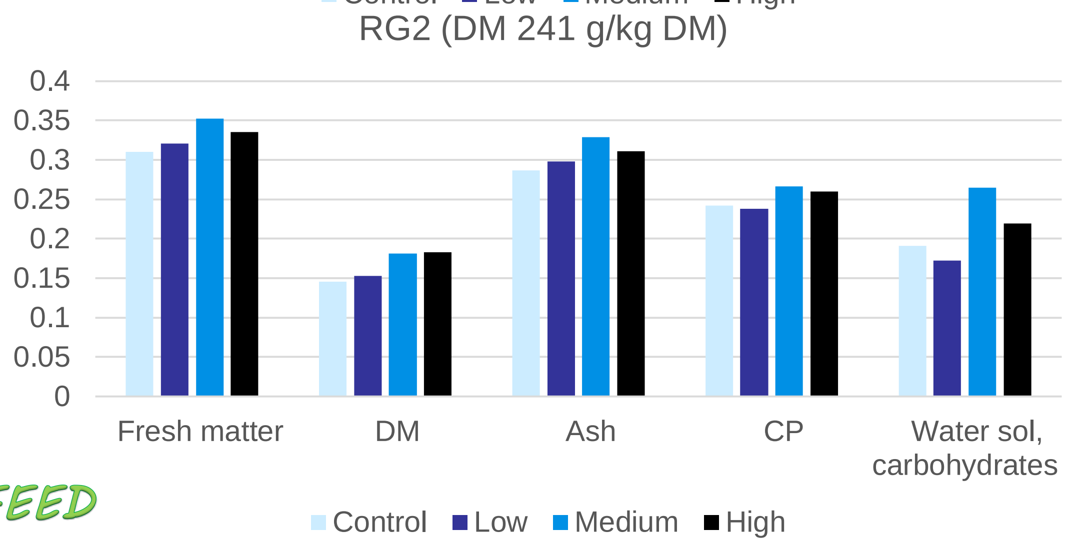
Fermentation quality of the silages, RG2

	Enzyme level				SEM[1)]	Statistical significance[2)]		
	Control	Low	Medium	High		L	Q	C
DM, g/kg	233	232	228	234	0.2	NS	NS	NS
pH	4.09	4.06	4.03	3.96	0.021	**	NS	NS
In dry matter, g/kg								
Crude protein	130	131	136	133	1.5	*	NS	NS
Neutral detergent fibre	509	493	465	447	3.6	***	NS	NS
Water sol. carbohydrates	22	19	25	25	4.1	NS	NS	NS
Ethanol	9	10	16	16	1.3	**	NS	NS
Lactic acid	103	102	117	124	3.5	***	NS	NS
Acetic acid	21	23	23	27	1.2	***	NS	NS
Ammonium N, g/kg total N	91	76	66	68	3.2	***	*	NS

formic acid content for RG2 it was only 0.016 g/kg FM (too low dosage)

Extraction results





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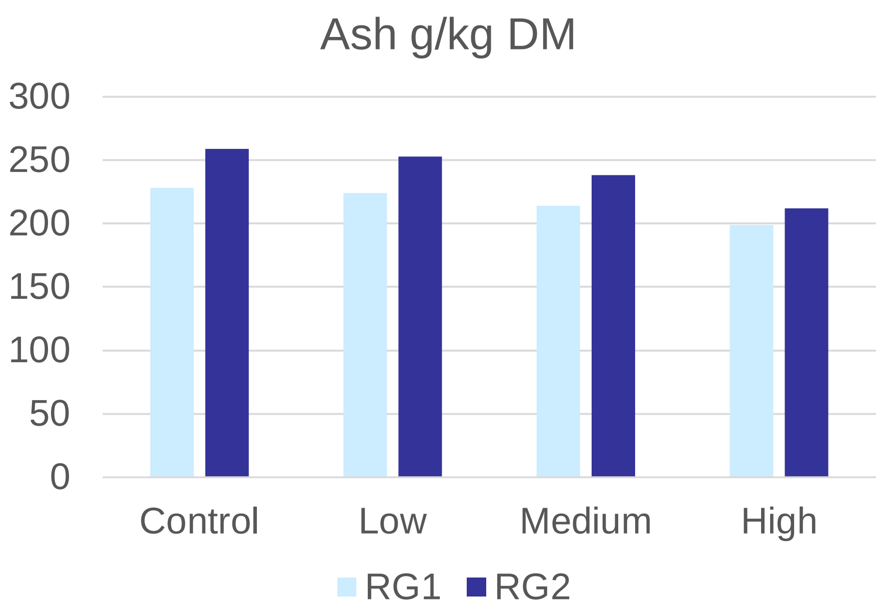
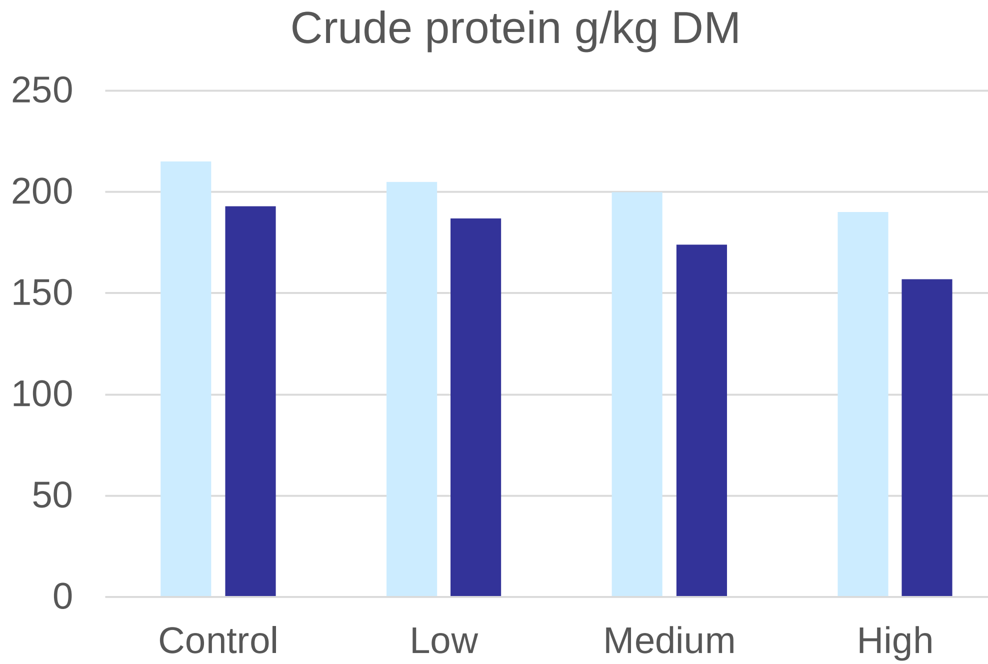
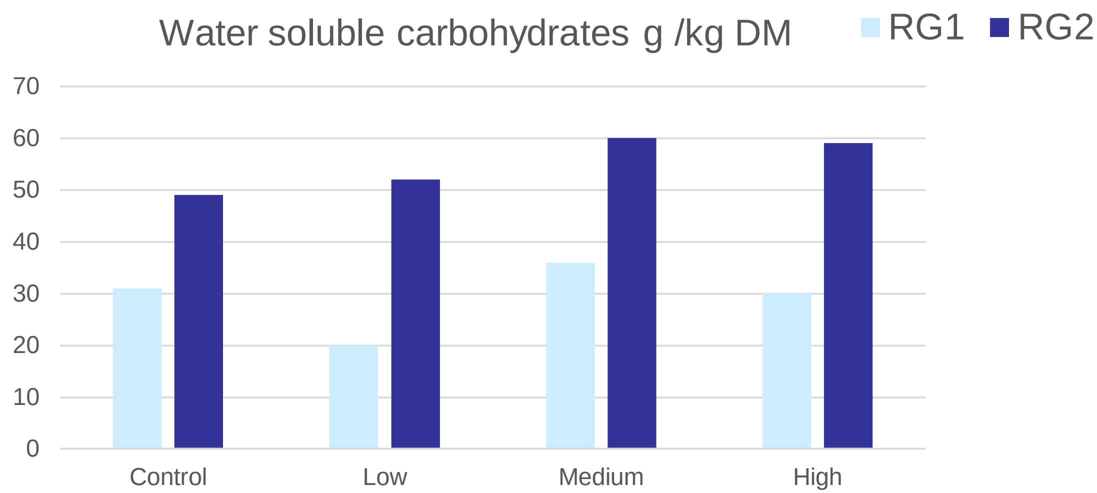
In press juice







Extraction efficiency

- The method of press-juice extraction used in the current experiment was rather inefficient as the proportion of FM extracted was on average 0.273.
- The efficacy can be much higher if e.g. screw type extraction is used.

Practical aspects related to on-farm liquid feed production

- Using the press-juice directly at the site of production, i.e. on-farm, either as part of total mixed ration for cattle or part of liquid feed for pigs, would minimize the costs of transportation.
- Lactic acid and volatile fatty acid concentrations increased with increasing enzyme application.
- Higher concentration of fermentation acids in the press juice could be considered a positive factor if used as a liquid pig feed, since organic acids are commonly used as feed additives to stabilize the feed and improve intestinal conditions.

Examples of juice extraction efficiences with different types of screws, silage DM 25 %

Device	Extracted juice, % of the silage mass
Angel Juicer	58.0
Mechanical compressor in Luke	28.8

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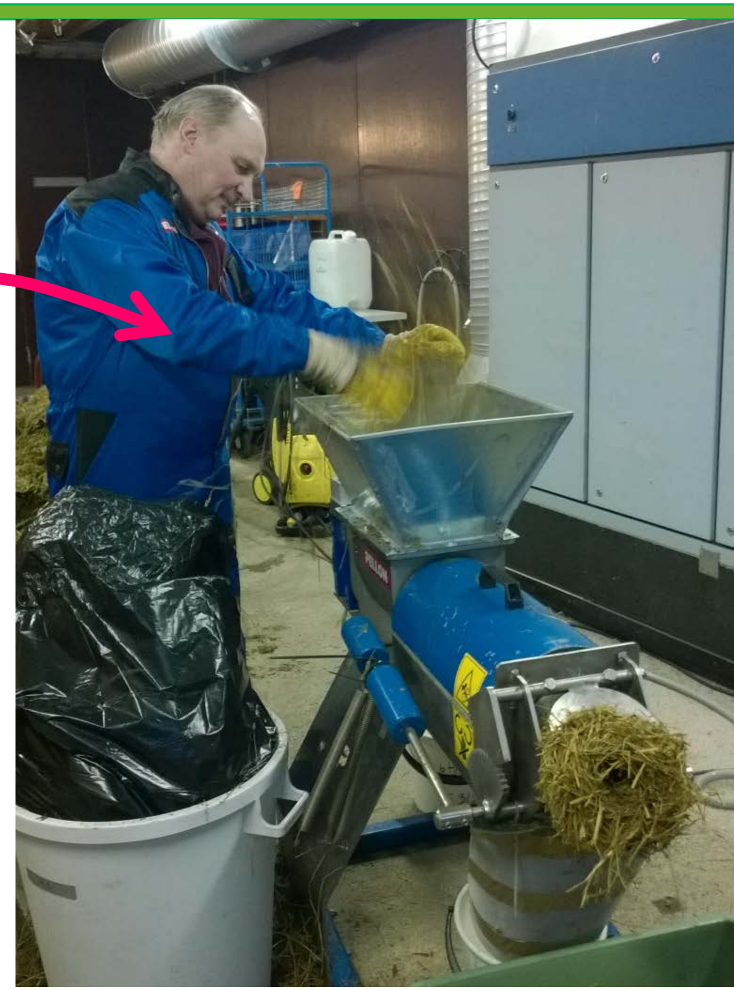
Examples of juice extraction efficiences with different types of scruves, silage DM 25 %

Device	Extracted juice, % of the silage mass
Press from Pellon Group	26.6
Haarslev	56.1



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Conclusions

- Silage fermentation quality was improved by the use of fibrolytic enzymes particularly with low FA application
- Press-juice yield and DM concentration increased by the use of enzymes
- In general, the effect of increasing level of enzyme application was linear and only very few quadratic and cubic effects were detected
- Optimal ensiling methodology can be seen as a pretreatment for a biorefinery process

Thank you!

More information about Innofeed project:

- Project homepage: https://www.ibcfinland.fi/projects/innofeed/
- Facebook: https://www.facebook.com/innofeedprojekti
- Earlier project: https://www.ibcfinland.fi/projects/protein-feed-from-grass-silage-b/
- Press release: http://www.vtt.fi/medialle/uutiset/nurmi-taipuu-biojalostamossa-uusiksi-rehutuotteiksi