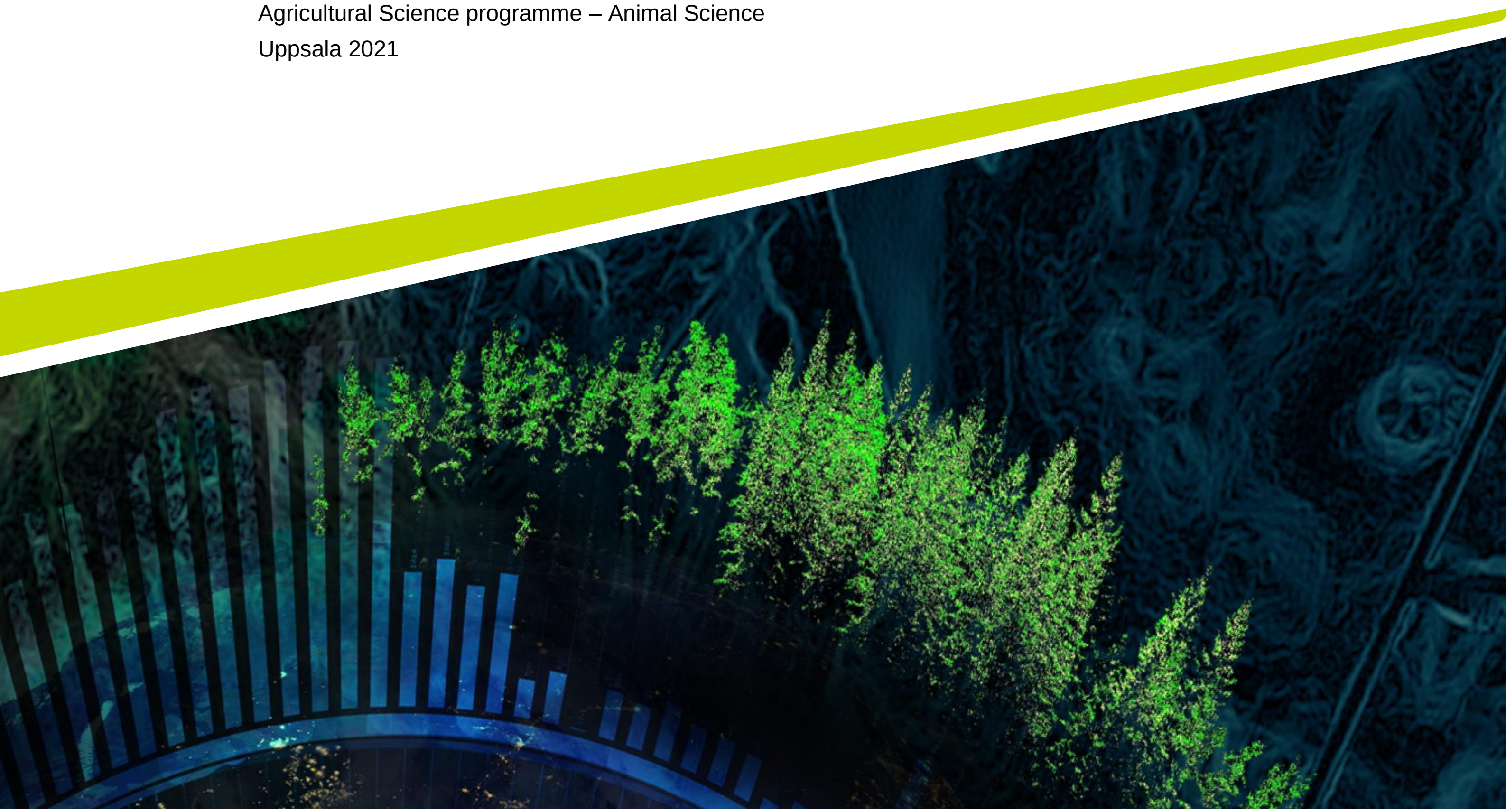
Performance of dairy cows fed grass-clover silage or biorefined silage pulp of grass-clover silage

Produktion hos mjölkkor som utfodras med gräs-klöver ensilage eller bioraffinerad presskaka av gräs- klöver ensilage

Matilda Larsson

Master's thesis • 30 hp
Swedish University of Agricultural Sciences, SLU
Department of Animal Environment and Health
Agricultural Science programme – Animal Science
Uppsala 2021



Performance of dairy cows fed grass-clover silage or biorefined silage pulp of grass-clover silage

Produktion hos mjölkkor som utfodras med gräs-klöver ensilage eller bioraffinerad presskaka av gräs-klöver ensilage

Matilda Larsson

Supervisor:	Dannylo Sousa, Department of Animal Environment and Health
Assistant supervisor:	Elisabet Nadeau, Department of Animal Environment and Health
Examiner:	Katarina Arvidsson Segerkvist, Department of Animal Environment and Health

Credits:	30 hp
Level:	Advanced A2E
Course title:	Master of Science in Agriculture
Course code:	EX0872
Programme/education:	Animal Science
Course coordinating dept:	Department of Animal Environment and Health
Place of publication:	https://stud.epsilon.slu.se/
Year of publication:	2021
Keywords:	biorefined silage, silage pulp, forage, milk production, dairy cow

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Sciences
Department of Animal Environment and Health

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file.

If you are more than one author, you all need to agree on a decision. Read about SLU's publishing agreement here: https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

Grass and legumes can do ecosystem services like no other crops and are one of the biggest contributions to protein in dairy cow diets. Biorefining of silage, makes it possible to produce a high-fibre silage pulp (SP) that can be fed to ruminants and a local high-quality protein feed (press juice) for monogastric animals. Earlier studies have shown inconsistent effects on milk production when cows were fed SP. This master thesis will focus on investigation of intake, milk yield and milk composition in dairy cows fed a diet containing grass-clover silage or its biorefined SP from silage. The hypothesis was that the cows would have a similar milk yield when fed a diet containing SP compared to a diet containing grass-clover silage, when both diets are supplemented with concentrates. The experiment was conducted on the organic dairy farm of Sötåsen Agricultural High School, Töreboda, Sweden as a part of a larger EU-project, Green Valleys. The forage was harvested on the farm and stored in a bunker silo and then fed as silage or the silage was biorefined and then fed as SP to the cows. The SP contained higher dry-matter and neutral detergent fibre concentrations, but lower concentrations of crude protein and water-soluble carbohydrates compared to the silage. Seventy-two dairy cows were allocated to two groups and fed a diet containing either silage or SP, supplemented with concentrate, during the whole experiment that lasted for 120 days. The results showed a numerically higher forage dry-matter intake (DMI) for cows fed silage compared to SP. For cows fed SP, the milk yield and the energy-corrected milk (ECM) yield were generally lower compared to cows fed silage. Yields of milk protein and milk fat were lower for cows fed SP compared to cows fed silage, and the milk lactose yield showed a similar tendency as the milk fat and milk protein yields. The milk composition was not affected by the diets. Body-condition scores and body weights of the cows were not affected by the treatments.

Keywords: biorefined silage, silage pulp, forage, milk production, dairy cow.

Sammanfattning

Gräs och baljväxter ger oss möjligheten att producera större mängder protein till mjölkkors foderstat och fler ekosystemtjänster jämfört med andra grödor. Genom bioraffinering är det möjligt att förädla vallen till en fiberrik presskaka (PK) som kan utfodras till idisslare och ett lokalt odlat högkvalitativt proteinfoder (pressjuice) som kan utfodras till enkelmagade djur. Resultat från tidigare studier där PK har utfodrats har antingen sett oförändrad eller ökad mjölkproduktion. Detta examensarbete har fokuserat på att undersöka foderintag, mjölkavkastning och mjölksammansättning hos mjölkkor som fått en foderstat som innehåller gräs / klöverensilage eller en bioraffinerad PK från ensilage. Hypotesen var att korna skulle ha en liknande mjölkavkastning när de utfodrades med en foderstat som innehöll PK jämfört med en foderstat som innehöll ensilage, när båda vallfodren kompletterades med kraftfoder. Studien genomfördes på den ekologiska gården Sötåsen Naturbruksskola i Töreboda, Sverige, som en del av ett större EU-projekt, Green Valleys. Vallfodret skördades på gården och ensilerades i plansilos för att sedan utfodras som ensilage, eller bioraffineras och utfodras som PK från ensilage till korna i experimentet. Det bioraffinerade PK hade högre halter av torrsubstans och totalfiber (NDF) men lägre halter av råprotein och vattenlösliga kolhydrater jämfört med ensilage. Sjuttiotvå mjölkande kor delades in i två grupper och utfodrades med en foderstat innehållande antingen ensilage eller PK, kompletterat med kraftfoder, under hela experimentet, som varade i 120 dagar. Resultatet visade på ett större numerisk torrsubstans (TS) intag hos kor som åt ensilage än kor som utfodrades med PK. För kor som utfodrades med PK var mjölkavkastning i kg mjölk och i energikorrigerad mjölk (ECM) generellt lägre jämfört med kor som utfodrades med ensilage. Mängden mjölkprotein och mjölkfett var lägre för kor som utfodrades med PK jämfört med kor som fick ensilage, och mängden laktos i mjölken visade en liknande tendens som mängden mjölkfett och mjölkprotein. Mjölksammansättningen påverkades inte av foderstaten. Kornas hull och kroppsvikt påverkades inte av om de hade utfodrats PK eller ensilage.

Nyckelord: bioraffinaderi, vallfoder, ensilage, mjölkko, mjölkproduktion.

Table of contents

List of tables

List of figures

Abbreviations

ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
BCS	Body condition score
CP	Crude protein
CPI	Crude protein intake
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
iNDF	Indigestible Neutral detergent fibre
IVOMD	In vitro organic matter digestibility
MY	Milk yield
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre digestibility
NDFI	Neutral detergent fibre intake
OMI	Organic matter intake
SP	Silage pulp
WSC	Water Soluble Carbohydrates

1. Introduction

In Sweden, 2019, 42% of the total agricultural land was ley and 18% pasture (Jordbruksverket, 2020), which makes a total of 60% of the agricultural land in Sweden cultivated for forage for ruminants. Forage is the most important feed for cows and is also one of the biggest contributions to protein in the feed for cows (Gustafsson et al., 2013). Grasses and legumes have the potential to produce a high protein yield per hectare and even higher compared to other protein feed such as soybean, peas, faba bean, lupin, and rapeseed (Gustafsson et al., 2013). The protein in grasses and legumes is today foremost fed to ruminants or not utilized (Santamaria-Fernandez and Lübeck, 2020). At the same time we import a large amount of protein feed to Sweden to give to our farm animals since the protein feed production is not big enough in Sweden (Gustafsson et al., 2013). Grassland also have the ability to increase soil carbon and do other ecosystem services unlike other crops (Fogelfors, 2016).

There is a growing interest in developing and establishing green biorefinery to produce food and feed, energy, chemicals, and materials from renewable feedstock to replace the oil refineries and fossil fuels (Santamaria-Fernandez and Lübeck, 2020). This thesis is a part of a bigger EU-project, Green Valleys, where they investigate how grass and clover can be converted into protein concentrate, forage, bioenergy, and raw material for future biomaterials using biorefineries.

Today it is mostly ruminants that eat grasses and legumes but by biorefining the forage there is a way to produce both a local high-quality protein feed for monogastric animals as press juice and a biorefined forage (SP; silage pulp) for ruminants using biorefined raw materials from the grass and clover (Agroväst, 2021). This can ensures and increase the total utilization of the grass-clover silage and its positive effects on the climate and environment (Hermansen et al., 2017).

Two earlier Nordic studies examined the milk production when feeding the SP instead of silage and they had an increased milk production (Kragbæk Damborg et al., 2019) or no change in milk production (Savonen et al., 2020).

This study will focus on evaluation of intake, milk yield and milk composition in dairy cows fed a diet containing grass-clover silage or its biorefined SP from silage, when both diets are supplemented with concentrate. The hypothesis is that the cows will have similar milk yields when fed a diet containing SP compared to a diet containing grass-clover silage.

2. Literature review

2.1. Green biorefinery

Grasslands have a positive impact that adds several different ecosystem services, unlike other crops (Fogelfors, 2016). Grasslands can be used as material in the green biorefineries and at the same time contribute to increased soil carbon that is bound from the atmospheric CO_2 (Contant et al., 2017). A green biorefinery is a collective name for different solutions, processes and techniques that convert biomass to different products that are more climate-friendly than fossil-based products (RISE, 2021). In this literature review the focus will be on grass-clover leys but different plant-based materials can be used.

Figure 1 shows schematically how biorefinery of grass-clover forage takes place and the products that can be made from it. The first step in biorefining process is pressing of the grass-clover silage or of fresh grass-clover forage in screw press or in another similar process. The biomass is then separated into two parts, SP, and press juice. The SP is characterized by an increased dry matter (DM) and fibre content compared to the grass-clover silage and can be used as feed for ruminants, biogas substrate or in lignocellulosic biorefining. Soluble carbohydrates, crude protein (CP) and ash contents can decrease in the SP but is found in higher concentrations in the press juice on a DM basis. The press juice can in the next step be processed and converted to a protein concentrate for animals and a rest juice that can be used as a biogas substrate or nutrient fertilizer in the fields. (Hermansen et al., 2017).

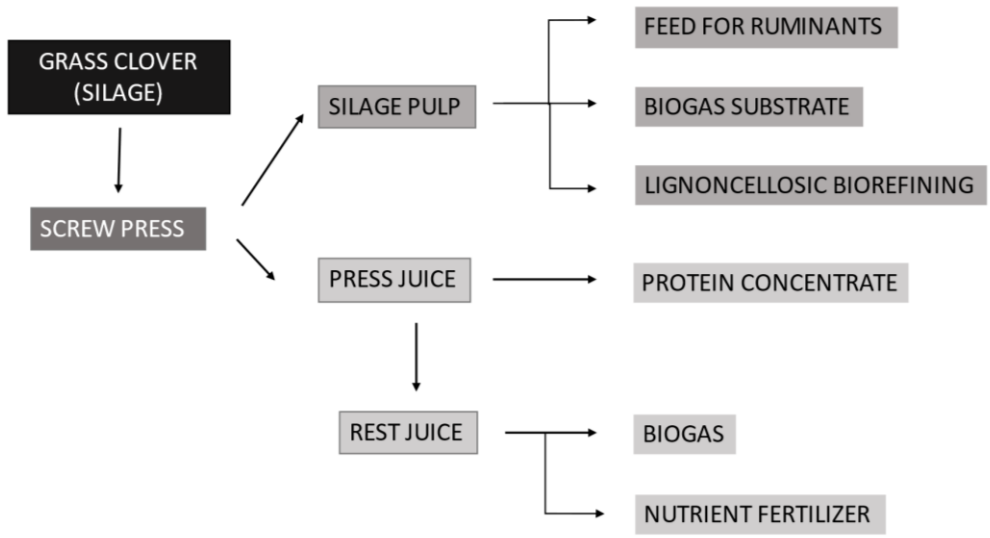


Figure 1. Schematically how the biorefining of the grass-clover works. (Adapted from Hermansen et al., 2017).

2.2. Feed value in biorefined forage

In the grass or clover, the composition and feed value is very variable and can, for example, depend on the species of the plant and how mature the grass or clover are at harvest (McDonald et al., 2011). Biorefinery of forage produces new feeds, foods, and other products, which multiply the use of cultivated forages. Silage pulp can be fed to ruminants, after processing of the grass-clover forage, the CP concentration can decrease in the SP and the neutral detergent fibre (NDF) increases. In the press juice, there is a higher CP content and lower NDF content, which is possible to feed to monogastric animals (Damborg et al., 2020).

In a study of Santamaria-Fernandez et al. (2018), they exanimated a fresh grass-clover mixture and its biorefined parts. In the mixture, there was a grass: clover ratio of 45:55 and the mixture of grass-clover forage was perennial ryegrass (*Lolium perenne L.),* hybrid ryegrass (*Lolium hybridum L.*), red clover (*Trifolium pratense L.),* and white clover (*Trifolium repens L.*) that was harvested between June 27 and July 1. Results of the nutrient composition of fresh grass-clover forage, biorefined pulp from fresh grass-clover and press juice found in the study can be found in table 1. In the pulp, the DM content increased compared to the fresh grass-clover forage, which is due to the extraction of water to the press juice. The CP was instead found in the press juice and increased compared to the fresh grass-clover forage, which shows that the protein extraction in the grass-clover forage was successful (Santamaria-Fernandez et al., 2018). There was an increase in fibres, NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL), in the pulp compared to the fresh grass-clover forage and this was expected because only the soluble nutrients would get washed into the press juice and then increase the fibre proportion in the pulp. In table 1 it is also possible to see that the ash content decreased in the pulp and increased in the press juice. Santamaria-Fernandez et al. (2018) believe that this is because the minerals e.g., K, P and Cl follow the liquid to the press juice where the ash content is higher compared to both fresh grass-clover forage and pulp. Even though the CP content decreases and the fibre content increases in the pulp compared to the fresh grass-clover it does not mean that the compounds that are potentially available for the ruminants are lower (Damborg et al., 2018). The digestibility of organic matter can be lower in pulp than in the original plant, however, the digestible organic matter is not different between the pulp and the original plant (Damborg et al., 2018). The soluble CP left in the pulp is lower, whereas the concentration of cell-wall bound protein increases, and the quality of the protein may be enhanced because of the physical processing. Much of the soluble protein is lost to the press juice and the proportion of rumen undegradable protein in the pulp is increased due to the physical processing of the grass-clover forage. This shows that the pulp has a composition that is suitable as forage for dairy cows (Damborg et al., 2018).

Table 1. Composition of fresh grass-clover forage, pulp of fresh grass-clover and press juice in the study of Santamaria-Fernandez et al. (2018).

Component	Fresh grass-clover forage	Pulp of fresh grass-clover forage	Press juice
DM	19.0	29.9	8.0
CP (%DM)	17.2	15.8	23.8
Ash (%DM)	10.0	8.1	15.7
NDF (%DM)	35.9	46.2	n.d.
ADF (%DM)	19.1	25.2	n.d.
ADL (%DM)	2.8	4.0	n.d.

n.d.= not determined

2.2.1. Feed intake

Feed intake in lactating cows is determined by different dietary factors (Allen, 2000). The primary limitation for milk yield (MY) is energy intake, which is determined by dry matter intake (DMI) and net energy content of the feed. However, the feed intake also can be restricted by many different factors and a combination between these factors (Allen, 2000). In the present study, it is interesting to take an extra look at the NDF because it increases in the SP compared to the silage and can therefore limit the DMI of the cows because of its filling effect on the rumen (Allen, 2000). Digestibility of the NDF (NDFD) is also one of the limiting factors for the DMI and contributes to the filling effects in the rumen, so the lower the NDFD in the feed the lower the DMI. In the pulp, the NDF are higher than in a fresh grass-clover forage (Santamaria-Fernandez et al., 2018) so the DMI of the pulp is expected to be lower than of a grass-clover silage (Kragbæk Damborg et al., 2019).

Two previous Nordic studies have investigated a similar hypothesis to this study and how the fibre fraction in biorefined forage affects milk production compared to cows fed silage (Kragbæk Damborg et al., 2019; Savonen et al., 2020).

One of the studies, performed in Denmark examined the effect on milk production with cows fed different diets with silage or biorefined pulp from fresh grass-clover forage that was ensiled after biorefining. The diet also had a partial substitution of soybean meal with green protein (Kragbæk Damborg et al., 2019). In this study, the grass-clover was harvested from June 29 to 1 July and then pressed in a twin screw and the pulp was then baled and wrapped. The press juice was fermented and then separated into green protein and brown juice. The control grass-clover was harvested around a week later than the biorefined grass-clover. The grass-clover had a grass: clover ratio at 45:55 and the mixture consisted of perennial ryegrass (*Lolium perenne* L.*),* hybrid ryegrass (*Lolium hybridum* L.), red clover (*Trifolium pratense* L.*),* and white clover (*Trifolium repens* L.).

The Danish study was conducted with 36 lactating Danish Holstein cows, 12 in first lactation and 24 multiparous cows. At the start of the experiment the cows were on average (mean ± SD) 72 ± 46 days in milk (DIM). The multiparous cows weighed (mean ± SD) 642 ± 59 kg and the primiparous cows weighed 545± 50 kg. Six diets were tested, three diets based on the pulp as a forage: low CP concentration (P), high CP with green protein and soybean meal (PGpS) and high CP with soybean meal (PS). The other three diets were with the silage as the forage: low CP concentration (G), high CP concentration with green protein and soybean meal (GGpS) and high CP with soybean meal (GS). Ingredients in the different diets can be found in table 2. The cows were fed individually, and *ad libitum* of the forage and the concentrate were individually assigned to each cow. It is possible to see the nutrient composition of the forage and concentrate in table 3. (Kragbæk Damborg et al., 2019).

Kragbæk Damborg et al. (2019) blocked the cows in 6 blocks according to parity and the DIM were randomly assigned to one of the six different diets. There were 6 x 4 incomplete Latin square designs with 6 diets, 6 cows and 4 experimental periods of 21 days. With a 2 x 3 factorial arrangement of treatments including 3 protein treatments and 2 silage types.

In the study of Kragbæk Damborg et al. (2019) the DM intake was not different between the cows that were fed pulp or grass-clover silage (table 4). In their study, the result showed that the protein concentration in the feed would affect DM intake. So, the DM intake for cows was lower when they received the low protein diet compared to high protein diets regardless if the cows had been fed pulp or grass-clover silage.

Table 2. Ingredients in the diets in the study of Kragbæk Damborg et al. (2019).

	Diets[1]					
Item	G	GS	GGpS	P	PS	PGpS
Ingredient (g/kg DM)						
Grass-clover silage	372	342	342	0	0	0
Silage pulp	0	0	0	372	342	342
Maize silage	177	163	163	177	163	163
Green protein	0	0	28.5	0	0	28.5
Soybean meal	0	69.2	50.9	0	69.2	50.9
Barley	132	122	122	132	122	122
NaOH Wheat	133	122	122	133	122	122
Rapeseed cake	111	102	102	111	102	102
Dried sugar beet pulp	62.0	69.2	59.0	62.0	69.2	59.0
Vitamin and mineral mix	12.4	11.3	11.3	12.4	11.3	11.3
Titanium dioxide	0.967	0.890	0.890	0.967	0.890	0.890
Chemical composition (g/kg DM)						
DM (g/kg fresh)	585	598	597	423	439	443
OM	923	924	921	925	927	925
CP	137	166	165	148	173	173
NDF	296	287	284	337	322	321

[1]Diets; G, grass-clover silage, and low protein; GGpS, grass-clover silage high CP concentration with green protein and soybean meal; GS, grass-clover silage and high CP with soybean meal; P, pulp and low protein; PS, pulp and high CP with soybean meal; PGpS pulp and high CP with green protein and soybean meal.

Table 3. Nutrient composition in the feed from the study of Kragbæk Damborg et al. (2019).

Feedstuff	DM (g/kg)	OM (g/kg of DM)	CP (g/kg of DM)	NDF (g/kg of DM)	OMD, % of DM	Starch (g/kg of DM)
Grass-clover silage	514±41	906±8.3	161±0.7	417±7.8	73.2±1.13	
Pulp	313±19	907±2.3	179±2.0	472±7.0	71.6±0.56	
Maize silage	344±9.9	972±1.1	69.2±0.0	431±20	79.2±0.21	306±2.9
Green protein	924±14	880±0.7	332±0.1	189±9.1	86.9±0.64	119±6.0
Soybean meal	900±1.5	926±0.3	576±0.9	96.5±1.53	91.3±0.21	12.9±2.81
Barley	883±4.5	978±0.1	117±1.18	246±20	85.4±0.34	688±28
NaOH wheat	820±16	939±0.9	121±0.7	81.8	90.1±0.25	645±27
Rapeseed cake	921±5.5	926±0.0	339±4.2	289±2.6	78.0±0.0	7.57±1.20
Dried sugar beet pulp	895±	947±6.2	98.7±2.92	461±26	86.8±2.85	7.07±2.74

Values are means ± standard derivation.

Table 4. Feed intake in the study of Kragbæk Damborg et al. (2019).

	Diets[1]							P-value[2]				
Intake per day (kg/day)	G	GS	GGpS	P	PS	PGpS	SEM	Treat	Sil	Level of prot	Source of prot	Level of prot x sil
DM	22.6	23.3	23.8	22.4	23.7	23.7	0.41	0.001	0.92	<0.001	0.42	0.53
Total CP	3.11	3.92	3.96	3.34	4.13	4.12	0.07	<0.001	<0.001	<0.001	0.72	0.59
OM	21.0	21.8	22.1	20.9	22.1	22.1	0.38	0.001	0.65	<0.001	0.56	0.50
NDF	6.76	6.75	6.82	7.64	7.69	7.66	0.14	<0.001	<0.001	0.715	0.86	0.96

[1]Diets; G, grass-clover silage, and low protein; GGpS, grass-clover silage high CP concentration with green protein and soybean meal; GS, grass-clover silage and high CP with soybean meal; P, pulp and low protein; PS, pulp and high CP with soybean meal; PGpS pulp and high CP with green protein and soybean meal.

[2]Treat=treatment, Sil = experimental silage, Prot = protein. Probability of contrasts; Sil = G, GS, and GGpS versus P, PS, and PGpS; Level of prot = G and P versus GS, GGpS, PS, and PGpS; Source of Prot = GS and PS versus GGpS and PGpS; Level of Prot × Sil = G, PS, and PGpS versus P, GS, and GGpS.

The other Nordic study was conducted by Savonen et al. (2020) in Finland. In this study, they examined the dairy cows response to diets based on a biorefined solid fraction of grass silage. In the study, they used 24 multiparous Nordic red cows. The average parity (mean ± SD) was 3.3 ± 1.07 and when the experiment started the cows were on average 125 ± 27.7 DIM. The cows had a body condition score (BCS) (mean ± SD) of 3.1 ± 0.28 and body weight (BW) of 665 ± 57.7 kg in the beginning of the experiment. The study design was an incomplete changeover (reduced 3x3 Latin square) design with two 21-day periods and three diets.

The grass was harvested on 21 and 22 June and baled. The silage was biorefined once a week during the experiment and then mixed with the silage and feed *ad libum* using the following treatments; only grass silage (P0), 75 % original grass silage and 25 % SP (P25), and 50 % original grass silage and 50 % SP (P50). The silage was a mix of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*).

Besides the silage and SP, the cows were fed 13 kg of concentrate per day. The basal concentrate was given in an amount of 7.8 kg per day and contained (g/kg): barley 202, oats 110, wheat 132, sugar beet expeller 120, rapeseed meal 411 and mineral and vitamin premix 25. In the milking parlour, another concentrate was given of 5.2 kg, and this contained (g/kg) barley 300, wheat 162, sugar beet expeller 128, rapeseed meal 187, soybean meal 193 and mineral and vitamin premix 30. The mineral and vitamin premix contained (g/kg); Na 110 and Mg 65; (mg/g) Zn 1600, vitamin E 1140, Mn 330, Cu 296, I 43, Se 29, Co 27; (IU/kg) Vitamin D_3 52 000 and Vitamin A 158 000. It is possible to see the nutrient composition of the forage and concentrate in table 5. (Savonen et al., 2020).

The feed intake in the study of Savonen et al. (2020) showed an increase in the DM intake when the diet contained 25% SP compared to diets with only silage or 50% SP (table 6). In the diets with only silage and 50% SP the DM intake was similar.

Table 5. Nutrient composition in feed from the study of Savonen et al. (2020).

Feed	Diets[1]			Pulp	Basal concentrate	Milking parlour concentrate
	P0	P25	P50			
DM (g/kg)	220	252	294	432	870	865
In DM (g/kg)						
Ash	69	63	57	42	76	79
CP	144	133	122	107	219	224
NDF	589	611	614	709	237	193
WSC	21.3	16.1	12.7	6.92		
IVOMD	0.725	0.719	0.708	0.696		

IVOMD – In vitro organic matter digestibility

[1] Diets; P0, only grass silage as forage; P25, 0.75 original grass silage and 0.25 silage pulp (F25); F50, 0.50 original grass silage and 0.50 silage pulp.

Table 6. Feed intake in the study of Savonen et al. (2020).

	Diets[1]				Statistical significance[2]	
	P0	P25	P50	SEM	Lin	Quad
Feed intake (kg DM/day)						
Total	24.4^{b}	25.4^{a}	24.1^{b}	0.21	0.354	<0.001
Forage	13.2^{b}	14.1^{a}	13.0^{b}	0.18	0.458	<0.001
Concentrate	11.1	11.3	11.0	0.07	0.354	0.072
Nutrient intake per day						
Organic matter	22.6^{b}	23.6^{a}	22.5^{b}	0.20	0.669	<0.001
CP (g)	4362^{a}	4370^{a}	4030^{b}	31.6	<0.001	<0.001
NDF (kg)	10.2^{b}	11.1^{a}	10.8^{a}	0.12	0.007	<0.001
Indigestible NDF (kg)	1.87	2.02	2.03	0.023	<0.001	0.019
Nutrient concentration in the diet (g/kg DM)						
Organic matter	928^{c}	931^{b}	934^{a}	0.1	<0.001	<0.001
CP	179^{a}	172^{b}	167^{c}	0.3	<0.001	0.042
NDF	419^{c}	437^{b}	446^{a}	1.5	<0.001	0.063
Indigestible NDF	76.8^{c}	79.7^{b}	84.0^{a}	0.45	<0.001	0.216

^{abc} shows significance at $P < 0.05$.

[1] Diets; P0, only grass silage as forage; P25, 0.75 original grass silage and 0.25 silage pulp (F25); F50, 0.50 original grass silage and 0.50 silage pulp.

[2]Lin= linear effect on silage pulp, Quad; quadric effect on the amount of silage pulp.

2.3. Milk yield and milk composition

The MY and the milk composition can differ based upon the diet that the dairy cow is fed (Huhtanen and Nousiainen, 2012). The earlier explained Nordic studies of Kragbæk Damborg et al. (2019) and Savonen et al. (2020) showed both an increase or no difference in milk yield for cows fed biorefined forage.

The MY in the study of Savonen et al. (2020) was not affected by the different diets, as seen in table 7. With increasing SP in the diet, the authors could see a tendency to a linearly decrease in ECM production. The diet did not affect the production of lactose. There was a linear decrease in milk protein production and a tendency to a decrease in the milk fat production with increasing SP in the diet. Even though the ECM production tended to decrease the authors believe that it is considered moderate, and the SP could be included in the cow diet if it has other benefits like reduced feed costs.

Table 7. Milk production and milk composition in the study of Savonen et al. (2020).

	Diets[1]				Statistical significance[2]	
	P0	P25	P50	SEM	Lin	Quad
Production per day						
Milk (kg)	37.2	37.5	36.3	0.39	0.116	0.173
Energy corrected milk (kg)	39.8	39.8	38.5	0.42	0.056	0.230
Fat (g)	1706	1703	1646	20.4	0.061	0.320
Protein (g)	1327	1330	1279	13.8	0.029	0.141
Lactose (g)	1620	1630	1589	18.4	0.264	0.293
Milk composition (g/kg)						
Fat	46.0	45.5	45.6	0.40	0.461	0.596
Protein	35.7	35.5	35.4	0.20	0.124	0.996
Lactose	43.5	43.5	43.8	0.10	0.102	0.341
Solids	136	135	135	0.50	0.650	0.589
Urea (mg/100 ml)	32.7	33.7	32.2	0.65	0.608	0.146
Energy-corrected milk kg/kg DM intake	1.64	1.57	1.60			

[1] Diets; P0, only grass silage as forage; P25, 0.75 original grass silage and 0.25 silage pulp (F25); F50, 0.50 original grass silage and 0.50 silage pulp.

[2]Lin= linear effect on silage pulp, Quad; quadratic effect on the amount of silage pulp.

Milk yield and milk composition from the study of Kragbæk Damborg et al. (2019) can be found in table 8. The cows that were fed pulp had a higher ECM per day than the cows fed with grass-clover silage. Milk protein concentration was lower when cows were fed high protein pulp diets compared with high protein grass-clover-diets, whereas milk fat concentration was higher with high protein pulp diet compared with high protein grass-clover-diets. The daily production of milk protein and milk fat were higher for cows receiving diets with pulp than with grass-clover diets. Milk yield production and yields of milk fat, milk protein or lactose were not affected by the protein source, in other words, it did not matter if the diet contained soybean meal or green protein. (Kragbæk Damborg et al., 2019).

Table 8. Feed intake in the study of Kragbæk Damborg et al. (2019).

	Diets[1]							P-value[2]				
Milk production per day	G	GS	GGpS	P	PS	PGpS	SEM	Treat	Sil	Level of prot	Source of prot	Level of prot x sil
Milk, kg	33.0	35.4	36.1	36.6	38.2	38.1	1.04	<0.001	<0.001	<0.001	0.50	0.19
ECM, kg	31.8	33.7	34.8	35.9	37.6	37.6	0.94	<0.001	<0.001	<0.001	0.32	0.40
Fat, kg	1.24	1.28	1.32	1.40	1.44	1.44	0.04	<0.001	<0.001	0.013	0.51	0.61
Protein, kg	1.1	1.22	1.25	1.25	1.33	1.34	0.03	<0.001	<0.001	<0.001	0.25	0.19
Lactose, kg	1.59	1.69	1.76	1.79	1.88	1.89	0.05	<0.001	<0.001	<0.001	0.19	0.43
Milk composition (g/kg)												
Fat	38.8	37.9	37.7	38.8	38.4	38.2	0.36	<0.001	0.038	<0.001	0.35	0.20
Protein	35.0	36.3	36.2	35.0	35.5	35.7	0.35	<0.001	0.011	<0.001	0.73	0.052
Lactose	50.1	50.3	50.8	49.9	50.2	50.4	0.52	0.19	0.38	0.043	0.16	0.052

[1]Diets; G, grass-clover silage and low protein; GGpS, grass-clover silage high CP concentration with green protein and soybean meal; GS, grass-clover silage and high CP with soybean meal; P, silage pulp and low protein; PS, silage pulp and high CP with soybean meal; PGpS silage pulp and high CP with green protein and soybean meal.

[2]Treat=treatment, Sil = experimental silage, Prot = protein. Probability of contrasts; Sil = G, GS, and GGpS versus P, PS, and PGpS; Level of prot = G and P versus GS, GGpS, PS, and PGpS; Source of Prot = GS and PS versus GGpS and PGpS; Level of Prot × Sil = G, PS, and PGpS versus P, GS, and GGpS.

3. Material and Methods

This study was part of a larger project, Green Valleys, which investigates how biorefined products of grass-clover forage can be used as animal feed and as substrates in biogas production. The dairy cow experiment started on 23 November 2020 and ended on 14 April 2021. The experiment was conducted at the organic dairy farm Sötåsen Agricultural High School, Töreboda, Sweden (N 58° 41', E 14° 8',) and the forage was harvested at the farm. The study was approved by the Gothenburg Research Animal Ethics Committee (case no. 003106, 5.8.18-09145/2020).

3.1. Experimental forages

The harvested leys were established in the years of 2017, 2018 and 2019. The sown mixture on one of the three fields was GEV Stabil (Scandinavian Seed), containing timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.), perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) where the grass: clover seed ratio was 80:20. Bete SW Intensiv (Lantmännen, Stockholm, Sweden), which was sown on another field and contained meadow fescue (*Festuca pratensis* L.), perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), red fescue (*Festuca rubra* L.) and white clover (*Trifolium repens* L.). In this mixture the grass: clover seed ratio was 90:10. The third mixture was Eco Pavo 23 (Lantmännen, Stockholm, Sweden) containing timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea*), lucerne (*Medicago sativa* L.) red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) with the grass: legume seed ratio of 65:35.

The forages were harvested in a 3-cut system in 2020. The first harvest was June 4 and the second and third regrowth were harvested July 13 and September 16. The harvested forages were wilted to around 30% DM and then chopped with a harvester (1060, JF, Milton Keynes, England) where an acid-based silage additive, containing formic acid and propionic acid, was added to the chopped forage. The forage was then transported to a bunker silo and properly compacted using a heavy tractor (Volvo L40B) in the bunker silo before it was covered. Each cut was individually stored in separate bunker silos. First cut silage was used throughout the study and mixed with either second or third cuts (50% of each cut, on DM basis). Silos containing first and third cut forages were opened on November 23 of 2020 and equally mixed until January 19 of 2021. The second-cut silo was opened on January 20 of 2021 and mixed with the first-cut silage until the end of the experiment.

To produce the SP and press juice, the silage from the bunker silo was transported to the biorefinery and pressed through the screw press (Cir-Tech, Skærbæk, Denmark) at 1.5 ton/hour on Mondays, Wednesdays, and Fridays during the whole experiment. The produced SP was then transported to the mixer wagon to be fed to the cows. Chemical composition of the silage and SP can be found in table 9. Values showed in Table 9 are average mean from the respective silage mixture (50% of each cut, as DM basis). Concentrations of propionic acid, butyric acid and propanol of the forages were not detected in the analysis.

Table 9. Chemical composition of the forage. (n=8).

Item	Silage	SP
DM, g/kg	310 ± 32.5	469 ± 12.4
Ash, g/kg DM	86.0 ± 13.5	61.8 ± 8.06
NDF, g/kg DM	457 ± 26.9	589 ± 19.8
ADF, g/kg DM	297 ± 38.8	394 ± 44.8
ADL, g/kg DM	47,3 ± 12.5	42.3 ± 15.7
iNDF, g/kg NDF	271 ± 37.5	279 ± 85.0
NDFD, g/kg NDF	729 ± 37.5	721 ± 85.0
IVOMD, g/kg DM	792 ± 50.6	749 ± 76.5
Crude protein, g/kg DM	138 ± 19.8	115 ± 8.85
Protein fractions[1], % of CP		
A	53.0 ± 3.78	36.6 ± 3.00
B_1	1,71 ± 1.03	3.09 ± 0.99
B_2	26.5 ± 2.30	36.3 ± 2.11
AB_1B_2	81.1 ± 3.07	76.1 ± 2.02
B_3	14.6 ± 3.14	16.9 ± 2.39
C	4.28 ± 0.88	7.01 ± 0.90
RUP_5	23.5 ± 1.62	34.6 ± 2.19
WSC, g/kg DM	103 ± 89.3	58.1 ± 36.7
pH	3.93 ± 0.12	3.97 ± 0.18
Lactic acid, g/kg DM	115 ± 1.30	54.2 ± 0.74
Acetic acid, g/kg DM	22.6 ± 0.49	12.1 ± 0.20
1,2-propandiol, g/kg DM	3.34 ± 0.17	1.23 ± 0.03
Ethanol, g/kg DM	3.93 ± 0.18	2.80 ± 0.06
Ammonium N, g/kg of total N	54.5 ± 5.08	32.2 ± 4.14

Values are average means ± standard derivation from the respective silage mixture (50% of each cut as DM basis).

SP=Silage pulp.

[1]A=non-protein nitrogen; B_1=buffer-soluble protein; B_2=neutral detergent-soluble protein; AB_1B_2=sum of CP fractions A, B_1 and B_2.; B_3=acid detergent-soluble protein; C=acid detergent insoluble nitrogen; RUP_5=Rumen undegradable protein at a passage rate of 5% per h. (Kirchhof et al., 2010).

3.2. Cows, experimental design, and diets

Seventy-two lactating cows (28 primiparous and 44 multiparous) of Holstein (49), Swedish red (11), Jersey (8), and mixed breeds (Swedish red x Ayrshire cattle) (4) were used in a completely randomized block design. Cows were blocked based on their lactation number, DIM and ECM yield and randomly assigned to one of the two treatments within block (n = 36). The cows were divided into two groups and kept in a loose housing system, one group receiving SP based-diet and the other group receiving silage based-diet. At the beginning of the experiment, the primiparous cows had an average (mean ± SD) MY of 25.5±7.9, ECM 28.7±7.3, DIM 127±117, BW 738±122 kg and BCS was 2.9±0.4. For multiparous cows the average (mean ± SD) MY was 34.4±10.7, ECM 37.9±9.7, DIM 124±125, BW 617±102 kg and BCS was 2.7±0.3. The average parity for all the cows was 2.1±1.1.

The adaptation period lasted for three weeks, where the cows were fed a diet containing 50% silage and 50% SP for 11 days, followed by 10 days with the respective experimental diet (silage or SP). After the adaptation period, the experiment lasted for 120 d with cows receiving the assigned diet continuously.

The cows' diets were formulated according to the regulations for organic production (KRAV, 2021) in the diet formulating programme IndividRAM by a dairy nutritionist from Växa Sverige. The diets were formulated based on the DIM of the cows and to have similar forage NDF concentrations between the treatments. The dietary ingredients and chemical composition of the diets averaged over the cows per treatment group throughout the experiment are presented in table 10. The dietary ingredient varied according to days in milk of the cows, in appendix 1 it is possible to see the average dietary ingredient according to DIM 1-100 or 101-365.

The cereals and faba beans were grown on the farm. The mix of cereals contained (g/kg) wheat (700) and barley (300). Minerals (MIXA TMR, Lantmännen, Stockholm, Sweden) and pellets (Sund Vässa Mix, Lantmännen, Stockholm, Sweden) were purchased. The pellets contained (g/kg) wheat or barley (117), soybean (331), soy expeller (421), molasses (30), rapeseed cake (101). The minerals composed of (g/kg); calcium (120), phosphor (60), magnesium (170), natrium (45), sulphur (30), and (mg/kg) cupper (1000), manganese (5000), zinc (6200), iodine (150), selenium (50), cobalt (50). Added vitamins (IE/kg), A (500000), D (120000) and E (8000).

Chemical composition of the mix of cereals, pellets and faba beans are presented in Table 11. The cows fed SP was also given limestone. Silage and SP were mixed with the minerals in separate TMR mixers (Cormall Feed Mixer-Multimix, Sønderborg, Denmark and GEA MVM 10 Mixer, Düsseldorf, Germany, respectively) and delivered to each group twice a day by an automatic feeding wagon (DEC SR, Rovibec Agrisolutions, Nicolet, Canada). Concentrates were individually fed in automatic feed stations (DeLaval feed station FSC400, Tumba, Sweden) placed in each pen. At milking the cows were offered 0.5 kg of pellets in the milking parlour which was includes in the total intake of pellets. The cows had free access to water and salt blocks

Table 10. Dietary ingredients and chemical composition for the treatments.

	Diet	
Item	Silage	SP
Dietary ingredient, % of DM		
Forage	62.2	52.5
Mix of cereals[1]	17.0	16.9
Faba bean	5.74	14.7
Pellets[2]	14.9	15.2
Minerals[3]	0.19	0.61
Chemical composition, g/kg of DM		
DM, g/kg as fed	533	671
NDF	336	376
Forage NDF	284	310
ADF	219	253
ADL	35.1	30.8
Starch	184	195
Ether extract	15.9	28.3
CP	164	170
WSC	81.3	58.1

SP = Silage pulp

[1]Mix of cereal composed of (g/kg); wheat (700) and barley (300).

[2]Sund Vässa Mix (Lantmännen, Stockholm, Sweden). The pellets contained (g/kg) wheat or barley (117), soybean (331), soy expeller (421), molasses (30), rapeseed cake (101).

[3]MIXA TMR (Lantmännen, Stockholm, Sweden) composed of (g/kg); calcium (120), phosphor (60), magnesium (170), natrium (45), sulphur (30), and (mg/kg) cupper (1000), manganese (5000), zinc (6200), iodine (150), selenium (50), cobalt (50). Added vitamins (IE/kg), A (500000), D (120000) and E (8000).

Table 11. Chemical composition of the concentrates. (n=2).

Item	Mix of cerals[1]	Pellets[2]	Faba bean
DM, g/kg	896 ± 8.68	912 ± 1.36	916 ± 0.38
Ash, g/kg DM	19.7 ± 0.32	56.7 ± 0.77	36.1 ± 0.13
NDF, g/kg DM	131 ± 1.35	153 ± 2.57	140 ± 14.0
ADF, g/kg DM	48.9 ± 1.03	129 ± 2.50	123 ± 7.59
ADL, g/kg DM	15.7 ± 1.32	32.4 ± 7.72	7.76 ± 4.70
iNDF, g/kg NDF	26.8 ± 0.03	23.6 ± 0.47	18.3 ± 0.79
NDFD, g/kg NDF	73.2 ± 0.03	76.4 ± 0.47	81.7 ± 0.79
IVOMD, g/kg OM	89.5 ± 0.25	79.3 ± 0.00	87.3 ± 2.00
Starch, g/kg DM	662 ± 1.14	115 ± 1.50	439 ± 9.20
Ether extract, g/kg DM	26.9 ± 0.04	152 ± 1.09	17.6 ± 1.15
Crude protein, g/kg DM	113 ± 1.26	345 ± 5.46	262 ± 5.63
Protein fractions[3], % of CP			
A	13.2 ± 1.06	4.42 ± 0.30	10.6 ± 0.45
B_1	23.1 ± 0.03	48.0 ± 10.4	45.8 ± 3.36
B_2	55.1 ± 1.06	44.2 ± 10.5	39.1 ± 2.04
AB_1B_2	91.4 ± 0.04	96.6 ± 0.25	95.5 ± 0.88
B_3	7.27 ± 0.14	1.98 ± 0.19	3.61 ± 0.71
C	1.38 ± 0.10	1.34 ± 0.06	0.93 ± 0.16
WSC, g/kg DM	47.4 ± 3.96	112 ± 7.12	19.5 ± 0.83

Values are means ± standard derivation.

[1]Mix of cereal composed of (g/kg); wheat (700) and barley (300).

[2] Sund Vässa Mix (Lantmännen, Stockholm, Sweden). The pellets contained (g/kg) wheat or barley (117), soybean (331), soy expeller (421), molasses (30), rapeseed cake (101).

[3]A=non-protein nitrogen; B_1=buffer-soluble protein; B_2=neutral detergent-soluble protein; AB_1B_2=sum of CP fractions A, B_1 and $B_{2.;}$ B_3=acid detergent-soluble protein; C=acid detergent insoluble nitrogen; RUP_5=Rumen undegradable protein at a passage rate of 5% per h. (Kirchhof et al., 2010).

3.3. Data and sample collection, and chemical analyses

Forage intake was evaluated on a group basis by weighting the total forage offered and the leftover in the next morning. Therefore, the total forage intake was divided by the number of cows in the group to estimate the average individual daily intake. The intake of the concentrates was individually recorded by an automatic system (DeLaval feed station FSC400) and offered based on the MY. As forage intake was measured at group level and concentrate was offered according to the MY of each block of cows, no statistical analysis was performed. However, the average mean of intake over time for each treated group was calculated and presented in Table

12. The intake data followed a normal distribution over time and was similar between silage and SP groups.

Body weight was collected by weighing the cows once a month, starting at the beginning of the experiment. Body condition score was evaluated at the beginning of the study and once a month on a 5-point scale, in 0.25-unit increments, where 1 = very thin and 5 = very fat, as described by Edmonson et al. (1989).

Milk yield was collected twice a day when the cows were milked at 0500 and 1500 h in a fishbone milk parlour. Milk composition was analysed every other week throughout the experiment. Milk samples for milk composition were collected at milking in the afternoon and the following morning and sent to the laboratory. The milk was analysed for fat, protein, lactose, urea, (MilkoScan FT, Foss) and somatic cell count (Fossmatic, Foss) by Eurofins Steins laboratory, Jönköping, Sweden. Feed efficiency was calculated by dividing MY or ECM by DMI and is presented in Table 12.

A sample of each of the feed ingredients of the diets was collected once a week and then stored in -20ºC until the end of the experiment, when samples were composed by month and analysed at LKS mbH (Lichtenwalde, Germany).

The DM concentration of forages, orts, and concentrates was determined by drying 150 g samples in a drying cabinet at 60 °C for 24 h at the Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara.

Samples of forages, orts, and concentrates were milled to pass through a 1-mm screen before laboratory analysis. Ash was determined by combustion at 525 °C for 16 h. Fibre components (NDF, ADF, and ADL) were determined by the fibre technology method, as described by Van Soest et al. (1991). The NDF analysis was modified by adding heat-stable α-amylase (Novozymes, Bagsvaerd, and Denmark) and omitting sodium sulphite. Concentrations of NDF, ADF, and ADL were corrected for residual ash after ADL treatment.

Indigestible NDF and NDFD were determined by *in vitro* incubation for 240 h (LKS FMUAA 223:2018-02). The IVOMD of silages was analysed by incubation at 38°C for 96 h of 0.5 g of dried, milled sample in 49 mL of buffer and 1 mL of rumen fluid (Lindgren, 1979, 1983). Ether extract concentration was determined according to EU Council Directive (1998) 64/1998/EC.

The concentration of N was determined by the Kjeldahl method and CP concentration were then calculated as total N*6,25. Protein fractions (A, B_1, B_2, B_3, and C) were determined according to Licitra et al. (1996) and is based on degradability characteristics according to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). Fraction A is non protein nitrogen and is nitrogen in the filtrate after precipitation with tungstic acid. Fraction B is degradable true protein and can be further divided into; B_1 that is soluble in borate-phosphate buffer at rumen pH and is rapidly degraded in the rumen; B_2 that is soluble in neutral detergent solution, but insoluble in borate-phosphate buffer and the rumen degradation rate is intermediate; B_3 that is soluble in acid detergent solution but insoluble in neutral detergent solution. Fraction B_3 is associated with cell walls and has a slower degradation rate than fractions B_1 and B_2. The C fraction is insoluble in acid detergent solution and is considered to be indigestible. The rumen undegradable dietary CP (RUP), at a 5% passage rate/h was calculated according to Kirchhof et al. (2010).

Water Soluble Carbohydrates (WSC) were determined by analysis of free glucose and fructose and followed by hydrolysis of sucrose and fructans to glucose and fructose before being summed to WSC (LKS FMUAA 194:2019-04). Fermentation characteristics (pH, acids, alcohols, and ammonia N) were analysed in the silage and SP. The pH was defined in a water extract of the silage and SP forages by a pH meter (Metrohm 654). The concentration of organic acids was determined according to LKS FMUAA 166:2019-10 method. Ammonia N was analysed as percentage of total N (VDLUFA III, 4.8.1, 1976).

3.4. Statistical analysis

A power analysis test was performed using JMP Pro (version 16, SAS) to estimate the sample size. The fixed parameters considered a level of significance in an F test of 0.05 and a standard deviation for ECM of the cows within the block of 3 kg. The expected difference to detect significance was 1 kg ECM. The test aimed to estimate the sample size for a power of 75%. The minimum sample size required in the power test was sixty-five cows. The present study used seventy-two lactating cows, which showed a statistical power of 80%.

Data on milk yield, ECM and milk components were analysed as a randomized block design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) with week as repeated measures using the covariance structure that provided the best fit according to Bayesian information criterion. Body weight and BCS data were analysed using the same model but did not include week as repeated measures.

The model included treatment, time, and treatment by time interaction as fixed effects, and block and cow within block as a random effect. The statistical model was:

$$Y_{ijkl} = \mu + F_i + T_j + FT_{ij} + B_k + C_l\,(B_k) + e_{ijkl}$$

where Y_{ijkl} is the observed response, F_i is the fixed effect of forages (i = 1 to 2), T_j is the fixed effect of time (j = 1 to 17 for MY; j = 1 to 8 for ECM and milk components; j = 1 to 4 for BCS and BW), FT_{ij} is the fixed effect of the interaction between forages and time, B_k is the random effect of block, $C_l\,(B_k)$ is the random effect of cow within block, and e_{ijkl} is the error term.

Degrees of freedom were calculated using the Kenward Rogers option. Means were determined using the least squares means statement and treatment means were compared using the PDIFF option with Tukey adjustment. Statistical significance was considered at $P \leq 0.05$ and tendency to significance at $0.05 < P \leq 0.10$.

4. Results

4.1. Feed intake

The average mean of intake over time is shown in Table 12. The forage intake was numerically greater for cows fed silage compared to SP, except for forage NDFI that was similar between groups. In general, concentrate intake was numerically greater for cows receiving SP than silage diet. The total intake was numerically greater for cows fed silage compared to SP, except for NDFI, for which cows fed SP diet showed greater intake. The feed efficiency based on MY was similar between groups, but when it was calculated using ECM, feed efficiency was numerically greater for cows fed silage than SP diet.

Table 12. Feed efficiency and average dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI) and neutral detergent fiber intake (NDFI) for forage, concentrate and in total diet.

Item	Silage	SP
Forage		
DMI, kg/d	13.7	10.7
OMI, kg/d	12.5	10.0
CPI, kg/d	1.89	1.23
NDFI, kg/d	6.26	6.30
Concentrate		
DMI, kg/d	8.28	9.54
OMI, kg/d	8.17	9.38
CPI, kg/d	0.71	1.05
NDFI, kg/d	0.44	0.63
In total diet		
DMI, kg/d	22.0	20.2
OMI, kg/d	20.7	19.4
CPI, kg/d	2.60	2.28
NDFI, kg/d	6.70	6.93
Feed efficiency		
MY/DMI, kg/kg	1.55	1.55
ECM/ DMI, kg/kg	1.68	1.61

SP = Silage pulp

4.2. Milk yield and milk composition

Milk yield and milk composition are presented in table 13. Daily MY had a treatment × time interaction (*P*=0.028). The MY was similar for the treatments until week 9, and on weeks 11 and 12 (Figure 2). During week 10 and after week 13 the MY was generally higher for cows fed silage compared to cows fed SP (Figure 2). In daily ECM yield, there was an effect of treatment (*P*=0.013) and a tendency to an interaction between treatment and time (*P*=0.093). As shown in figure 3, the ECM production was similar between the treatments in week 1 and after that, the cows that were fed silage generally had a higher ECM production than cows fed SP.

There was no interaction between treatment and time or main effect of treatment, when averaged over time, regarding milk composition. However, there was a main effect of time, when averaged over treatments, for percentages of fat and lactose in the milk where the fat and lactose decreased over time for cows eating SP. The total yield of milk protein per day showed an effect between the treatments (*P*=0.011), where the cows eating silage produced more milk protein than cows fed SP. There was a treatment effect on the total produced milk fat per day (*P*=0.035) where the cows fed silage produced more fat in the milk than cows fed SP. Cows fed silage also tended to produce more lactose in the milk per day (*P*=0.053) than the cows fed SP. There was a main effect of time, averaged over treatments for the milk fat yield, where the fat yield decreased over time.

Table 13. Milk yield, milk composition, body condition score (BCS) and body weight (BW). (n=36).

Item	Treatments		SEM	P-value		
	Silage	SP		Trt[1]	Time[2]	Trt[1]*Time
MY, kg/d	34.0	31.3	1.63	0.165	0.030	0.028
ECM, kg/d	37.0	32.5	1.72	0.013	0.012	0.093
Composition, %						
Protein	3.44	3.52	0.06	0.240	0.362	0.597
Fat	4.38	4.56	0.14	0.233	0.005	0.598
Lactose	4.90	4.92	0.04	0.754	<0.001	0.232
Yield, kg/d						
Protein	1.20	1.05	0.05	0.011	0.182	0.398
Fat	1.53	1.36	0.07	0.035	0.001	0.306
Lactose	1.72	1.53	0.10	0.053	0.608	0.206
BCS	2.68	2.73	0.04	0.379	0.195	0.216
BW kg	679	679	19.1	0.336	0.027	0.506

SP= Silage pulp, SEM = standard error of the mean

[1]Trt=treatment

[2]Time were evaluated differently for each variable. Milk yield and concentrate were evaluated for intake every week; ECM, milk composition and yield every other week; BCS and BW once a month.

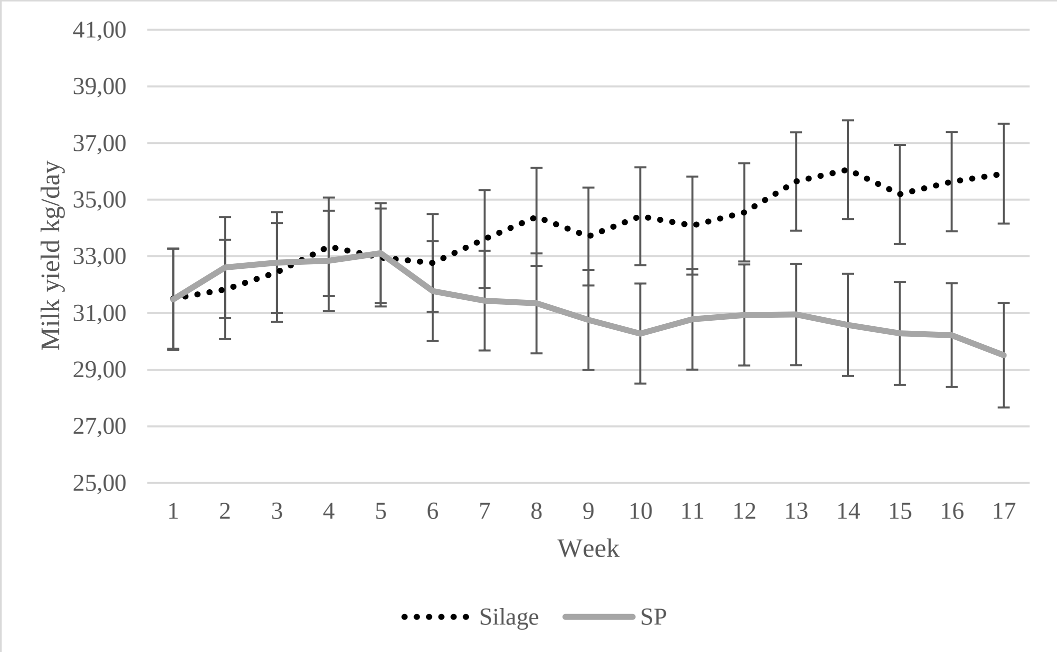


Figure 2. Milk yield kg, per day, for cows fed silage or silage pulp (SP). Error bars indicate standard error of the mean (SEM).

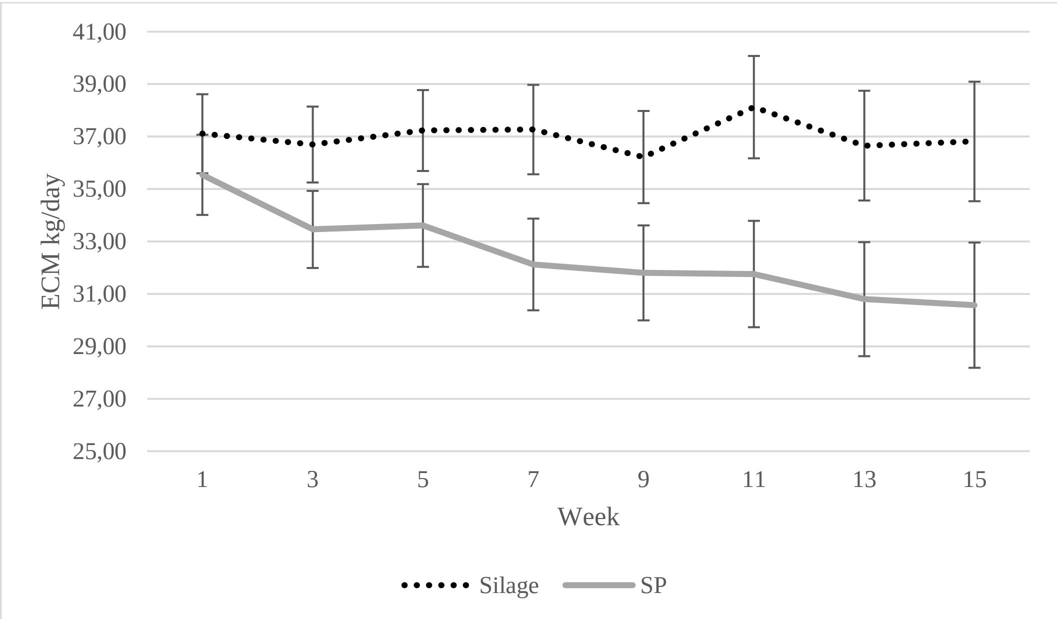


Figure 3. Energy corrected milk (ECM) kg per day for cows fed silage or silage pulp (SP). Error bars indicate standard error of the mean SEM.

4.3. Body weight and body condition score

There was no effect of treatments or its interaction with time on BCS (*P*=0.379 and *P*=0.216, respectively) and BW (*P* = 0.336 and *P* = 0.506, respectively) in this study (Table 13). There was a main effect of time on BW (*P*=0.027) averaged over treatments, the effect showed both an increase and decrease in BW over time and could be the variation in the heard itself.

5. Discussion

5.1. Feed composition and feed intake

The difference in the chemical composition of SP compared to silage was expected and was also seen in earlier studies (Kragbæk Damborg et al., 2019; Santamaria-Fernandez et al., 2018; Savonen et al., 2020). After pressing the silage in the screw press, the DM increased and the WSC concentration decreased in the SP because the water and WSC were extracted to the press juice. A decrease in crude ash is seen in this study and in other studies (Damborg et al., 2018; Savonen et al., 2020), because the minerals e.g. K, P and Cl follow the liquid to the press juice (Santamaria-Fernandez et al., 2018). The increase in fibres, NDF, ADF, in the SP was expected since only the soluble nutrients would be washed out into the press juice and therefor the other nutrients in the SP would increase (Damborg et al., 2018).

The CP in the SP decreased compared to the CP in the silage as it was washed out to the press juice. Even though the CP is partially extracted during the pressing, Damborg et al. (2018) explained that by physical processing the CP quality of the SP may be enhanced as the protein originally bound to the fibre will be released and can be used by the dairy cows. The proportion of the rumen soluble protein fractions (AB_1B_2) decreased in the SP compared to the silage and the cell-wall bound protein fractions B_3 and C and the rumen undegradable protein RUP_5 increased. The proportion of AB_1B_2 most likely decreased because fraction A was washed out to the press juice during the processing of the silage and then the proportions of the other fractions increased. Non protein nitrogen (fraction A) cannot be totally utilized in the rumen and can be converted into ammonia and be lost as urea-N in the urine (Givens and Rulquin, 2004). In the ensiling process of the grass-clover forage, fraction A increases because of proteolysis of true protein (Givens and Rulquin, 2004). So, a decrease in rumen degradable protein and an increase of rumen undegradable protein in the SP is desired when it comes to forage for cows. This is because the rumen undegradable protein can be digested in the intestine instead of the rumen and this reduces the risk of lost urea-N in the urine (Givens and Rulquin, 2004).

The average DMI of forage were numerically lower for cows fed SP compared to cows fed silage, except for the forage NDFI that were similar between the treatments. In the present study, forage intake of the silage diet was expected to be higher than for the SP diet. Diets were formulated aiming to have similar NDF concentration, however, due to the higher NDF concentration of SP, the SP diet resulted in higher NDF contents, likely limiting forage DMI by rumen fill. Similar forage NDFI between the treatments suggest that intake was limited by the filling effect in the rumen (Allen, 2000). The DM were higher for the SP than the silage and the workers on the farm experienced that the cows were not so fond of the SP and were often seen trying to reach the silage because it was more appetizing. So, the DMI could also be limited because of the higher DM in the SP since the cows seemed not so fond of the SP compared to the silage. In the earlier study of Kragbæk Damborg et al. (2019) the DMI for forage did not differ between silage and pulp but in their study, the silage was harvested one week later than the pulp because of rainy weather so the feed is not comparable. In the study of Savonen et al. (2020) the DMI decreased when the diet contained 25% SP of forage DM but did not differ when the cows were fed only silage or 50% SP and 50% silage of forage DM.

The forage OMI was numerically lower for cows fed SP compared to cows fed silage, which is expected since the DMI were lower for cows fed SP compared to cows fed silage. Since the cows eat less forage, the forage OMI should decrease with the forage DMI. The concentrate DMI were numerically higher for cows fed SP than for cows fed silage. This was expected since the calculated diet contained more concentrate in the diet for cows eating SP than for cows eating silage.

The total DMI, OMI and CPI in the diet were numerically higher for cows fed silage than for cows fed SP and the total NDFI were on average higher for cows fed SP compared to cows fed silage. Even though the average DMI was numerically higher for cows fed silage diet, the feed efficiency MY/DMI was similar and when calculated using ECM numerically higher for cows fed silage compared to SP diet. Feed efficiency is determined by the conversion of feed nutrients into milk production, and it is affected by the quality of the dietary ingredients, the diet formulation, and the nutrient absorption by the animal. Cows fed the silage diet receiving a diet that allowed cows to eat more, likely converting more nutrients into milk production.

5.2. Milk yield and milk composition

In the present study, there was an interaction between treatments and time for the daily MY. The MY was similar between the treatments until week 9, and also on weeks 11 and 12 (Figure 2). However, on week 10 and after week 13, MY were higher for cows fed silage compared to cows fed SP. There was also a similar pattern for the ECM, but the ECM were only similar in week 1 (Figure 3) and then the ECM were generally higher for cows fed silage than for cows fed SP. In the study of Kragbæk Damborg et al. (2019) the ECM were higher for cows given pulp instead of silage, but in this study, the silage was harvested a week later than the pulp, which makes the forages not comparable between each other, so it is hard to compare results on milk production to the present study. In the study of Savonen et al. (2020) the MY and ECM were not different between the treatments. Even though there was no statistical difference the author could see a numerically lower MY for cows eating SP compared to silage, they could also see a tendency to a linear decrease in ECM with increasing SP in the diet. The cows in the study of Savonen et al. (2020) were given SP in a proportion of 25 and 50 % of their total forage DM. This might be the reason why they did not get the difference in ECM and MY as we did in the present study, but they could see a tendency to a linear decrease in the ECM and a numerically lower MY in their study. So, based on the results of this study and the results of Savonen et al. (2020) it appears that the cows MY and ECM decrease when replacing all the silage with SP.

There was no effect on the milk composition (protein, fat and lactose) between the treatments in this study, but there was an effect on the yields of protein and fat in the milk. The cows eating silage produced more protein and fat (kg/day) than cows eating SP. There was also a tendency to more produced lactose (kg/day) when the cows were fed silage compared to SP. In earlier studies, the MY was not affected (Savonen et al., 2020) or higher (Kragbæk Damborg et al., 2019) for cows eating biorefined forage compared to cows eating silage. This might be the reason why the earlier studies did not affect the total produced protein, fat, and lactose in the milk. Even though in the study of Savonen et al. (2020), the total production of fat and lactose did not differ between the treatments but they could see a linear decrease in the total produced milk protein with increased SP in the diet. This is a similar linear decrease as the MY and ECM in their study, which resulted in decreased production of milk protein with increasing SP in the diet. Since the forage for the cows fed the SP diet only consisted of SP in the present study, it seems that the linear decrease seen in the study of Savonen et al. (2020) is visible in this study. As the cows eating SP had a lower MY than the cows eating silage, they were producing less protein, fat and lactose in the milk compared to cows eating silage.

The interaction between treatment and time on MY and ECM can be explained by the time factor. At the beginning of the experiment, the cows have had an adaptation period where they were eating the same diet to have similar milk yields between the treatments at the start of the experiment. Then at week 10 and after week 12 the MY decreased for cows fed SP and increased for cows fed silage, but this trend could already be seen at week 6 (Figure 2). The ECM were lower already after week 1 but the milk composition did not change over time between the treatments, which means that the change in ECM between treatments is most likely caused by the change in MY without affecting the composition of the milk.

The reason for the decrease in MY and ECM in this study is probably because of the numerically lower DMI for cows eating SP compared to cows eating silage. Even though the calculated diets have a similar nutrient composition cows eating SP had a numerically lower OMI and CPI and higher NDFI than cows eating silage (Table 12), which might indicate that the energy intake decreases for cows eating SP. The reason for the numerically lower DMI might either be because of the rumen filling effect of the increasing NDF in the diet, so the energy intake is suppressed, or it could be because of the lower energy concentration or utilization of the SP in the diet. But further studies must be made on the digestibility of the SP to deepen the understanding of how the SP affects ruminants.

The forage that was fed to the cows came from the same bunker silo and the SP were pressed every other day over time during the experiment. So, the effect of the SP compared to silage in terms of milk production should be accurate in this study.

5.3. Body condition score and body weight

In this study, there was no treatment effect or interaction with time on the cows BCS and BW. Since registration of BCS was done over time it is possible to get information whether that the cow´s nutrient intake was relative to the cow´s requirement (Roche et al., 2009). No significant treatment x time interaction on BCS in this study shows that the cows were milking on the feed that they were given and not from their body reserves when they were producing the milk regardless of which diet they were given.

5.4. Future research with biorefined fibre fraction to cows

By biorefining the silage, it is possible to produce both a forage feed (SP) to ruminants and a locally grown protein feed (press juice) to monogastric animals. This could then be a solution to replace imported protein feed, such as soybean, with green protein sources to monogastric animals and at the same time use the SP as a forage feed to ruminants. Since ley has a positive effect on the climate and environment it would be favourable to use its potential.

In this study, there was an interaction between treatment and time for the MY and ECM where the milk production was lower for cows fed SP compared to cows fed silage. Since the milk production was lower in the present study it might not be advantageous to only give SP to the cows, but an alternative might be to mix silage with SP to reduce the cost of the diet and maintain milk production. In the study of Savonen et al. (2020), there was no difference in MY and ECM when giving the cows 25% and 50% SP. Mixing SP and silage in the diet could also reduce the dietary NDF concentration that limited the forage DMI in this study.

But there is more to take into consideration before changing the diet to SP, for example, is there an increase in concentrate fed to the cows and how and where are the concentrate grown. In this study, one of the protein feed, faba beans (grown on the farm), was fed an amount on almost three times higher for cows fed SP than for cows fed silage. The increase of concentrate in the diet can also affect the total price of the diet because concentrate is usually more expensive than forage and this increase the total price of the diet to the cows. But if it is possible to sell the press juice as a locally grown protein feed to monogastric animals the total price for the diet might break even or even generate income to the farm.

For continued research it would be interesting to see more studies like this but where they might compare different proportions of SP in the diet and how it effects milk production. But it would also be interesting to see an economical evaluation and life cycle assessment of how SP and silage relate to each other.

6. Conclusions

Dry matter intake was numerically higher for cows receiving silage than SP diet. Milk yield and ECM were generally lower for cows fed SP compared to cows fed silage. The milk composition was not affected by the treatments, but the yields of milk protein and milk fat were lower for cows fed SP compared to cows fed silage. The lactose yield tended to be lower in cows fed SP compared to cows fed silage. The BCS and BW of the cows were not affected by the treatments. Further research has to be made in this area both on the milk production and the sustainability for the environment and the economy on the farm if biorefined silage for dairy cows is going to be implemented on the farms.

References

Allen, M.S., 2000. Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. J. Dairy Sci. 2000, 1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2

Contant, R.T., Cerri, C.E.P., Osbourne, B., Paustian, K., 2017. Grassland management impacts on soil carbon stocks: a new synthesis. Ecol. Appl. 27, 662–668. https://doi.org/10.1002/eap.1473

Damborg, V.K., Krogh Jensen, S., Weisbjerg, M.R., Adamsen, A.P., Stødkilde, L., 2020. Screw-pressed fractions from green forages as animal feed: Chemical composition and mass balances. Anim. Feed Sci. Technol. March 2020, 114401. https://doi.org/10.1016/j.anifeedsci.2020.114401

Damborg, V.K., Stødkilde, L., Jensen, S.K., Weisbjerg, M.R., 2018. Protein value and degradation characteristics of pulp fibre fractions from screw pressed grass, clover, and lucerne. Anim. Feed Sci. Technol. 2018, 93–103. https://doi.org/10.1016/j.anifeedsci.2018.08.004

Fogelfors, H., 2016. Vår mat. Författarna och Studentlitteratur, Lund.

Givens, D.I., Rulquin, 2004. Utilisation by ruminants of nitrogen compounds in silage-based diets. Anim. Feed Sci. Technol. 114, 1–18. https://doi.org/10.1016/j.anifeedsci.2003.09.005

Gustafsson, A.H., Bergsten, C., Kronqvist, C., Lovang, M., Månsson Lindmark, H., Lovang, U., Svensson, C., 2013. Närproducerat foder fullt ut till mjölkkor - en kunskapsgenomgång (Forskningsrapport No. 1).

Hermansen, J., Jørgensen, U., Laerke, PE., Boelt, B., Jensen, SK., 2017. Green Biomass – protein production through bio-refining (No. 093), DCA raport.

Huhtanen, P., Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Livest. Sci. Volume 148, 146–158. https://doi.org/10.1016/j.livsci.2012.05.023

Jordbruksverket, 2020. Jordbruksmarkens användning efter sördområde/riket och gröda, Serie JO - Jordbruk, skogsbruk och fiske.

Kirchhof, S., Eisner, I., Gierus, M., Sudekum, H., 2010. Variation in the contents of crude protein fractions of different forage legumes during the spring growth. Grass Forage Sci. 65, 376–382. https://doi.org/https: / / doi .org/ 10 .1111/ j .1365 -2494 .2010 .00756 .x

Kragbæk Damborg, V., Krogh Jensen, S., Johansen, M., Ambye-Jensen, M., Weisbjerg, M.R., 2019. Ensiled pulp from biorefining increased milk production in dairy cows compared with grass–clover silage. J. Dairy Sci. 102, 8883–8897. https://doi.org/10.3168/jds.2018-16096

KRAV, 2021. Regler för KRAV-certifierad produktion. 318 sidor. [WWW Document]. URL https://overbliq-cloud-images.s3.eu.cloud-object-storage.appdomain.cloud/regelboken/files/kravsregler_utgava2021_webb.pdf Nedladdning 2021-08-12

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 1996, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal nutrition, 7th ed. Pearson Education Limited, Harlow.

RISE, 2021. Vad är ett bioraffinaderi? [WWW Document]. RISE. URL https://www.ri.se/sv/berattelser/vad-ar-ett-bioraffinaderi (accessed 3.19.21).

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stanfford, K.J., Berry, D.P., 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92, 5769–5801. https://doi.org/10.3168/jds.2009-2431

Santamaria-Fernandez, M., Ambye-Jensen, M., Damborg, V.K., Lübeck, M., 2018. Demonstration-scale protein recovery by lactic acid fermentation from grass clover – a single case of the production of protein concentrate and press cake silage for animal feeding trials. Biofuels Bioprod. Biorefining 13, 502–513. https://doi.org/10.1002/bbb.1957

Santamaria-Fernandez, M., Lübeck, M., 2020. Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals. Anim. Feed Sci. Technol. 268, 114605. https://doi.org/10.1016/j.anifeedsci.2020.114605

Savonen, O., Franco, M., Stefański, T., Mäntysaari, P., Kuoppala, K., Rinne, M., 2020. Grass silage pulp as a dietary component for high-yielding dairy cows. Animal 14, 1472–1480. https://doi.org/10.1017/S1751731119002970

Sniffen, C.J., O'Connor, J.D.O., Van Soest, P.J., Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 1992, 3562–3577. https://doi.org/10.2527/1992.70113562x

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. 1991, 3583–3597. https://doi.org/https: / / doi .org/ 10 .3168/ jds .S0022 -0302(91)78551 -2

Appendix 1

Dietary ingredients according to days in milk.

	DIM			
	Silage		Silage pulp	
	1-100	101-365	1-100	101-365
Dietary ingredient, % of DM				
Forage	61	50	73	60
Mix of cereals	18.8	11.6	20.3	13.3
Faba beans	5.1	3.7	14.0	14.4
Pellets	14.4	10.8	14.4	10.8
Minerals	1.2	1.2	1.2	1.2

Acknowledgements

This project was part of the EU Interreg project Green Valleys, with funding from Interreg Öresund-Kattegatt-Skagerrak and Region Västra Götaland. I would like to give a special thanks to my supervisors Dannylo Sousa and Elisabet Nadeau for their help and support on my thesis. I would also thank the team in the cow stable at Sötåsen Agricultural High School for answering my questions and collecting the samples and data for this project. Thanks to Karin Stålnert and Torbjörn Lundborg at Växa Sverige for the help with calculations of the diets. Lastly, I also would like to thank my family and friends for supporting me during the writing of my thesis.

Popular science summary

Grasses and legumes can contribute to several ecosystem services and increase soil carbon. In Sweden, large parts of the cultivated land are ley and pasture and can today mainly be used by ruminants. Biorefinery of grass - clover silage, makes it is possible to use the ley for both ruminants and monogastric animals such as pigs and chickens.

In a green biorefinery, the silage is run through a screw press which presses the silage into a liquid part (press juice) and a fibre part (silage pulp). Concentrations of fibre and dry matter increase in the silage pulp, while much of the soluble protein and the water-soluble carbohydrates are extracted into the press juice. The locally produced press juice can replace other protein feeds, such as soybean meal, which are imported to Sweden. This study is part of the EU-project Green Valleys, which investigates several aspects of how to utilize biorefined forages.

In this study, a total of 72 dairy cows at the organic farm at Sötåsen Agricultural High School, Töreboda, Sweden, were divided into two different groups and fed either grass-clover silage or silage pulp from the grass-clover silage during the indoor period 2020–2021. The silage pulp was produced during the entire experiment from the same bunker silos, where the silage was taken. The forage was supplemented with mix of cereals (wheat and barley), pellets (Sund Vässa Mix, Lantmännen) and faba beans.

Results of this experiment showed generally a lower milk yield and ECM yield in cows fed the silage pulp diet than cows fed the silage diet. Milk composition did not differ between the diets but yields of milk protein and milk fat were higher for cows fed silage compared to cows fed silage pulp. Also, the lactose yield in the milk tended to decrease when the cows were fed silage pulp. During the experiment, the body condition score and body weight were measured, and these were constant throughout the study. The intake of silage pulp was lower, and the concentrate intake was higher when fed silage pulp compared to the silage. This was expected because the fibre content (NDF) in the silage pulp increased so much that it was limiting how much silage pulp could be included in the diet. An opportunity for the future, that has been seen in a previous study from Finland, is to some extent include the silage pulp in the diet and then be able to balance the diet to avoid large amounts of concentrate and avoid the lower milk production seen in this study.