Green biomass - protein production through bio-refining

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BIOVALUE SPIR

GREEN BIOMASS - PROTEIN PRODUCTION THROUGH BIO-REFINING

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Preface

The aim of this report is to summarize our present knowledge on the bio-technical as well as economic issues in relation to value creation of green biomass in Denmark. This includes many types of knowledge from the different types of actors included in activities going on at present in this field. To start the work, a kick-off workshop was held in Copenhagen in January 2016, where a range of stakeholders from many fields enthusiastically expressed their views and ideas as regards what to include and take into account in the report. We have tried to include these as far as possible. Thus a number of persons have contributed directly in the writing process whereas as others have contributed with particular overall insight.

Thus Uffe Jørgensen, Poul Erik Lærke, Kiril Manevski, Birte Boelt and Torben Asp has mainly contributed to Chapters 2 and 3; Morten Amby-Jensen, Mette Lübeck and Erik Fog mainly to Chapter 4; Søren Krogh Jensen and Martin Weisbjerg mainly to Chapter 5, Trine K Dalsgaard and Marianne Danielsen to Chapter 6; Mikkel Vestby Jensen, Morten Gylling, Claus Grøn Sørensen to Chapter 7; John E Hermansen to Chapter 7, 8 and 9, Jane Lindedam and all authors to Chapter 10.

Summary

Utilization of ‘Green biomasses’ for producing high quality feed proteins has been proposed as a mean to substitute other protein sources for monogastric animals and at the same time obtain environmental benefits when the production of green biomass substitutes cereal production. The aim of this report is to summarize our present knowledge on the bio-technical as well as economic issues in relation to value creation of green biomass in Denmark focusing on the resource base for producing and obtaining green biomass, the environmental impacts related to the production hereof, the concepts for bio-refining, the quality of the products produced and possible business cases.

Considering availability and quality of green biomass, grasses and grass-clover crops grown in rotation on ‘arable’ land shows a huge potential to deliver high yields of biomass as well as protein with an appropriate amino acid profile. For pure grasses the protein yield increases significantly with increased N fertilization without impairing protein quality. In grass-clover mixtures the importance of N fertilization is much lower. New initiatives on plant breeding to increase production and in particular protein production or persistence are going on, but the outcome of these initiatives is yet not clear. Grass from unfertilized permanent grassland may represent an opportunity if focus is on the fibre part of the grass. However if focus is on the protein part, it is required that the permanent grass is fertilized with nitrogen, which in some cases may counteract other environmental issues. For cover crops to be an attractive supply of biomass new production systems needs to be developed, eg by an earlier harvest of the main crop and use either fertilized or legumes cover crops in order to have a sufficiently high production to cover harvesting costs.

There is clear evidence that changing from winter wheat or maize to either grass-clover or fertilized ryegrass result in a decreased N- leaching and decreased green-house gas emissions, taken the difference in soil carbon storage into account. Only in the situation with very high N-fertilization to longer lasting grass field these benefits disappear or become less pronounced. The environmental benefit of using permanent wet grassland for production remains to be documented.

It is estimated that by the present technology for bio-refining 45% of the protein present in the green biomass can be recovered in a protein concentrate paste having protein content in the range of 47% of dry matter, similarly to the protein content of soya bean meal. In addition a fibre fraction containing 17% protein in dry matter can be produced and used for ruminant feed or energy production or even further bio-refined into chemical blocks or used for bio-materials.

Based on laboratory assessments, the protein concentrate is expected to be able to replace traditional protein sources for monogastrics, like pigs and poultry, but this is not confirmed in full scale feeding trials so far. Likewise, based on the chemical composition of the fibre fraction this seems suitable for

ruminant feeding replacing other types of silage, but this also remains to be documented in feeding experiments, which, however, is currently being performed.

There are major uncertainties in the economic assessment of establishing a full scale bio-refinery based on the concept mentioned above. Major obstacles are transportation costs and uncertainty in running cost for the bio-refinery. It will be very important that the energy use in the refinery can be partly or fully covered by the energy production based on the residual dry matter (mainly sugars) not present in the protein concentrate or the fibre fraction.

At the national scale it is estimated that there are obvious bio-technical options to produce green biomass that in turn can cover 25% of the Danish need for imported feed protein. Within the organic sector it is estimated that there are options to produce feed protein based on green biomass to cover three times the nutritional requirements for the Danish organic pig and poultry sector, thus representing a possibility for export.

A range of initiatives is taking place at the moment as private public co-operation in Denmark and other European countries in order to optimize the bio-refinery concept.

1. Introduction

In 2015 the Danish Center for Food and Agriculture published the report ‘Green biomasses’, highlightening some perspectives on producing high quality feed proteins from green biomass to substitute other protein sources for monogastric animals (Termansen et al., 2015). Subsequently, The National Bio-economy Panel published their recommendations on new value chains based on green biomasses, and the need for a broad update and evaluation of the present concepts and experiences on value creation based on green biomass. This is needed in order to qualify the knowledge and debate among central stakeholders in Denmark from authorities to business and NGO’s, and ultimately to qualify the future policy development within this area. While the aim is to evaluate the perspectives in a Danish context, relevant international experiences will also be included.

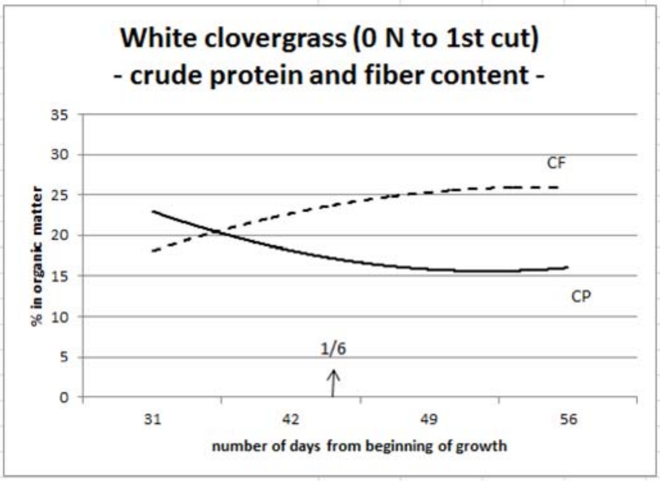
Thus, the aim of this report is to summarize our present knowledge on the bio-technical as well as economic issues in relation to value creation of green biomass in Denmark focusing on the resource base for producing and obtaining green biomass, the environmental impacts related to the production hereof, the concepts for bio-refining and the quality of the products produced.

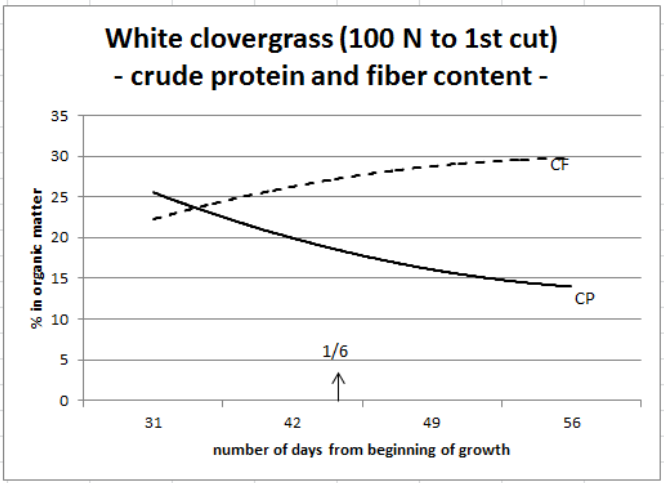
We limit the considerations to green biomass in the form of grasses and legumes harvested before maturity, where it is the vegetative parts of the biomass that are used for further value creation.

2. Availability and quality of green biomass

2.1 Characteristics of green biomass of importance for the biorefining

The chemical composition of green biomass changes significantly depending on the maturity of the vegetation. In grasses and clover the fiber content in dry matter increases while protein content decrease with increasing stage of development of plants. The changes are most pronounce in the beginning of the growth season. Fig 1 shows examples for white clover and grass.





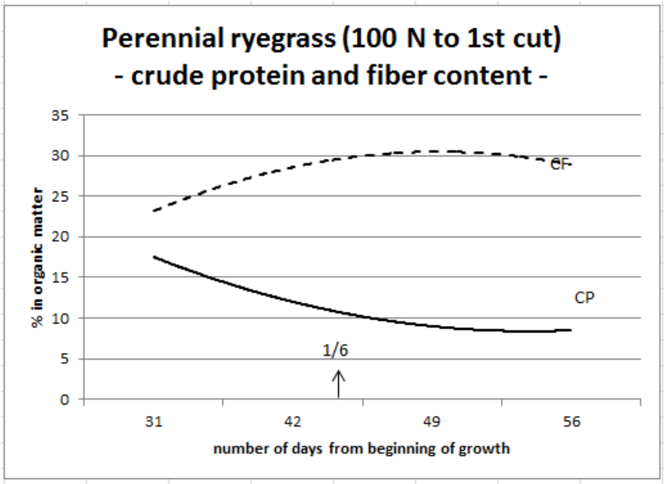


Figure 1. Changes in crude protein and crude fiber content by increased maturity of rye grass and grass-white clover with no N-fertilizer or fertilized with 100 kg N at the beginning of the growth season. (After Pedersen and Møller 1976).

The chemical composition and in particular the protein content depends on N fertilization. In Figure 2 is shown an example on the combined effect of N- fertilization and number of cuts (more cuts mean harvested at an earlier development stage) on biomass and protein yields over an entire season.

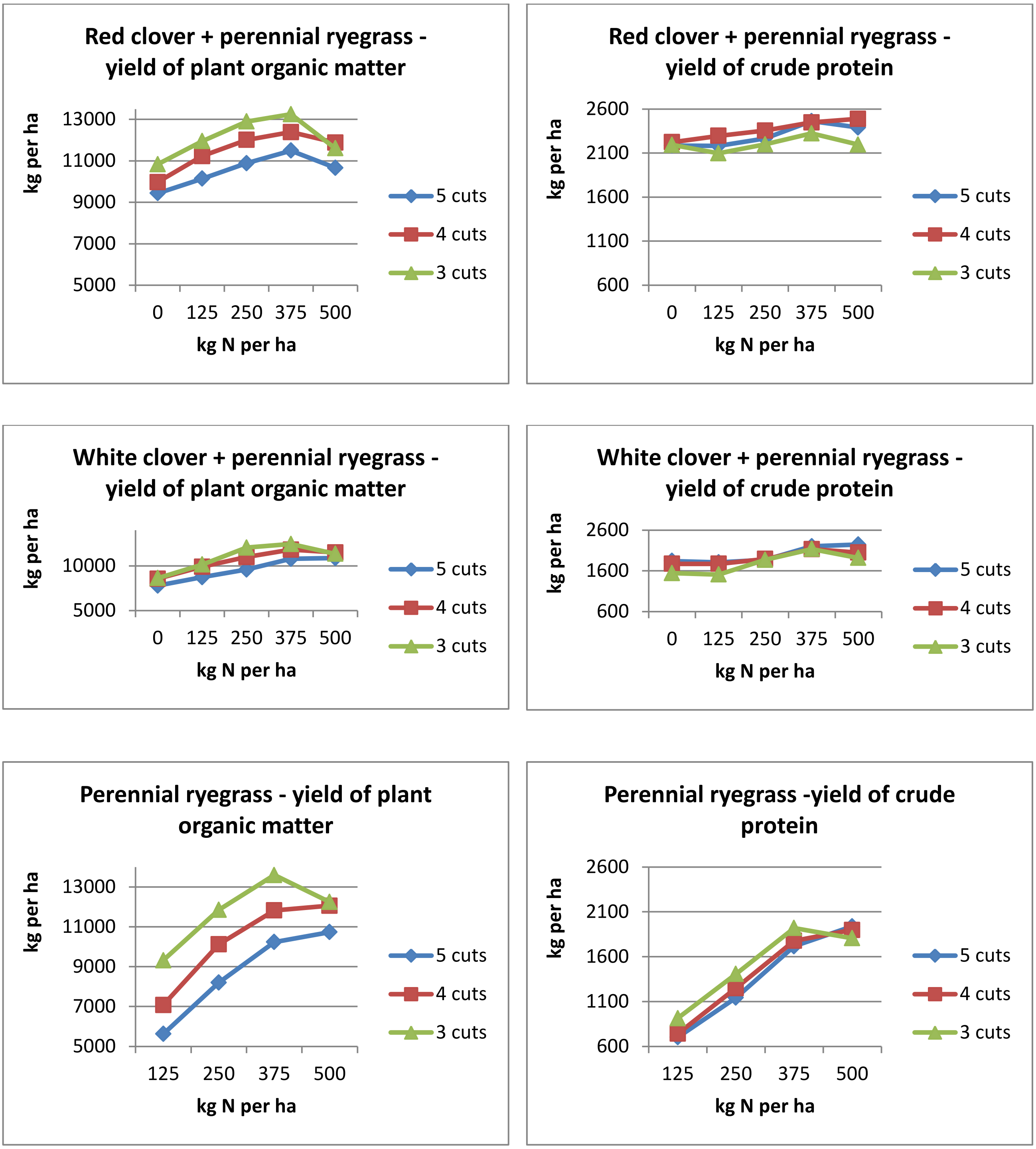


Figure 2. Yield of biomass and protein in a red grass-clover mixture and perennial ryegrass depending on N fertilization and number of cuts (After Pedersen and Møller 1976).

It appears that yield of biomass over an entire season does not depend very much on number of cuts, though 3 cuts typically yield the highest biomass. Likewise crude protein yield does not vary much dependent on number of cuts although it tends to be higher with 5 cuts in highly fertilized perennial ryegrass compared to three cuts. Also, while total protein yield are not influenced very much by N- fertilization in clover grass mixtures, the yield of protein in ryegrass is very much increasing following increased N-fertilization. Thus, the protein to carbohydrate ratio is high in grasses that are cut frequently and supplemented with N fertilizer, while protein content in clover grass only varies a little depending on N fertilization.

Sørensen and Grevsen (2016) investigated the influence of number of cuts in unfertilized crops of red grass-clover mix and white clover on total biomass and N yield over the season. Four cuts compared to two cuts per year resulted in a slightly higher N yield and a lower C:N ratio in the harvested biomass. Thus the C:N ration in red clover and clover-grass was reduced from 17 to 13 with four compared to three cuts. In white clover the changes were smaller.

The changes in chemical composition as illustrated above are important to take into account when deciding the production strategy for green biomass and considering what it is aimed for in the bio- refinery process.

When the focus is on achieving high value protein for food and feed protein from green biomass, the fraction of soluble and precipitable protein is the most important constituent. The influence of the production strategy on this fraction is not completely understood. However, Solati at al. (2016) showed that the proportion of soluble true protein in total protein did not change much over a large span of maturity, where total protein changed from 30 to 15% of dry matter, although the proportion was slightly reduced. More important was the type of crop, where red clover showed a significantly lower proportion of soluble true protein than did white clover, lucerne and perennial ryegrass. As appears from Figure 2 - and which is confirmed in more recent work – total protein yield per ha is typically higher in red clover than in white clover and moderately fertilized perennial ryegrass, but from a protein extraction point of view this may be counteracted by the lower solubility.

The work of Pedersen and Møller (1976) presented previously showed that the true protein fraction of total N also did not change much depending on fertilization and cutting strategy, though fewer cuts and a high N-fertilization tended to reduce the proportion of true protein to total N (2-4% units). The aspect of protein characteristics with different management is going to be investigated in more detail during 2016 at Arhus University and University of Copenhagen with the purpose to determine the relationship between plant development, plant chemical composition and yield with respect to precipitated protein, pulp and remaining soluble' s (brown juice).

The optimal composition for precipitated protein and pulp depends on several factors including plant material processed and processing efficiency and still needs final optimization, but roughly the precipitated protein contains 40-50% protein and around 40% carbohydrates of which the majority belongs to fiber carbohydrates. Likewise the composition of the pulp depends on the same factors and the chemical composition of this fraction is even more dependent on the composition of the starting material as variations in protein and fiber content is highly expressed in the pulp. Thus, low protein and/or fiber in the starting material give low protein in the pulp and vice versa. In the precipitated protein variations in starting materials is more reflected in the general yield of the fraction.

But for feed purposes not just the amount of protein is relevant: pigs have specific requirements for the amino acids, lysine, cysteine and methionine, whereas the poultry has a high requirement for the sulfur-containing amino acids, methionine and cysteine. Preliminary studies have shown that extracted protein concentrate from grass, clover, and lucerne have a favourable content of lysine and methionine, but a lower content of cysteine. The higher content of methionine compensates – in a nutritional perspective – for the lower content of cysteine. Thus the protein concentrate can as regards amino-acid composition substitute soy meal for broilers and laying hens (Table 1) providing a potential advantage of grass derived protein over soy. This has a big advantage in organic production systems where the use of synthetic amino acids is prohibited and today's widespread use of conventional potato protein concentrate is under pressure due to the coming requirement for 100% organic feeding. In this production system there is a huge undersupply of protein feeds with a high content of especially methionine and lysine (around 50% within EU) and only few organic produced protein feeds can meet the required composition. In this context grass and forage based protein concentrate has the possibility to fulfil this gap.

Table 1. Content of lysine, methionine and cysteine as % of total amino acid content in soy bean, compared to protein concentrate of white clover, red clover, lucerne, and ryegrass. Unpublished results from AU under the BioValue SPIR project (Vinnie Damgaard, Søren Krogh Jensen)

Amino acid (% of total AA)	Soy bean	White clover protein concentrate	Red clover protein concentrate	Lucerne protein concentrate	Ryegrass protein concentrate
Lysine	5.9	6.1	6.1	6.4	5.9
Methionine	1.1	1.9	1.9	1.8	2.1
Cysteine	1.6	0.7	0.9	1.0	0.9

2.2 Grass legume crops from arable land

Since arable land is a scarce resource globally a key issue is the land required to produce the feedstock for the bio-refining. Potentially, grass can produce more than annual crops due to their longer growing season and thus higher radiation capture in green foliage. This seems to be confirmed by Pugesgaard et al.(2015) where a grass-clover produced a mean yield of 14.8 tonnes/ha DM over 3 years, while the mean yield of winter wheat (grain + straw) was 10.7 tonnes/ha. In ongoing experiments grass yields have reached above 20 tonnes/ha DM, while annual crops have produced between 9 and 19 tonnes/ha DM (Jørgensen & Lærke, 2016; Jørgensen et al., 2016; Manevski et al., 2016). The higher interception of photosynthetically active radiation (iPAR) in grasses than in annual crops is shown in Fig. 3.

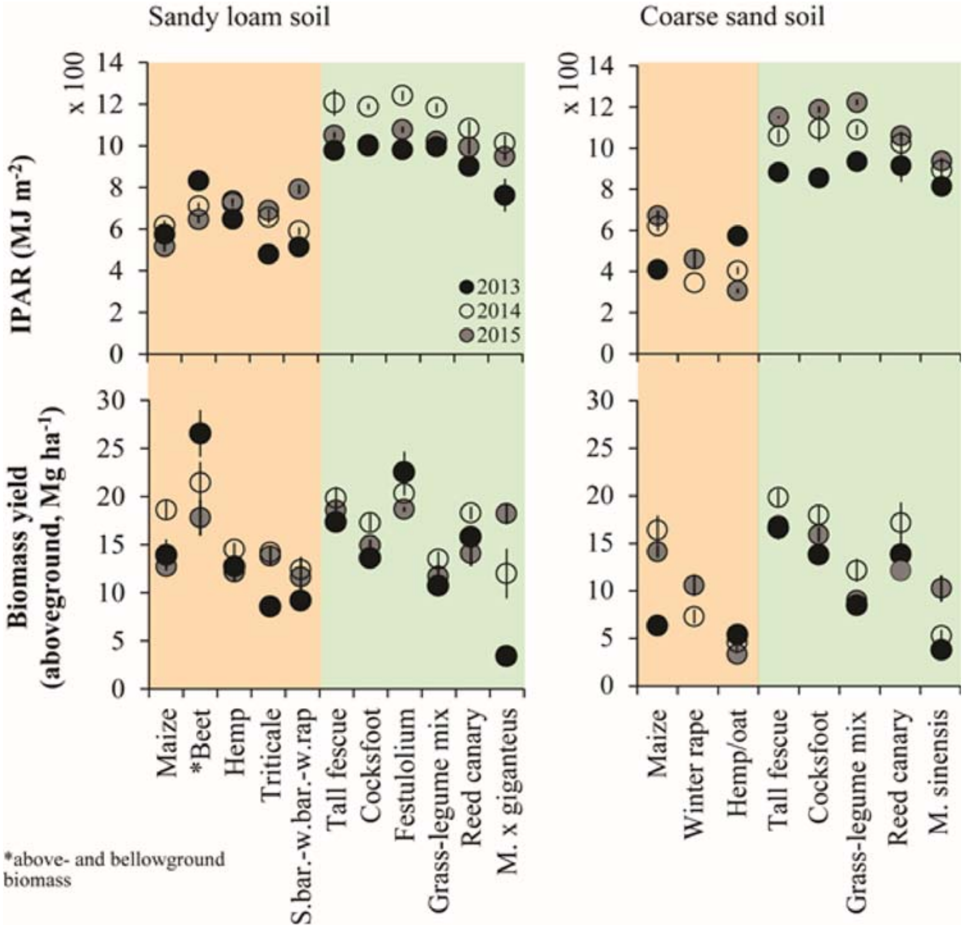


Figure 3. Interception of photosynthetically active radiation (IPAR) in annual (orange shade) and perennial (green shade) crops during 2013-2015 on two soil types at AU (from Manevski et al., 2016).

However, in practical agriculture grass crops are not always more productive than annual crops, which has a number of causes. Some reasons may be changed if grasses are to be used for biorefinery instead of direct animal feeding, while others may be difficult to change. In the following an overview of current yield correlations in agriculture is given.

Estimates of yield levels in Denmark of grass-clover (mixture 45 consisting of ryegrass, red clover, white clover and festulolium) and pure grass (ryegrass) are given in Table 2. These estimates are based on data from trials that are adjusted to yield levels in practice. Nitrogen response is based on recent fertilizer trials in the National Field Trials and at experimental stations (Madsen and Søegaard, 1991; Søegaard, 1994; Søegaard, 2004), and the yield level is set to norm yield at 2015 fertilization norms.

The level of yield is likely in many cases to increase in pure grass with 1-2 tons of DM/ha if other grass species than perennial ryegrass are produced, for example tall fescue or festulolium.

Grass yields most often decrease with number of years of age as also indicated in Table 2. How much yield is reduced over time is, however, very variable, and can be attributed to the species mix, weather conditions, fertilization and cutting frequency (Søegaard and Kristensen, 2015). In some cases only very little yield reduction is seen with time (Eriksen et al., 2004). There is a need for better understanding these processes, and to develop recommendations to sustain productivity over time.

Table 2. Dry matter yields of grass under a 4-cut strategy at different fertilization levels and at different ages of the grassland under practical farm conditions. Numbers represent net yield, i.e. net dry matter removed from the field (Olesen et al., 2016).

	Fertilisation (kg N/ha)	Yield 1st-2nd year (t DM/ha)	Yield 3rd-8th year (t DM/ha)
Grass-clover (mix DLF 45)	0	8.9	6.9
	240	11.5	9.5
grass (ryegrass)	150	9.1	7.1
	300	11.1	9.1
	450	12.5	10.5
	575	13.0	11.0

All studies behind Table 2 were conducted in plots where there was no tractor driving, but in practical grass-clover production at farms much traffic takes place through the season. Søegaard and Kristensen (2015) estimated a yield reduction of 1.2 tonnes DM/ha due to the traffic on farm grassland. Recent recommendations from the agricultural advisory service are therefore to try to run the traffic in grass fields on fixed trails. The effect of traffic on the annual decline of net grass yield has not been studied.

The grass-clover in the example in Table 2 is chosen to be DLF mixture 45, which is the most used highly productive mixture, and it includes both white and red clover. Red clover is not permanent, so the lower producing white clover will take over after a few years. This in itself will reduce the yield as white clover and grasses cannot compensate for the high red clover productivity. There is no basis for a more detailed estimation of yield decline over time. We have set it to be 0.7 t DM / ha for each year after the second year of use.

For comparison, Table 3 shows the standard yields for winter wheat and silage maize at the economic optimum fertilization level.

Table 3. Dry matter yields of winter wheat and maize whole crop by economic optimum level. There is no deducted after-effect of cover crops in the economically optimum level of nitrogen for maize. Based on Knudsen (2015).

Crop	Soil type	Fertilisation (kg N/ha)	Grain (t DM/ha)	Straw (t DM/ha)	Whole crop (t DM/ha)
Winter wheat	Sand (irrigated)	191	6.1	3.3[b]	
	Clay	207	7.5	4.1[b]	
Maize whole crop	Sand (irrigated)	192			13.2[a]
	Clay	174			13.5[a]

a) Total including straw. Calculated by a relation of 1.2 kg DM / FE
b) Calculated as 55% of the grain yield

It should be acknowledged that winter wheat typically is the highest yielding cereal and it only constitutes 40-45% of the total area with cereal. The average cereal yield in the period 2013-2015 was 5.7 t DM ha in grain (DS 2015).

Likewise, it is difficult to obtain good data on yield of forage crops in practical farming. Kristensen (2015) compared the realized yield at cattle farms of grass-clover crops and maize with the standard yield used for environmental planning. While there was a good agreement for grass-clover grass (realized yield approx. 400 kg DM per/ha lower than standard), for maize the realized yield was approx. 1600 kg DM lower per ha than standard. This probably reflects that yield of maize show a higher variation between years and dependent on local climate conditions than grass-clover, and thus for practical planning conditions the standard maize yield in Table 3 may be too high.

Except for white-clover and mixed crops containing white-clover the dry matter yield per ha typically decreases with the number of cuts (Figure 4). This is particularly the case with tall fescue showing the highest yield of the investigate species. However, at the same time the feed quality increases, which several studies have documented within the range of 3-7 cuts per year. Tests have shown that the optimal number of cuttings to produce a high quality feed for dairy cattle is five for mixtures containing red clover and festulolium or tall fescue, and four for mixtures that do not contain the aforementioned species (Videncenter for Landbrug, 2013).

As the optimum quality characteristics for bio-refinery are still unclear and total dry matter yield is also an important parameter this interaction needs further study, and is already part of ongoing research at Aarhus University and University of Copenhagen. The main aspect is whether the biomass is to be used for lignicellotic biorefining or for protein refining as discussed in chapter 4. With regards to protein refining the first result on protein quality variation as a function of cutting time and species are now published (Solati et al., 2016a) and submitted (Solati et al, 2016b) as well as on the variation in yield potential between cropping systems (Manevski et al., 2016). However, this need coupling with estimates on best performance set-up of bio-refinery concepts in order to be able to prepare full chain evaluations of optimal combinations.

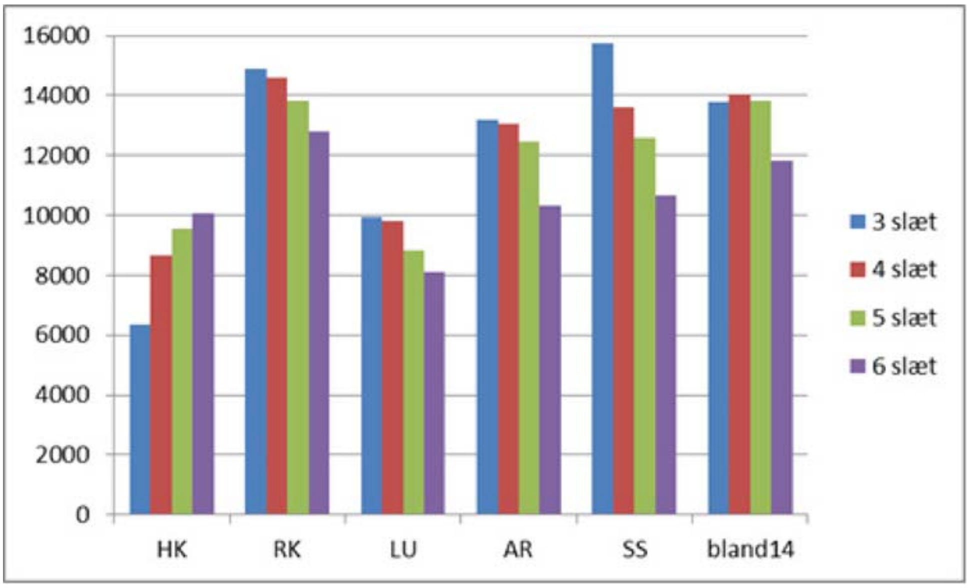


Figure 4. Dry matter yields (kg/ha) of grass and clover species with cut strategies from 3 to 6 cuts per season. HK: white clover, RK: red clover, LU: Preliminary results from ongoing results at AU-Foulum (Karen Søegaard, pers. Comm.).

2.3 The potential of cover crops

While growing grass or clover as a main crop on arable fields competes with other types of production, an alternative option could be to use cover crops in-between the cereal crops. It is mandatory in some cases to include unfertilized cover crops in the crop rotation as a mean to reduce nitrate leaching and this could be considered as a biomass resource for bio-refining. The present inclusion of cover crops in Denmark is approx. 200.000 ha. Several studies show, however, that, with current yields in practice (usually well below one ton of dry matter per ha), it is not profitable to harvest cover crops (Hvid, 2012).

Nonetheless, it may be possible to turn cover crop production into a business opportunity rather than just a legal obligation, which holds several perspectives (Jørgensen & Olesen, 2013):

- Farmers might be more focused on good cover crop establishment if the crop is to be harvested and used, resulting in a better function of the cover crop in relation to reduction of N leaching risks

- Total productivity of Danish agriculture will be increased, as today the cover crops are an unused biomass resource, albeit it has a nutrient value for the subsequent crop in the crop rotation
- New research indicates that the incorporation of cover crops into the soil releases significant amounts of the potent greenhouse gas nitrous oxide. Harvesting the top will likely reduce this problem.

It might be considered to fertilize the cover crop in order to increase dry matter yield. If cover crops with improved productivity are harvested and removed, their fertilization is unlikely to increase nitrate leaching. A short term study even indicated that a reduction in nitrate leaching may be achieved if the main crop is harvested early leaving 3 weeks longer growing season for the cover crops – even if the cover crop is fertilized (Jensen et al., 2016).

Another option could be applying N-fixing catch crops. This was investigated by Askegaard & Eriksen (2007) who tested a range of legume crops in comparison with a few non N-fixing crops (chicory, ryegrass, sorrel and fodder radish) (Table 4). There were significant differences between the above-ground catch-crop DM and N production. Dry matter production and N uptake in the catch crops were highest in Persian clover, kidney vetch, red clover, and black medic, and lowest in the non-legume group. Thus a significant harvest of dry matter could be achieved with the legumes crops, but with relatively low protein content (around 16%) No significant differences in N-min as an indicator of risk for nitrate leaching was observed in the work, but overall the legumes showed similar N-min as no catch crop, whereas the non-legumes showed lower concentration.

Table 4. Aboveground DM and total N as well as apparent N_2 fixation in the catch-crop species measured at the beginning of November, the corresponding N_{min} of the 0–100 cm soil layer, and the nitrate-N share of total N_{min} (average of years)(Askegaard & Eriksen, 2007).

	DM	Total N	N_2 fixation	N_{min}	Nitrate-N
Crop	($T\ ha^{-1}$)	($kg\ ha^{-1}$)	($kg\ ha^{-1}$)	($kg\ ha^{-1}$)	(% of N_{min})
No catch crop				24	66
Persian clover	2.7	64	52	25	44
Kidney vetch	2.6	67	56	16	29
Red clover	2.3	61	50	20	39
Black medic	2.0	61	49	16	29
White clover	1.8	55	44	22	32
Lupin	1.2	33	21	18	41
Rye/hairy vetch	1.0	39	28	19	37
Chicory	0.8	12		10	25
Ryegrass	0.6	13		13	31
Sorrel	0.5	10		12	28
Fodder radish	0.4	11		12	28
$LSD_{0.05}$	0.7	19	22	n.s.	13

In order to achieve the full potential of cover crops probably new overall growing concepts needs to be developed. This could be earlier harvesting of the main crop, fertilize or use of N-fixing catch crops for the cover crop, or increase distance of rows in the main crop in order to support the development of the cover crop when undersown. Earlier harvest of the main crop will require gas-tight storage of grain because the water content in the main crop is higher than at normal harvesting time. Additionally, it may be advantageous to apply strip harvest for the early harvest. By this method of ears and kernels are stripped from the straw (Madsen, 2000), which can then be harvested shortly after or later (Jørgensen et al. 2013). This will reduce harvesting costs and can provide a better feed quality. Strip harvesting is less dependent on the weather, and total yield of feed units are usually larger than if the grain harvested at full maturity with combine harvester.

Li et al. (2014) tested the effects of harvesting catch crops late October compared with the usual practice in an organic cropping system with no modification of the grain harvesting system. The apparent N recovery in the following spring barley varied significantly with type of catch crop (leguminous or not) and with harvest. Such effects of modified catch crop strategies will need to be implemented in the N-regulation where currently a general residual N-effect of catch crops in the following crop is given.

So far, the focus of using catch crops has mainly been on their use for biogas and, if protein extraction is to be pursued, more knowledge of content and extractability across cover crop species and management options must be achieved. A particular concern could be the presence of straw residues from the main crop that might impact on the juice extraction.

2.4 Biomass from non-productive areas

17% of Danish agriculture fields are located on lowland areas of which 108.000 ha are soil types with more than 6% organic carbon (Greve et al. 2014) and can be characterized as peatland. Because of climate changes with more precipitation (or changed precipitation patterns) and because of subsidizes to protect the peatland from drainage, it is an increasing challenge to use the lowland areas for traditional agricultural crop rotations and these areas may represent an opportunity for biomass supply for bio-refining.

2.4.1 Yield of fertilized permanent lowland grassland

The attainable yield of permanent lowland grassland depends on type of species and cultivars, age of stand, number of cut per year, and fertilization rates. On well-drained land fertilized permanent grassland is for a number of years after establishment expected to produce the same yield as grass in rotation. However, if not well drained, typically dry matter production is estimated to between 70 and 80% of grass in rotation (Nielsen 2012). However, if species tolerant to temporary flooding are cultivated in lowland areas, the yield may even be larger as no water constraints are limiting production. Recent experiments at shallow ground water table and temporary flooded conditions documented annual yields of reed canary grass, festulolium and tall fescue of 16-19 t/ha DM from two or three cuts in a year (Kandel et al., 2013; Kandel et al., 2016).

However, constraints in timing of harvest may limit actual yields as harvest equipment on soft lowland soil types are still at the developmental stage. Efficient harvest equipment has been developed by two Dutch companies (De Vries Cornjum, http://www.devriescornjum.nl/ and Hanze wetlands http://www.hanzewetlands.com/en), but more contractors are needed for reduction of harvest cost on the Danish market (Hyttel, 2015).

Nitrogen and protein content depends on availability of nitrogen during growth, developmental stage of the crop and thus number of cuttings in a season as described earlier. Frequent harvestings may counteract the decreasing nitrogen concentration at increasing developmental stage provided additional N fertilizer is applied after each harvest.

2.4.2 Yield of permanent grassland without N and P fertilization

If the grass sward is not fertilized, only a very moderate dry matter yield of 2-4 t/ha can be expected after a few years of harvest (Nielsen, 2012). In addition, grass from unfertilized meadow has normally low nitrogen and protein concentration and is therefore not suitable for protein extraction. In a study on biogas production, Dubgaard et al. (2012) found that without area support revenue and cost of producing biogas were roughly balanced, and they concluded that biogas plants were not able to pay a price for grass silage, which exceeds the calculated cost of harvest, transport and storage. Therefore the owners of meadow land have no immediate financial incentive to produce grass biomass for biogas production.

More recent investigations have shown slightly more positive results when relatively dry biomass (20-30% water content) were harvested on meadow areas with conventional harvesting machines for biogas production (EU Interreg projektet (BioM)). However, this type of grass is probably not suitable for protein extraction.

In conclusion, grass from unfertilized permanent grassland may represent an opportunity if focus is on the fibre part of the grass. However if focus is also on the protein part, it is required that the permanent grass is fertilized with nitrogen, which in some cases may counteract other environmental issues.

2.5 Harvesting and storage – impact on quality in relation to biorefining

From a protein perspective we are aiming at the highest possible content of soluble but precipitiable protein. The storage of unprocessed forage will therefore be a challenge, since protein degradation initiate within few hours after harvest and will continue until protein has been broken down into free amino acids and small peptides and protein residues, which cannot be precipitated. These substances will end in the brown juice from where - with present technology - they cannot easily be extracted. However they still represent a resource that can be exploited using other technologies than at present for precipitation.

Likewise ensiling, which is the common preservation method in traditional forage handling aimed for ruminant feed, will initiate the same degradation processes of the protein and will have the same drawbacks as mentioned before. However, this options is at present being explored in a large Finnish research initiative INNOFEED (http://www.ibcfinland.fi/projects/biorefining-ensiled-grass-into-i/) through new technologies. In the project, grass silage will be used to produce various products that can be used as feed, such as protein-rich and sugar-rich “feed juice” and single-cell protein. The cellulose of the silage will be broken down into sugars that will be used for producing feed protein with the help of *Paecilomyces variotii*. The nutritional value and preservability of feed juice products will be improved with the help of lactic acid bacteria. A range of applications are sought for a fiber-rich side stream from the production of biogas, for example, which enables energy production and returning nutrients to fields.

It is not possible for now to judge the practical prospects of this technology.
Another way to cope with the capacity challenges related to the seasonal production of grasses might be press the juice immediately and conserve the rest of the biomass, which is the main part, by ensiling for later processing.

2.6 Improvement potential by new varieties

So far the breeding of grasses have focused mainly on grass yield and feed quality for ruminants. This has led to a development of high-sugar grasses with a high uptake-efficiency in dairy cows. There is a need for a new focus on the variation in protein content and quality for monogastric animals as well as the extractability of the protein content in a biorefinery. The genetic variability of protein content, composition and extractability is so far only superficially analysed across the potential green biomass crops for biorefinery (Losche et al 2010; Maamouri et al 2015).
With the traditional breeding techniques 10-12 years of development are often required before a new variety is approved. Further, complex traits of importance for bio-refining such as protein production, drought tolerance and persistence are hard to improve by the traditional breeding techniques.

2.6.1 Improved protein production

The key to the creation of new crop varieties with improved protein production through bio-refining lies in the systematic exploration of genetic variation and the selection of new phenotypes. Traditional plant breeding relies on phenotypic selection for identifying individuals with the highest breeding value, but phenotypic selection has made little progress for complex traits, e.g. protein production, due to challenges in measuring phenotypes.

Genomic selection (GS), introduced in 2001 by Meuwissen et al (2011) presents a new alternative to traditional plant breeding that has enormous potential to actually improve gain per selection in a breeding program per unit time, and thus breeding efficiency. In a GS breeding schema, genome-wide DNA markers are used to predict which individuals in a breeding population are most valuable as parents of the next generation of offspring. These estimated values, termed the genome estimated breeding values (GEBVs), are output from a model of the relationship between the genome-wide markers and phenotypes of the individuals undergoing selection. The GEBVs are then used to select the best parents for making new varieties.

The advantage of GS over the widely-used traditional pedigree breeding method is thus one of breeding efficiency. Gain from selection during GS is proportional to GEBV accuracy. As a result, when GEBV accuracy is high enough, GS can reduce breeding time by increasing the proportion of high-performing offspring in a breeding population, thus accelerating gain from selection (Bernado, 2010; Heffner et al 2009). In plant breeding, Genotype x Environment interactions present a challenge, as does the presence of structure within and between breeding populations, but GS still holds the potential to improve breeding efficiency. In crops GS is expected to accelerate gain from selection per unit time.

GS thus provides a breeding strategy for improved protein production for bio-refining. The information is available not just for a single gene or trait, but for all genes and all traits at the same time, enabling a dramatic increase in the genetic progress for the development of improved varieties. Currently DLF and Aarhus University is actively pursuing this goal in a number of collaborative research projects.

2.6.2. Improved persistence and stress tolerance

The symbiotic interaction of endophyte/host holds a potential for improved bioresource production. The term 'endophyte' by definition includes all organisms that, during a variable period of their life, colonize the living internal tissues of their hosts asymptomatically. Fungal species of *Neotyphodium* are found as asymptomatic endophytes of temperate grasses in natural habitats, and they are transmitted via the seeds. Endophytes have been demonstrated to protect their host against aphids, beetles or insects (Scott & Schardl, 1993), increases performance under abiotic stress (Schardl et al., 2004) and some endophytes offered considerable protection against various plant diseases (Clarke et al 2006). Further there have been many reports on endophyte-induced effects on vegetative growth and production such as enhanced biomass production, tiller numbers, seed production, and root growth in many of the cool season grass species.

Currently, four classes of alkaloids are known from *Neotyphodium* spp. Of these, the ergot alkaloids and the lolitrems have a long-standing association with grazing animal toxicosis (Young et al.,2006; 2009) while peramine and loline alkaloids have received attention for their anti-insect properties (Schardl et al., 2007).

Grass varieties containing endophytes with no adverse effects on animals have been commercialized in recent years. Over a period of the last 10-15 years, this plant/endophyte interaction has developed commercially to such an extent where most grasses sown in New Zealand and Australia contain endophytes. Grass varieties containing endophytes are generally not in use in Denmark but they hold a perspective to increase productivity and persistence in grasses for biomass production due to their general protection against drought and against insects.

In Denmark DLF is actively breeding new grass varieties containing endophytes for increased stress tolerance and endophyte containing varieties are commercially available.

It can be concluded that grass and clover holds a great potential for the production of green biomass and the current development in new breeding techniques implies that complex traits such as protein production can be improved in future varieties according to the needs in the bio-refining industry. The potential of endophyte containing grass varieties is, however, until now un-explored in Denmark.

3. Environmental impacts related to growing

3.1 Grass and legumes in rotation

3.1.1 Leaching of nitrate

Pure cut-grass under unfertilized conditions has a marginal leaching (<5 kg N / ha) and by adding up fertiliser to the economic optimum for plant growth nitrate leaching is still quite low (<20 kg N/ha). Thus, Whitehead (1995) refers a number of studies that by adding up to 500 kg N/ha/year for grass showed no leaching above the above-mentioned low level. It agrees well with the Danish studies where leaching in the 4th-5th year ryegrass with supply of 300 kg N/ha was 12-20 kg N/ha (Eriksen et al. 2004). With increasing age of the pasture there was a tendency for increased leaching and the leaching in the 6th-8th ryegrass year was on average 38 kg N / ha in the same experiment.

In cut grass-clover, leaching under unfertilized conditions is found to be in the range of 15-20 kg N/ha, and not differing significantly with the age of the crop (Eriksen et al. 2004, 2015). Fertiliser application within the economic optimum for plant growth has only limited effect on nitrate leaching - in the range of 2-3 kg N/ha (Eriksen et al. 2015; Wachendorf et al. 2004). The more fertilizer that is applied to a grass-clover the smaller will become the clover content and nitrate leaching will approximate that of pure grass.

From the above, Table 5 summarises an estimated N leaching. It should be emphasized that this is an estimate, since there are no Danish experiments with the determination of nitrate leaching by increasing fertilizer application to grass or grass-clover with current agronomic practices. It is expected that the effects of soil type on leaching is only limited for grasses.

Table 5. Expected nitrogen leaching (kg N/ha/year) from cut grassland at different fertilisation and age (Olesen et al., 2016).

Pure grass			Grass-clover		
N-fertilisation	1.-2. year	3.-8. year	N-fertilisation	1.-2. year	3.-8. year
0	5	5	0	15	15
150	15	15	120	20	20
300	20	30	240	20	30
450	25	35			
575	55	70			

For comparison nitrogen leaching from grain and maize is shown in Table 6. The crops chosen to compare with are winter wheat and maize grown continuously. It is assumed that maize is grown with a cover crop, but often cover crop does not develop well in maize. The calculations in Table 7 are made with NLES4 using the same method as in Jensen et al. (2016). There is no data for maize in combination with cover crops in NLES4. It is not reasonable to assume the same effect of cover crops as in a cereal crop, since a cover crop in maize is not developed to the same level of N-uptake as in a cereal crop. Instead, the model calculations anticipate that the cover crop in maize has a similar effect as has a winter cereal crop in winter. The calculation includes the statutory pre-crop effect of cover crops of 25 kg N/ha to be subtracted from the following years N allocation.

Table 6. Nitrogen leaching in winter wheat and maize by economically optimal fertilization level (Olesen et al., 2016).

Crop	Soil type	Fertilisation (kg N/ha)	Leaching (kg N/ha)
Winter wheat	Sand (irrigated)	93 + 140*	79
	Clay	109 +140*	69
Maize	Sand (irrigated)	69 +140*	103
	Clay	44 + 140*	81

* Total N with manure

Comparing table 5 and table 6 it is clear, that grass production in almost all circumstances brings about significantly less nitrate leaching than the production of wheat and maize. Only, care should be given to reduce fertilisation levels of pure grass to the level of crop removal when the crop is older than 3 years.

Another issue is when the grass or grass-clover sward is ploughed after end use or for reseeding. At this point there is a significant risk for a substantial nitrate leaching, probably in particular for grass-clover swards. Eriksen et al (2013) showed however, that this risk could be reduced substantially if the grass-clover sward was followed by an unfertilized barley crop with under sown catch crop. Thus, the nitrate leaching was reduced by 66 – 80 % when the catch crop was included compared to no catch crop and an intensive tillage after harvest of the barley crop. The maximum reduction in nitrate leaching was obtained if the barley crop was harvested before maturity allowing the catch crop to develop better. In this case the leaching was reduced to approximately 10 kg N per ha. Therefore, in order in order to obtain the foreseen reduction in nitrate leaching at crop rotation or farm scales, the grassland need to be long-term, and/or very efficiently followed by catch crops when ploughed.

If a bio-refinery is established in a nitrate sensitive area it will be logical that much of the area is more or less permanently cropped with grass. We imagine that the grass is grown for 5-8 years, depending on how well yield reduction can be controlled. Then it is ploughed in spring, and spring barley with a ley crop of grass is established in order to enter a new grass cycle. We expect that such a system will be very efficient in keeping nitrate leaching low.

3.1.2 Nitrous oxide emission

Agriculture contributes 90% to the total Danish emissions of nitrous oxide (Nielsen et al., 2014). The emission is mainly due to the cycling of nitrogen in agricultural soil, where fertilizer, manure and crop residues are direct sources of nitrous oxide emissions, while ammonia and leached N are indirect sources. In the following assessment on what land use change means for these emissions, the latest revision of the methodology recommended by the International Panel on Climate Change (IPCC, 2006) is applied, and it is also the starting point for the national inventory of greenhouse gas emissions.

Emissions of nitrous oxide in a given year are linked to the land use (crop), fertilizer type (mineral or manure), nitrogen amount and method of application (manure), with a limited number of fixed emission factors linked to the various items.

The mineralisation of crop residues is an important source of nitrous oxide, and grasses develop a larger root biomass than winter wheat and maize. With perennial grass, however, the average annual contribution from this source becomes less important since only a limited part of the roots turn over each year. For the calculation of the contribution of plant residues, data from Mikkelsen et al. (2014) is used, and the amount of nitrogen from grass in rotation and grass outside the rotation is applied, respectively for 1-2 years of grass and 3-8 years of grass production.

The emission of nitrous oxide for winter wheat, maize, clover grass, and rye grass in Table 7 are calculated on the fertilizer levels as set out in Tables 5 and 6. A change in land use from cereals or maize to grass can, depending on the fertilizer level, lead to increased nitrous oxide emissions. The small increase in annual nitrate leaching with increased pasture age (Table 5) will give rise to a greater indirect emission of nitrous oxide, but it is offset by the less direct emissions from crop residues due to less frequent reestablishment.

Table 7. Emissions of nitrous oxide from the cultivation of different crops at different fertilization levels measured in both nitrous oxide N and CO_2 equivalents (Olesen et al., 2016).

Crop		Fertilisation (kg N/ha)	kg N_2O-N/ha/year	Ton CO_2-eq/ha
Winter wheat	sand (irrigated)	As in table 6	2.9	1.4
	Clay		3.0	1.4
Maize	sand (irrigated)		2.7	1.3
	Clay		2.3	1.1
Grass-clover	1-2 år	0	0.3	0.2
		240	2.8	1.3
	3-8 år	0	0.1	0.1
		240	2.6	1.2
Ryegrass	1-2 år	150	1.8	0.9
		300	3.4	1.6
		450	4.9	2.3
		575	6.3	3.0
	3-8 år	150	1.6	0.8
		300	3.2	1.5
		450	4.8	2.2
		575	6.2	2.9

Nitrous oxide emissions may be reduced by application of nitrification inhibitors. Meta-analyses have shown an average reduction of emission by 40-45% (Akiyama et al., 2010; Qiao et al., 2015). The cost of application together with fertiliser or manure is approx. 200 DKK/ha annually (H.S. Østergaard, personal communication). As this is a rather limited cost, which will have a large effect at the high levels of N-fertiliser necessary to support high protein production in high-yielding pure grasses, this can be an attractive measure to keep climate impact low even at high fertilisation and productivity. On the other hand, if grass clover mixtures or pure clover can deliver appropriate yields of total biomass and of protein with no or limited N-fertiliser, this will be the most environmental benign production method.

3.1.3 Carbon storage

By a transition from grain cultivation to grass there will be a rapid accumulation of carbon in the soil over the first few years, after which the rate will fall and the rate will be more constant. This is because, especially in the first year, there will be a very large build-up of carbon in the grass root system. Taghizadeh-Toosi and Olesen (2015) calculated an annual accumulation of carbon in the entire soil profile below productive grass around 2 tonnes C/ha/year in the first two years after conversion, but this slowed to an annual accumulation of approximately 0.6 tonnes C/ha/year in subsequent decades. The greater build-up of carbon in the soil in the first few years is not permanent, since it mainly consists of easily degradable material. Carbon accumulation in common productive pastures can be set to 0.6 t C/ha/ year. The annual build-up of carbon under the grass will continue over a very long period (over 100 years), and the measured carbon content in permanent grassland is typically 50 to 100% higher than for land with annual crops in rotation (Soussana et al., 2004).

Little is known about the effect of the composition of grassland, their fertilisation and cutting systems on carbon. The above mentioned carbon storage will probably apply to clover regardless of fertilization level, whereas carbon storage is estimated to be lower (half) at a low fertilization level in pure grass because production here is smaller and thus the supply of carbon to the soil also smaller (Table 8).

Table 8. Carbon storage in grass (t C/ha/year) at different fertilization levels and at different ages of grassland (Olesen et al., 2016).

	Fertilization (kg N/ha)	Year 1-2	Year 3-8
Grass-clover (DLF mix 45)	0	0.6	0.6
	240	0.6	0.6
Pure grass (ryegrass)	150	0.3	0.3
	300	0.6	0.6
	450	0.6	0.6
	575	0.6	0.6

Earlier there has been a common understanding that tillage was an important factor in soil carbon turn-over, and that its absence was one of the main causes of the higher carbon storage below perennial than annual crops. Although there may still be a small effect of tillage, there is now a growing consensus that this effect is very limited, and that the annual carbon input to the soil in crop residues and animal manure is the main determining factor for the soil carbon balance. Likewise the claimed positive effect of no-till farming on soil carbon seems rather to be a difference in carbon distribution across the soil profile than a difference in total carbon content (Powlson et al. 2014).

3.1.4 Changes in climate and environmental profile by growing grass

Table 9 shows the calculated change (based on the former tables) in yield, N- leaching and greenhouse gas emissions on clay soils by replacing winter wheat with grass of different types and varying age under current production conditions for cattle feed. Only by cultivating pure grass with 450 kg N/ha or more, higher yields are obtained in the grass than in winter wheat (grain and straw accumulated). In general a reduction of N-leaching of 40-50 kg N/ha is obtained, except at the very highest levels of N-fertilization in pure grass, in which case there is no reduction in N-leaching. The reduction in greenhouse gases is about 2 tonnes of CO_2-eq / ha, but falls at the very highest level of nitrogen in the pure grass if not nitrification inhibitors are applied. Nitrous oxide emissions are less from clover and therefore the reduction in greenhouse gas emissions here are about 2 tonnes of CO_2-eq/ha greater.

Table 9. Changes in annual dry matter yields, N- leaching and net emissions of greenhouse gases (carbon storage and nitrous oxide) by changing from winter wheat (grain + straw) on clay to grass (Olesen et al., 2016).

Crop		Fertilisation (kg N/ha)	Change in DM yield (tonnes/ha)	Change in leaching (kg N/ha)	Change in GHG emission (tonnes CO_2-eq/ha)
Grass-clover	1-2 år	0	-2.7	-54	-3.4
		240	-0.1	-49	-2.3
	3-8 år	0	-4.7	-54	-3.,5
		240	-2.1	-49	-2.3
Ryegrass	1-2 år	150	-2.5	-54	-1.6
		300	-0.5	-49	-2.0
		450	0.9	-44	-1.3
		575	1.4	-14	-0.6
	3-8 år	150	-4.5	-54	-1.7
		300	-2.5	-39	-2.1
		450	-1.1	-34	-1.3
		575	-0.6	1	-0.7

As mentioned earlier the average yields of cereals in Denmark are approx. 15% lower than that of winter wheat corresponding to 1.5 ton/ha, and e.g. spring barley, that is grown on approx. 500.000 ha, will typically have a yield of 3.0 ton total biomass per ha less than winter wheat. Thus compared with spring barley, the dry matter yield will in most cases be superior in the fertilized grass or grass- clover.

Table 10 shows the calculated change in yield, N- leaching and greenhouse gas emissions on sandy soil by replacing whole crop maize with grass of different types and varying age under the present production conditions for cattle feed. The high yield in maize caused it in all cases to give higher yields than grass. There is a general reduction in N- leaching of 70-80 kg N/ha, except at the very highest N level in pure grass where the reduction is only half of that. The reduction in greenhouse gases is about 2 tonnes of CO_2-eq/ha, but falls at the very highest level of nitrogen in the pure grass if not nitrification inhibitors are applied. Nitrous oxide emissions are less of clover and therefore the reduction in greenhouse gas emissions here is about 2 tonnes of CO_2-eq/ha greater.

Table 10. Changes in annual dry matter yields, N leaching and net emissions of greenhouse gases (carbon storage and nitrous oxide) by changing from whole crop maize to grass on sandy soil.

Crop		Fertilisation (kg N/ha)	Change in DM yield (tonnes/ha)	Change in leaching (kg N/ha)	Change in GHG emission (tonnes CO_2-eq/ha)
Grass-clover	1-2 år	0	-4.3	-88	-3.3
		240	-1.7	-83	-2.1
	3-8 år	0	-6.3	-88	-3.4
		240	-3.7	-83	-2.2
Ryegrass	1-2 år	150	-4.1	-88	-1.5
		300	-2.1	-83	-1.9
		450	-0.7	-78	-1.2
		575	-0.2	-48	-0.5
	3-8 år	150	-6.1	-88	-1.6
		300	-4.1	-73	-2.0
		450	-2.7	-68	-1.2
		575	-2.2	-33	-0.6

As mentioned earlier (section 2.2) these calculations are on norm yields, which might be overestimated for maize (approx. 1 ton DM per ha) due to the higher climate dependent variability. Nevertheless, the main benefits for the change in production would be reduced leaching and GHG emissions and a higher protein production.

It should be noted, that the above calculations are with current yields of crops in practical agriculture. There seems, however, to be a higher yield difference between the most productive grasses and grain crops, which is not captured by the current management strategies in agriculture. Accordingly, ongoing experiments at AU have shown approx. twice as high yields in pure grass as in wheat and barley in some years, and so far higher yields in the grasses than in grain crops in all years, while maintaining a reduced risk for nitrate leaching (Fig. 5).

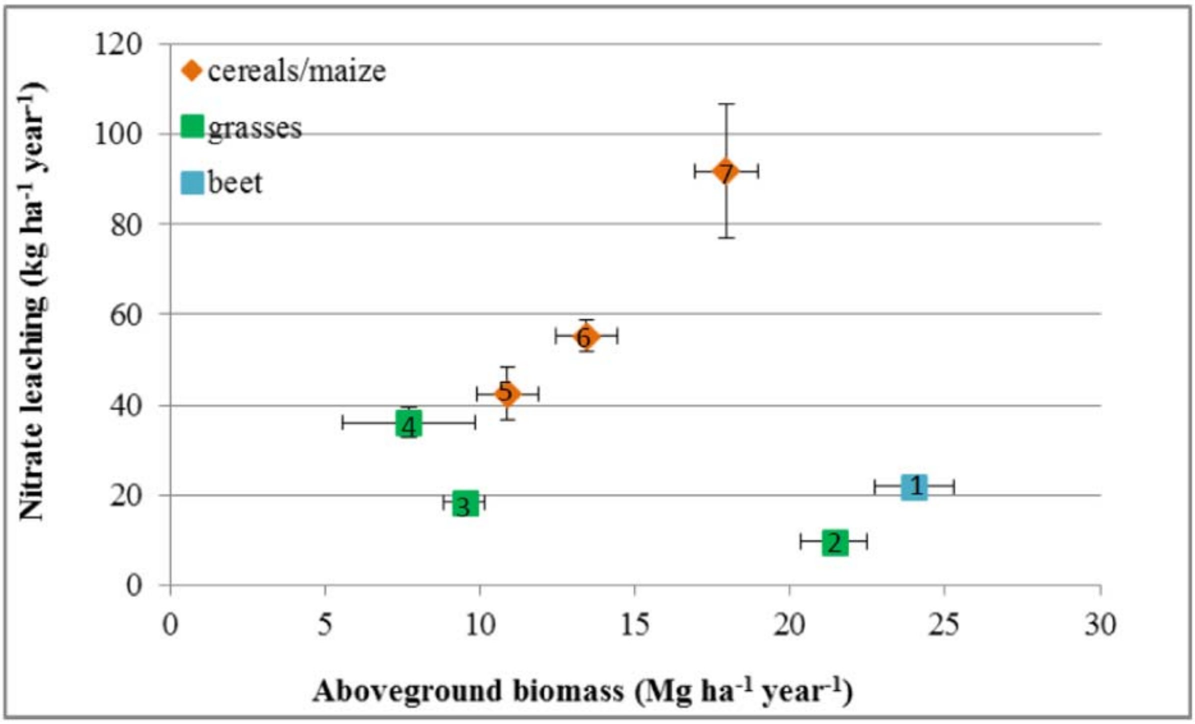


Figure 5. Mean nitrate leaching (based on 2013-2015) versus biomass yields for 1) beet, 2) festulolium, 3) grass-legume mix, 4) miscanthus, 5) barley, 6) wheat/triticale, 7) maize, for sandy loam soil at AU Foulum. Bars indicate +/- SE (unpublished results).

3.1.5 Pesticide use

Plant protection measures for both cereals and grasses minimize yield losses in relation to weed, pest and disease management. Due to the fewer natural pests, grasses require fewer pesticides compared to grain cereals and maize. According to the European Environmental Agency, perennial grasses grown for industrial purpose pose rather low environmental risk in relation to pesticide pollution of soils and water, whereas maize and some grain cereals are estimated to pose a moderate-to-high level of environmental risk (EEA, 2007).

In Denmark the mean pesticide treatment index for agricultural crops was 3.17 in 2011, covering over a variation from 0.20 in grass and clover to 17.82 in vegetables (Jørgensen et al., 2013). Rapeseed has so far been the main energy crop in Denmark used for biodiesel production, and it had a treatment index of 3.83 in 2011. Beets may be interesting for energy production due to their high productivity, but they had a pesticide index of 3.79. Grass and clover are thus the by far less pesticide treated agricultural crops today, and they can quite easily be grown organically if so wished.

A study on miscanthus from Germany concludes that the crop requires intensive care during its early years of establishment, and much less pesticide inputs thereafter, though it is also pointed that miscanthus may serve as a refuge or host for some important pests for cereals (Bunzel et al., 2014).

3.2 Permanent grassland on drained peatland

3.2.1 Without raising the water table

When the biomass is harvested and removed, nutrients are exported from environmental sensitive areas. The actual effect on leaching of N and P to water bodies is unknown but will depend on water flow in the area.

It is very difficult to establish uniform responses of fertilisation of lowland grassland on nutrient losses. The areas are very inhomogeneous, where some deliver a high amount of nutrients from peat mineralisation and fertilisation should be restricted. However, often lack of Potassium is restricting growth and application of only this has increased yield significantly and as well the correspondent removal of N and P with the biomass (Nielsen et al., 2013).

From well-drained lowlands there may be a risk of nutrient losses, but grassland with well-balanced fertilisation is probably the best option for reducing this risk. From less well-drained lowland areas there is most often significant denitrification taking place, which will reduce nitrate leaching in any case. However, significant P-mobilisation may occur under anaerobic conditions (Kjaergaard et al., 2012) and the best way to remove this is by growing a productive crop (Jørgensen & Schelde, 2011). In conclusion, it is impossible to give general estimations of losses from lowland areas, and each site needs to be evaluated separately.

The GHG emission from drained peatland is estimated to 25 ton CO_2-eq ha^{-1} y^{-1} (Nielsen et al., 2015). If these areas were to be used for perennial grass production there are no indications that GHG emissions will be significantly reduced from the land.

3.2.2 Raising the water table of drained peatland

Paludiculture is the term for a production system that combines rewetting and biomass production with flooding tolerant crops (Tanneberger & Wichtmann, 2011). Rewetting of formerly drained peatlands is a suggested mitigation option in terms of reducing CO_2- emissions and restoring the ecosystem carbon sink function (Joosten et al., 2012). In this context, rewetting of drained peatlands has been included as a potential target for climate change mitigation in the Kyoto protocol (IPCC, 2014). Paludiculture has further been suggested as a promising option to reduce anthropogenic CO_2 emissions from peatlands, while at the same time facilitating continued agricultural biomass production (Tanneberger & Wichtmann, 2011). In a Danish context it is estimated that rewetting of drained peatlands will reduce GHG-emission by approx. 13 ton CO_2-eq ha^{-1} y^{-1} (Nielsen et al., 2015).

In addition to effects of rewetting and paludiculture on GHG emissions, associated effects on potential nutrient discharges to water bodies are likely. The environmental effects of a raised water table will lead to changes in leaching of nutrients as soil redox conditions are decreased due to restricted oxygen (O_2) diffusion. In this context nitrogen (N) and phosphorus (P) biogeochemical processes are of special interest.

Anaerobic conditions favor denitrification, i.e., microbial removal of nitrate (NO3-), possibly in competition with plant NO^3- uptake (e.g., Kaye and Hart, 1997). On the other hand, anaerobic conditions decrease the adsorption of P to iron (Fe) and manganese (Mn) oxides due to microbial reduction of these minerals (Hoffmann et al., 2009). Consequently, P may be released to the soil solution and discharged to downstream vulnerable recipients. Indeed, paludiculture may encounter the same environmental problems as agricultural soils being re-established as pure wetlands, which is due to the elevated amounts of nutrients in the topsoil (Geurts et al., 2008; Kjaergaard et al., 2012). Yet, the P uptake by harvested and exported crops in paludiculture may mitigate the high P mobilisation at least during the growing season (Zak et al. 2014). However, the processes of denitrification and P mobilisation both need further quantification to address the environmental sustainability of drained peatland areas turned into paludiculture.

In conclusion the environmental impacts of using permanent grassland for production on wet areas remain to be documented.

3.3 Environmental effects of increasing productivity and harvesting catch crops

The large areas of cover crops that are currently not used may be an interesting additional biomass source in the case that greater yields by earlier harvesting of the main crop can be obtained and that possibly fertilization of the catch crops are allowed (Kristensen & Jørgensen, 2012). In the analyses behind the "+10 million. tons plan", it was assumed that the earlier establishment of the catch crops, fertilization in some cases, and harvesting the aboveground biomass, overall will not change the nitrate leaching compared to today's practice (Jørgensen, 2012), but this remains to be documented. Some results though point to the fact that leaching may be even reduced, when earlier growth of undersown cover crops is obtained by early harvest of main crop and fertilisation of the cover crop (Jensen, 2016).

Increased productivity and utilization of biomass crops would also affect the various contributions in the greenhouse gas accounts for cover crops. Nitrogen in crop residues from cover crops contributes to significant nitrous oxide emissions when the crops are ploughed under (Olesen et al., 2013) and these emissions offset or exceed the reduction in nitrous oxide emissions, which are calculated as a result of reduced nitrate leaching (Table 11). However the nitrous oxide contribution from cover crop residues may be reduced by harvesting the aboveground crop, which may though in turn reduce soil carbon build up. By increasing the productivity of cover crops the amount of root biomass will be increased as well, and it is difficult to assess what the net result on greenhouse gas emissions will be.

The above complexity is exemplified by the results from Li et al. (2014), who, surprisingly, did not measure a decrease in nitrous oxide emissions after harvesting catch crops late October compared with usual spring ploughing. This may be due to root leakage of N and C after harvest, which supports nitrous oxide emissions. This shows that more detailed process understanding needs to be obtained, as well as further optimization of crop management systems.

Table 11. Reduction in GHG emissions (kg CO_2 eq / ha / year) calculated for cover crops on sandy and clay soils at the current practice and without harvesting of biomass (from Jørgensen et al. (2013)).

Process	**Sand**	**Clay**
Nitrous oxide from saved N-fertilisation due to reduced N-norm	94	94
Nitrous oxide from reduced ammonia evaporation (due to reduced N-norm)	1	1
Nitrous oxide from crop residues	-323	-155
Nitrous oxide from reduced nitrate leaching	115	55
Total nitrous oxide reduction	-113	-5
Soil carbon storage from cover crop biomass	733	733
Total greenhouse gas reduction from cover crops	**620**	**728**

4. Bio-refining

4.1 Idea

The most productive grass species can utilize approx. double the solar radiation annually compared to annual grain crops, and thus at least theoretical produce approx. a much higher amount of biomass per ha. Thus, the major challenge is to extract desired components from the green biomass in a cost-efficient way, and to valorize all side streams of the refinery as well. While the idea of utilizing leaf-protein-concentrates as a protein source for animal or human consumption is not new (Pirie, 1987; Chiesa & Gnansounou, 2011; Houseman & Connell, 1976; Näsi & Kiiskinen, 1985; Pisulewska et al, 1991), recent advances in bio-refinery technology may now allow for efficient logistics, fractionation and extraction, and at the same time exploit new valuable components in the biomass creating an overall viable process.

Figure 6 shows schematically how processing of fresh grass can take place and produce a spectrum of different products. The process involves fractionating fresh grass into a juice and a fibre fraction, wherefrom high quality protein concentrate for the monogastric livestock industry can be extracted from the juice, and a grass fibre fraction that can be used for ruminant feed, biogas, or further biorefined into chemical building blocks or used for biomaterials. All of these products are in high demand of suitable, affordable, and environmentally sustainable feedstocks with documented interest by the respective target industries.

An example of a high value product from the fibre is xylooligosaccharides (XOS) with a prebiotic effect in food/feed applications. The effect of XOS depends on the length of the oligosaccharides and such a product has been shown to be refined from the fibres using a specific pretreatment process and has in pig gut simulation trials shown very promising results with respect to up concentration of healthy gut flora (Jurado & Ahring, unpublished results).

Since as the residual fibres can be considered as lignocellulosic biomass (see figure 6), the applications are similar to other such biomasses that can be pretreated and enzymatically hydrolysed to generate a sugar platform for fermentation into different products, including bioethanol, other fuels, biochemicals and so on (Amore et al. 2016) . The remaining lignin from such processes can also be regarded as a resource from which several products can be obtained. This is further investigated in the Danish SPIR project BioValue.

The first fractionation is performed by pressing the green biomass using screwpress technology. This will separate the fresh grass and grass/clover into a press juice containing soluble proteins and other soluble plant components and a fibre fraction characterised by increased dry matter and reduced protein, soluble carbohydrate and ash content. The proteins in the juice can be precipitated by heat coagulation and/or decreasing pH and separated by centrifugation or filter separation technologies producing a wet protein paste with a dry matter around 30% and a protein content of 35-45%.

Through the Danish OrganoFinery project, a fermentation technology using addition of a specific lactic acid bacterium for precipitation of the proteins in the juice was developed (Kiel et al. 2015). A potential benefit of this technology is that the resulting protein paste also contains 5-7% of lactic acid reported to be beneficial for the gut health of poultry and pigs as well as reports have indicated that some of these mild organic acids can lower the total amount of feed needed with same growth (Jørgensen et al., 2001). The protein paste can potentially be fed directly into wet feeding systems, or can be dried to a stable storable protein product suited for formulation and distribution in the global feed/food market. Further processing of the protein paste, where e.g. other plant components are removed, would lead to even higher protein concentration in the product, increasing feed quality and thus product value.

The residual juice containing primarily water soluble carbohydrates, organic acids and minerals can be utilized as an easily digested biogas substrate and subsequently used for fertilization alternatively it could also be spread on the field as it is. The latter is currently advertised by the company Biofabrik (www.biofabrik.com). The residual juice could also serve as a nutritional substrate for different fermentation applications, such as for lysine production (Thomsen et al. 2004), or potentially used for direct extraction of valuable compounds such as vitamins, phytoestrogens and other active biochemicals for health or cosmetic purposes (Azmir et al. 2013).

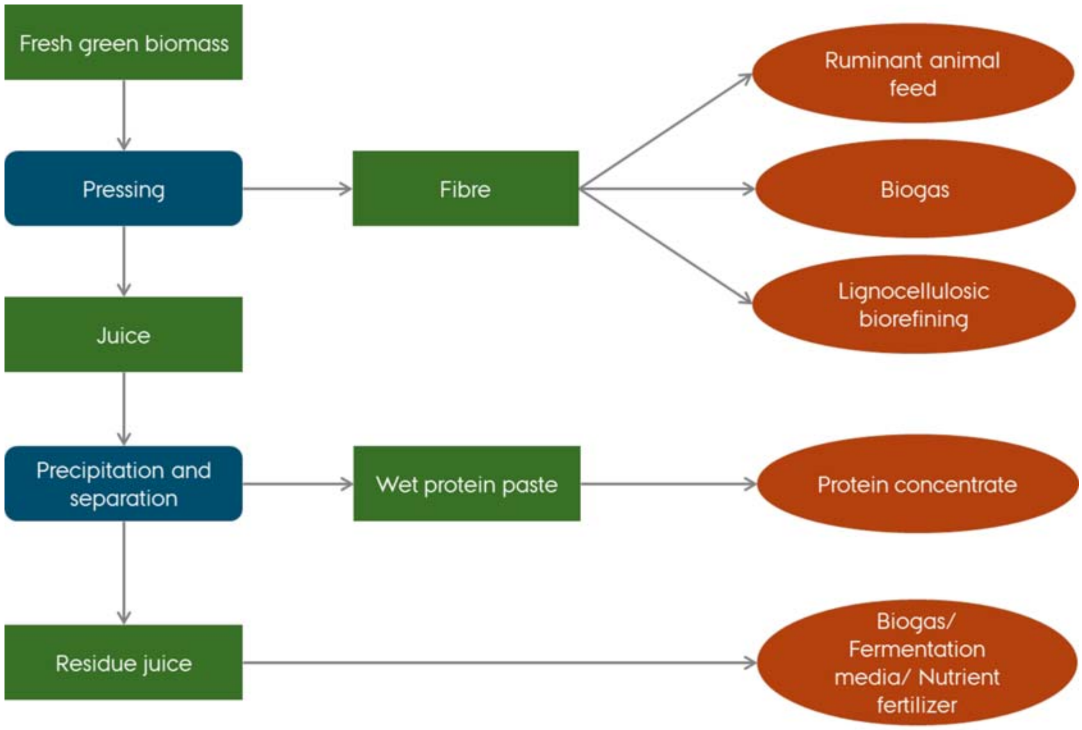
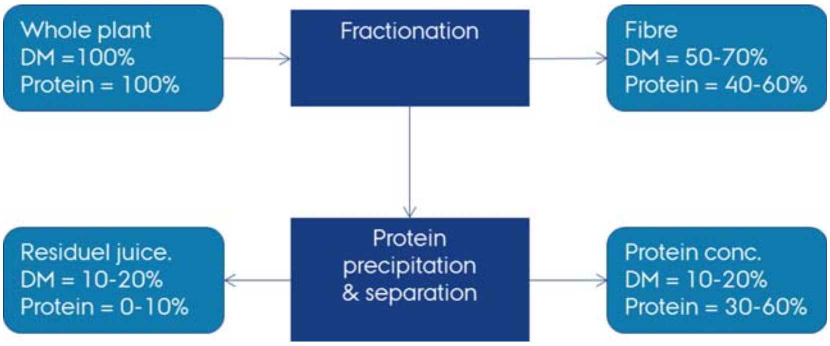


Figure 6. Schematic overview of possible products from biorefining of fresh green biomass

4.2 Example with focus on protein for monogastrics and fiber fraction for ruminants

The protein content of grass biomass depends on type of crop, plant maturity at harvest and N fertilization. When the focus is on achieving high value protein for food and feed protein from green biomass, the fraction of soluble and precipitable protein is an additional important characteristic. As mentioned earlier the influence of the production strategy on this fraction is not completely understood, but it seems that the proportion of soluble true protein in total protein did not change much over a large span of maturity where total protein changed from 30 to 15% of dry matter, while red clover compared to most other crops had a lower proportion of soluble true protein (Solati et al 2016).

Figure 7 shows an example of the typical range of yield of different fractions following a separation process. Depending on the efficiency and technology used in the plant, between 50 and 70% of dry matter and 40-60% of protein will be retained in the fiber fraction, while the rest is pressed out in the liquid fraction. Following precipitation, 10-20% of the original dry matter and 30-60% of the original protein can be found in the precipitated protein rich fraction, while the rest will be present in a residual juice. These ranges of mass and protein distribution are not ultimate, but illustrates the possibilities for optimization of the process according to what the desired outcome is. E.g. if the goal is to have maximum protein yield in the protein concentrate, one has to optimize the fractionation and press more protein out of the biomass, but also optimize the precipitation and separation reducing loss of proteins to the residual juice.



Figur 7. Typical distribution of dry matter and protein in the different fractions following a bio-refinery process

Figure 8 shows a theoretical example of mass and energy balance for a green biomass processing a fresh green biomass with a dry matter content of 18% and a protein content of 20% of dry matter. The mass and energy balance is based on laboratory tests and expected yields as presented in Figure 7.

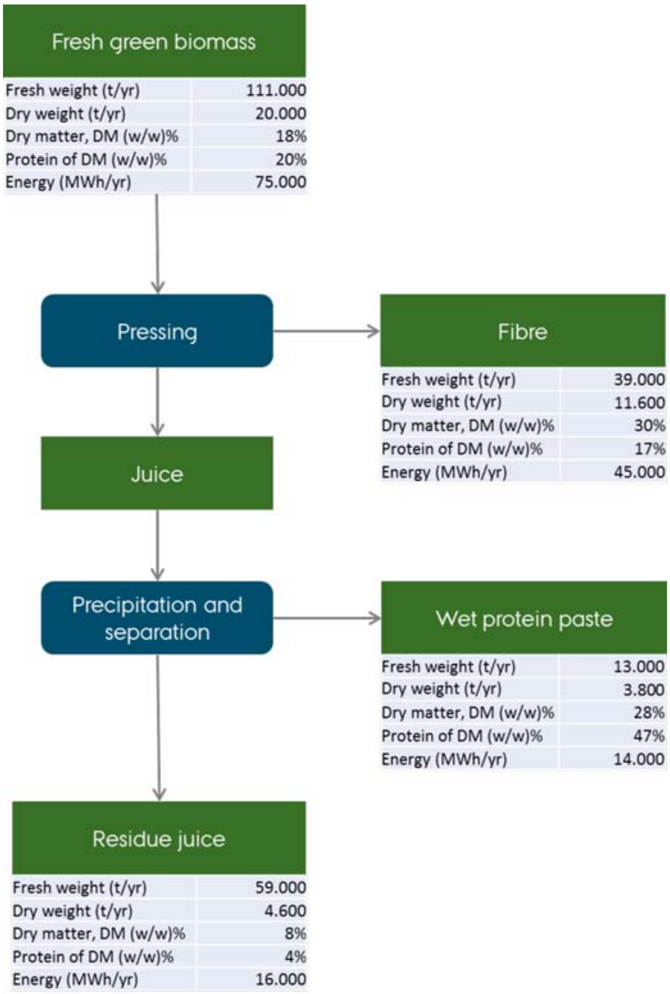


Figure 8. Mass and energy balance over the pressing and the protein separation. The calculation is based on a simulation of a decentral processing plant with a biomass input of 20.000 dry weight/yr. The weight percentage of dry matter and protein (w/w) is the concentration of each component in the separated biomass fraction (Ambye-Jensen, 2015)

In this example the wet protein paste contains 28% dry matter with a protein content of 47% in dry matter. Following a drying this fraction thus has protein content close to soybean meal.

Taken the example from table 2 with ryegrass fertilized with 450 kg N per year, a dry matter yield of 12.5 ton/ha with a protein content of 20% in dry matter can be expected. Following the distribution of fractions in Figure 8, the green biomass from 1 ha will thus result in:

- 7250 kg dry matter fiber rich feed with a dry matter content of 30% and a protein content of 17% in dry matter
- 2375 kg dry matter in protein rich feed with a protein content of 47% in dry matter
- 2875 kg dry matter to be used for biogas

The fiber rich feed is expected to be able to store as silage. Also the technology to dry the wet protein rich paste into a storage stable feed is developed but is relatively energy demanding. A particular challenge is the very low dry matter content in the residual juice (after protein extraction) that can make it difficult to use efficiently in a traditional biogas plant.

4.3 Experiences from pilot and demonstration scale experimentation

Up-scaling of the green biorefinery process is of great importance to the further development and implementation of the technology. There are several initiatives in Northern Europe including GRASSA in The Netherlands, BioPos in Germany, and the Green Biorefinery in Utzenaich, Austria, each with slightly different approach and process technology focus. It is however not possible yet to evaluate the overall results from these initiatives.

In Denmark, a pilot scale facility at Foulum, Aarhus University, has been established during 2015 and 2016 - the AU Grass Refinery. The scale of the pilot plant is 600-1200 kg fresh biomass input per hour, depending on biomass, dry matter and cutting lengths. The products are pressed fibre and wet protein paste. The initial experience from the pilot plant has revealed both challenges and opportunities in terms of up-scaled production. E.g., while biomass handling and fractionation is well-functioning, the separation of precipitated protein requires further development. Optimization of the pilot plant is ongoing. Preliminary laboratory results from double pressing of the biomass have shown good result yielding up to 70% protein extraction from the biomass. The possibility of double pressing is therefore being installed at the pilot plant.

In order to produce enough protein concentrate and fibre for larger scale animal feed experiments the scale of the AU pilot is still too small. Thus, a demonstration scale experiment (10 x AU pilot) was planned and executed during 2016. It involved several university- and industry- partners and was financed by two current research projects, Bio-Value SPIR and OrganoFinery, wherein animal feed experiments (poultry, pigs and cows)

Partners involved in demo-scale experiment 2016

- BioTest APS
- RUNI A/S
- Nybro Tørreri A.M.B.A.
- Sønderhøjgaard I/S
- KMC
- J. Chr. Koldkur A/S
- Bounum Maskinstation
- KU (BioValue SPIR)
- AAU (OrganoFinery)
- SEGES
- AU(BioBase)

are planned late autumn 2016. The demo-scale experiment was carried out in the last week of June 2016, and was running 24hr operation for 5 days. 400 tonnes of organic grass/red clover were processed, producing 7 tonnes of protein concentrate from the juice and 223 tonnes of silage wrap bales from the fibre. The separation into juice and fibre fraction took place at a commercial plant for dried grass and legume pellets, Nybro Tørreri A.M.B.A., while the further separation and drying of the protein took place at the potato starch and potato protein producer KMC A/S. The experiment was overall a big success, while, also here, it became apparent where focus in development and optimization is required, namely the separation of precipitated protein.

The main lessons learned from the experiment were:

- The logistics and unit operations needed for processing fresh grass in large amounts was definitely possible to upscale.
- The screw-press capacity went above 10 tonnes/hr, which was unexpectedly high
- Continuous lactic acid fermentation using addition of a specific bacterium inoculum (the technique developed in the OrganoFinery project) worked very well - efficiently lowering pH to 3.8 and precipitating the protein
- Proper handling of foam is an issue that needs further development
- Pumps needs to be over dimensioned and robust to handle days of production
- Separation and drying of precipitated juice has to be optimized in large scale

4.4 Minor but high value constituents in green biomass

Many plants contain minor components, often called secondary plant metabolites. These groups of compounds include biological valuable components as vitamins, colouring agents, antioxidants, as well as nutraceuticals and even pharmaceutical active compounds like morphine, digoxin, cannabinoids and saponins. Other plants contain biological active compounds with unwanted biological activity, commonly referred to as ANF's (**A**nti **N**utritional **F**actors). In combination with biorefining processes it may economical feasible to purify and isolate biological interesting minor components from certain plant species. However, this area is still very underexplored.

5. Feeding value

5.1 Proteins for monogastrics

The nutritional quality of the plant juice and of the protein-rich precipitate for monogastrics has been evaluated in earlier studies focusing mainly on chickens, pigs, or rats; however results are not consistent. Improper processing of plant protein has been suggested to be one cause of the inconsistency (Houseman, 1976). Improvements in methods for green plant processing and protein extraction combined with the increasing need for animal protein fuel new attempts to produce high quality protein alternatives to soy protein for animal feed.

Table 12 shows the amino acid profile of proteins from green plant processing compared with typical value for soya bean meal. It appears that the amino acid profile is very similar to dehulled soybean meal in most cases. A particular benefit is the higher proportion of the essential amino acid methionine compared to lysine in the green products compared to soya bean meal which in particular in poultry production makes it easier to fulfil the nutritional requirement.

Table 12. Amino acid composition in protein fractions from white clover, red clover, lucerne and perennial ryegrass (g/16 g N) (Damborg et al., 2016).

Amino acid	White clover	Red clover	Lucerne	Perennial ryegrass	Dehulled Soybean Meal*
Lysine	5.4	5.4	5.7	4.9	6.2
Methionine	1.6	1.6	1.7	1.8	1.4
Cysteine	0.7	0.7	0.9	0.7	1.5
Threonine	4.5	4.4	4.5	4.3	4.0
Histidine	2.1	2.1	2.3	1.8	2.7
Isoleucine	4.9	4.7	4.8	4.5	4.5
Leucine	8.3	7.8	8.1	7.9	7.7
Phenylalanine	5.5	5.2	5.5	5.3	5.1
Valine	5.9	5.8	5.8	5.7	4.8
Arginine	5.5	5.2	5.5	5.3	7.4
Serine	4.5	4.5	4.5	4.2	5.2
Proline	4.4	4.3	4.3	4.3	5.2
Alanine	5.7	5.4	5.5	6.2	4.4
Glycine	5.1	4.8	4.9	5.1	4.3
Asparagine/ Aspartic acid	10.3	10.3	10.9	8.2	11.6
Glutamine/ Glutamic acid	10.0	9.5	9.8	9.2	18.0

*Data obtained from VSP: Notat No. 1130

In the current projects within biorefining of green biomass digestibility experiments with pigs and broilers are planned autumn/winter 2016 and so far digestibility experiments with rats has been performed (Stødkilde-Jørgensen et al. 2016; manuscript). Results from these experiments reveal digestibility of protein up to 85% and show a clear positive correlation with the protein content in the protein concentrate. The results in the present study demonstrated that screw-press processing does not induce major quality impairing changes in proteins with respect to digestibility in monogastrics. The process of biorefining method for extraction of the proteins from the green juice of the grass might though impact of the nutritive value. It is expected that risk for denaturation is less when protein are precipitated using lactic acid fermentation techniques only, than when heat treatment is involved.

It remains to be documented in production trials that the green protein concentrate results in the same performance results as traditional protein sources like soya bean meal for pigs and poultry, but based on our present experience and preliminary experiment results, we would expect equivalent production results in practical framing. In conclusion, green plants can be an important source of protein for animal feed and human consumption (see below), thereby contributing to solving the increasing demand for protein worldwide.

5.2 Fiber feed for ruminants

For ruminants the main focus is on evaluating the feed value for the fiber rich pulp fraction originating after screw pressing of the green biomass. Around half of the crude protein are located in the pulp, and the composition of amino acids in this fraction are similar to the composition in the whole plant (Damborg, et al.,2016). As a considerable proportion of the protein retained in the pulp is expected to be fibre-bound, the pulp is expected to be suitable for ruminants.

The first results conducted with this fraction showed that the pulp remaining after juice extraction had higher DM concentration than the plant, similar crude protein concentration and lower crude ash concentration (Table 13).

Table 13. Chemical composition of red clover and perennial ryegrass plant and pulp. Mean of two seasons (June and September 2014).

Plant Species	Fraction	Dry matter [g/kg]	Crude protein [g/kg DM]	Crude Ash [g/kg DM]	In vitro digestibility [g/kg OM]	DOM [g/kg DM]
Red clover	Plant	156	213	98	681	614
	Pulp	424	213	72	636	589
Perennial	Plant	218	153	84	722	661
ryegrass	Pulp	456	150	48	684	652
P-value	Fraction	<0.001	N.S.	0.001	0.093	N.S.

For perennial ryegrass the crude ash content in the pulp was nearly half of the content in the plant. The *in vitro* digestibility tended to be lower for the pulp, as expected due to a large proportion of soluble organic matter being removed upon juice extraction. When expressed as digestible organic matter (DOM) as proportion of DM, though, no major difference was observed, due to the decrease in ash concentration.

Table 14 shows the changes in fibre fractions between the original biomass and the fibre pulp (Damborg et al., 2016). As expected the concentration of neutral detergent fibre (NDF), hemicellulose, acid detergent fibre (ADF), cellulose and acid detergent lignin (ADL) in both red clover and perennial ryegrass increased in the pulp compared to the original biomass. However as explained above the digestibility of the organic matter for ruminants was not significantly influenced. The fibre-associated crude protein was located in the hemicellulose, cellulose and lignin fractions indicating variable availability for ruminants.

Table 14. Content of NDF, hemicellulose, ADF, cellulose and ADL in red clover and perennial ryegrass plant and pulp. Mean of two seasons (June and September 2014).

Plant Species	Fraction	NDF [g/kg DM]	Hemicellulose [g/kg DM]	ADF [g/kg DM]	Cellulose [g/kg DM]	ADL [g/kg DM]
Red clover	Plant	369	132	238	194	44
	Pulp	552	194	358	289	70
Perennial	Plant	498	246	252	239	14
ryegrass	Pulp	706	357	349	321	28
P-value	Fraction	<0.001	0.008	0.002	0.001	0.030

Presently the feeding value of the fibre fraction for dairy cows is evaluated in ongoing production experiments with dairy cows as part of the BioValue project.

6. High value proteins for food

6.1 Issues concerning high quality protein for human consumption

The most abundant protein on earth is the respiratory protein in green plants, rubisco. In green materials, this protein constitute up till 50% of the whole protein pool in the plants. The amino acid composition of rubisco fulfills the need for essential amino acids for humans to the same extent as proteins from other sources (van de Velde, 2011). If the many green sources of protein (grasses, clover, lucerne, and waste products e.g. leaves from carrots and beets) could be exploited, not only for feed but also for food, it would contribute to solving an enormous need for protein to feed the fast growing world population.

For human consumption, we meet the same benefits and drawbacks as for monogastric animals concerning exploitation of protein from green biomass. Besides amino acid composition and the content of anti-nutritional factors (ANFs) e.g. fibres, process induced changes, e.g. heat, pH and the effect on bioavailability and functionality needs investigation. Among the most important ANFs that have not, previously, been discussed in this report are polyphenols and their oxidation.

In addition, heat-induced changes can function as ANFs, making processing optimization crucial. Another issue is getting white proteins instead of green. Currently, we are working on optimization of producing white protein from green biomass, thus reducing both content of chlorophyll and browning reaction.

6.2 Browning reaction and anti-nutritional factors in proteins from green biomass

When extracting protein from green materials, polyphenols and the redox enzyme; polyphenol oxidase (PPO; EC 1.14.18.1 or EC 1.10.3.1), which are separated in the living plant, are able to react, thus facilitating unwanted browning. The overall polyphenol oxidase activity has been determined in different plants showing highest activity in red clover > spinach > ryegrass > white clover (Møller et al., unpublished data). Many different phenolic compounds are present among others polyphenols (Amer et al., unpublished data), which are easily oxidized into quinones by a PPO catalyzed reaction (Figure 9). *o*-Semi-quinones are highly reactive compounds that react with the nucleophilic functional groups e.g. sulfhydryl, amine, amide, indole and imidazole group through the 1,4 Michael addition reaction and Strecker degradation (Bitter 2006). These compounds will influence color, taste, aroma and digestibility of food. So far, browning has been controlled by addition of inhibitors (e.g. sulfite) for polyphenol oxidase (Amer et al., unpublished). However, another possibility is to bind polyphenols during protein extraction, which is, currently, under investigation.

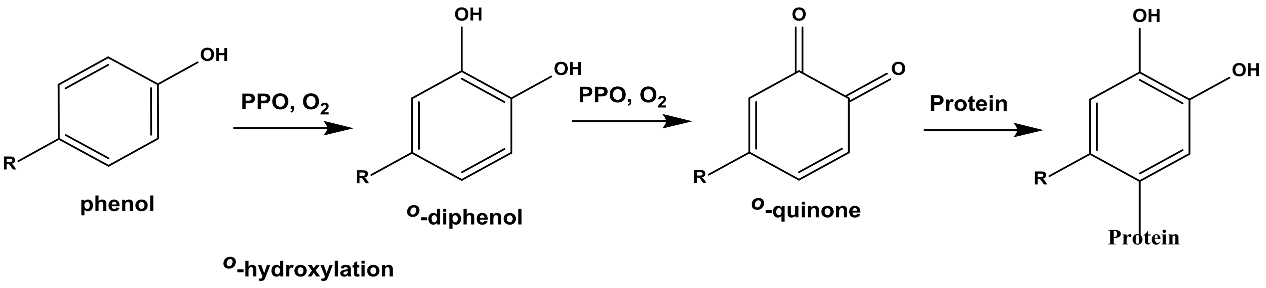


Figure 9. Polyphenol oxidase (PPO) catalyzed reaction of phenolic compound (o-diphenol) into an o-semiquinone and its reaction with protein.

Beside the oxidative changes occurring while processing heat-induced changes are also taking place. Currently, we are developing a MS-based method for the quantification of heat-induced changes measured as the Maillard products: furosine, carboxymethyl-lysine, carboxyethyl-lysine and lysinoalanine, which can be used as markers in the optimization of extraction and drying processes. Furthermore, the differentiation between L- and D-enantiomers of amino acids are indeed important for the nutritional value of proteins, therefore a MS-based method of their analysis has been developed (Danielsen et al., unpublished).

6.3 White protein without chlorophyll

An important issue regarding consumer's perception to take into account is the color of the proteins. For human consumption, we need to consider the chlorophylls in the protein samples in order to obtain high fractions of colorless proteins (Figure 10).

Figure 10. Juice with different levels of chlorophyll from left to right: high, none, and medium content (Amer et al., unpublished data).

Besides the color, chlorophyll is a highly potent type II photosensitizer, which may initiate production of reactive radical species and first of all singlet oxygen, which readily oxidizes unsaturated bonds in proteins and lipids giving raise to off-flavor formation and lower nutritional value. Hence, both from a visual and flavor perception for the consumer we wish to produce high level of white proteins from the green materials. Currently, we are optimizing a pre-heat treatment process to get rid of the chlorophyll.

The yield may suffocate from the pretreatment needed to obtain higher yield of white protein, however the waste fraction will be used for animal feed.

6.4. Proteins from green biomass as food ingredients

Plant proteins may have a huge potential as food ingredients either as only plant protein or in protein blends. Cheap proteins with gelling, foaming and/or emulsifying properties have potential in the food industry as ingredients. In preliminary studies, proteins from spinach along or in co-precipitates with whey or casein showed interesting functional properties (Romeih, unpublished data), which may be similar for protein from clovers and grass as rubisco is the major protein in all three plants. Hence, these are some of the future prospective that need further investigation.

6.5. What is next in exploitation of protein from green biomass in food

The challenges to provide colorless protein from green biomass are in principle known. The biggest challenge for developing food grade proteins is, however, the technological development of an economical method for isolating large (kilogram) quantities of the plant protein. Currently, the Netherlands are main contributors but also they are struggling with the upscaling from lab-scale to pilot- and full scale (van de Velde, 2011). Thus, there is a need for a substantial technological development to contribute with this significant source of protein to fulfil the increasing need for proteins.

7. Operational costs

Since no large scale bio-refinery plants for green biomass are established yet, there are no concrete economic assessments based on practical implementation. However, some scenario work has been done in relation to the supply of green biomass and considerations on the operational costs. Also, additional information on societal costs and benefits are presented in Appendix 1.

7.1 Supply of green biomass

A recent study examined the economic consequences of scenarios, where bio-refining of agricultural supplied biomass were implemented on a large scale in Denmark. The economic consequences were analyzed in a partial equilibrium model of the Danish farm sector and hence enabling an assessment of distributional effects between different farm types based on cost minimization theory (Jensen, 2016). One scenario was extraction of high-value protein from green biomass to be used for e.g. pig feeding (feed scenario). The study considered 15 farm types. The farms are distinguished according to main production, farm size, soil type, organic status and full-time/part-time status. The analysis suggests some variation across the farm types in terms of adoption of biomass production for industrial purposes. Conventional part-time farms represent almost half of the biomass production in the protein feed scenario. Other major contributors to the biomass production includes conventional crop farms on sandy soils, conventional pig farms and to some extent conventional cattle farms (Jensen, 2016). Considering the scenario specific production goals as the demand side, this implies that the biomass price will have to be adjusted to ensure that supply meets demand. The analysis suggests a price increase of 0.30 DKK per kg biomass dry matter on top on production costs.

7.2 Production costs of green biomass

In the background paper for the publication of the updated version of the "+10 mio. tonnes study" (Gylling et al., 2016), the operational costs of growing green biomass has been calculated both for a scenario with a high yield and for a scenario with a yield corresponding to meadowlands. The two scenarios were calculated for two geographical areas, one with primarily sandy soil classification and one with primarily clayey soil classification (Bojesen *et al.*, 2016).

The costs of producing a hectare of green biomass from the high yield (15 tonnes of dry matter (DM) per hectare) scenario were 12,411 DKK for the clayey soil classification and 12,384 DKK in the sandy classification (Bojesen *et al.,* 2016). This corresponds to 827 DDK and 826 DKK per tonne DM. In the high yield scenario a higher fertilization of 450 kg N per hectare has been applied. This is done on experimental basis regardless of what is determined as economic optimal (Olesen *et al.,* 2016).

The costs of producing a hectare of green biomass from the meadowland scenario on sandy soil with a production of 7,260 kg of DM were 5,758 DKK, corresponding to 793 DKK per tonne of DM. The production cost of a hectare of green biomass from the meadowland scenario on clayey soil with a production of 8,250 kg of DM is 5,996 DKK, corresponding to 727 DKK per tonne of DM (Bojesen *et al.,* 2016).

In Bojesen *et al.* (2016) the cost of harvesting green biomass is based on chopping and either direct delivery by truck or storage in a silo. The grass is cut, spread and raked before chopping. This is done 4 times per year which equals a harvest cost of 6,370 DKK per hectare per year in the high yield scenario (Bojesen *et al.,* 2016). Other methods of harvesting/storing green biomass could be to wrap large bales in plastic film. However this is a more expensive method on a large scale.

7.3 Transport and treatment costs

With a micro economic model based on data from The Danish Knowledge Center for Agriculture and The Danish Agrifish Agency and using Geographic Information System the cost of transporting green biomass has been estimated in Bojesen *et al.* (2016). The calculations were based on a model where a biomass area of 50,000 hectares is determined by GIS based on average field size, average distance to nearest neighbor and soil classification. The cost of transporting biomass from field to a facility was estimated by Bojesen *et al.* (2016) to be 1.97 DKK per tonne per kilometer plus a cost of 46 DKK per tonne for on- and off-loading procedures. These estimated are well in accordance with model calculations by Sørensen et al. (2010) underpinning the importance of transport for the total economic result.

Compared to other costs, the cost for establishing and running the bio-refinery is very difficult to estimate because of the lack of data from existing plants. The best estimate is from Termansen *et al.* (2015) where the refinery costs were estimated to 750 DKK per tonne DM for a central plant with a capacity of 150,000 tonnes of DM per year and delivering dried protein concentrates ready for inclusion in commercial feed mixtures. In the decentralized plant with a capacity of 20,000 tonnes of DM the cost was estimated to 236 DKK per tonne DM, where the protein product was a wet product to be used in a local feed situation.

The above mentioned costs are summarized in Table 15 for a case based on high yielding grasses.

Tabel 15. Example of costs based on production of high yielding grasses, DKK per tonne dry matter harvested.

Costs	***DKK***
Growing and harvest	*825*
Transport 10 km [1]	
33 vs. 20% dry matter	*198 - 330*
Remuneration premium needed	*300*
Refining costs (decentralized vs. centralized)	*236 - 750*
Refining costs (energy costs excluded)	*146 - 450*
Total per tonne dry matter	*1,469 – 2,205*
Value of products	
Protein concentrate (3,300 – 4,000 DKK/tonne dry matter)	*660 – 800*
Silage (1,000 – 1,250 DKK/tonne dry matter)	*580 – 725*
Residual juice	*0*
Total per tonne dry matter	*1,240 – 1,525*

1) 2 DKK/ton/km + 46 DKK/ton loading and unloading

The costs were compared to the estimated value of products sold. It appears that there is only a small window where – based on the present assumptions – the operational economy becomes positive. This situation is when the residual juice can be utilized for energy generation as indicated in figure 6 and thus alleviating energy costs at the bio-refinery.

There is a need to explore in more detail how operational cost can be reduced. Presently a model is under development by the partner SEGES in the BioValue SPIR project. Through multiple choices of yield per hectare, harvest methods, transport means, pretreatment, storage options, the total cost for biomass acquisition can be calculated. The model calculates "i) Total cost for acquisition of biomass, ii) Harvest cost on different soil types and nutrient supply, iii) Storage cost & storage loss for different storage options, iv) Cost depending on transport form, loading equipment, and distance. Likewise, this area is under research at the Department of Engineering at AU under the BioBase project with particular focus on harvest from unconventional areas.

Also there is a need to explore more in detail how the synergy with an energy producing plant can be optimized since this seems to be very critical for the economy. In addition, utilization of the fiber rich fraction for more valuable products that feed may change the picture as will be the case if the valuable minor components of the green juice can be extracted and utilized.

8. Example of scenario for implementation of green biomass refinery

The extent to which green biomass refinery concepts can be implemented can be considered in relation to the present land use. Table 16 shows land use for agricultural activities.

Table 16. Land use in Danish agriculture 2015 (Landbrug & Fødevarer2016)

Type of crop	1000 ha
Cereal, total	1453
• *Winter wheat*	*617*
• *Spring barley*	*512*
Oils seeds	193
Root crops	73
Pulses	12
Seed crops	71
Grass and forage in rotation	501
• *Maize*	*183*
Other	331

The major crops are winter wheat and spring barley in almost equal proportion. Considering an example where the grass fiber fraction is to be used for ruminant feed, focus could be on Jutland where the major part of the dairy cattle is kept. In Jutland there is (in round numbers) 900.000 ha on arable and pig farms, of which almost 700.000 ha is grown with cereals, and 500.000 ha on dairy farms, of which 100.000 ha is grown with cereals either for maturity or whole crop silage.

From a crop rotation perspective there is plenty of room to include a grass or grass-legume crop on the arable and pig farms. Including 200.000 ha grass out of the 900.000 ha in Jutland in addition to the 200.000 non-cereal crops at present would allow a cereal inclusion of 50 – 60% in the rotation compared with 75% at present, which will benefits overall fertility and probably allow a higher yield/ha of cereals and/or reduced use of inputs for the cereal production.

We earlier estimated the produced output from a bio-refinery process that was fed with biomass from 200.000 ha of green biomass using mass balance assumptions very close to those given in Figure 8 and in Termansen et al (2015), and which is reproduced in table 17.

*Table 17. Bio-*refinery *output from 200.000 ha of green biomass grown as current and optimised (more productive species and higher fertilization) grass production or as grass clover with no nitrogen fertilization.*

	Moderate fertilization	Highly fertilized	Unfertilized grass-clover
Produced biomass dry matter	200.000 ha 10.5 t/ha	200.000 ha a 15 t/ha	200.000 ha a 7 t/ha
	2.1 mill. ton	3 mill. ton	1.4 mill. ton
Yields from bio-refinery			
Protein concentrate (soy bean meal quality)	0.42 mill ton	0.60 mill ton	0.28 mill ton
Fibre-rich feeds for ruminants	1.20 mill ton	1.70 mill ton	0.91 mill ton
Biogas	0.48 mill ton	0.70 mill ton	0.21 mill ton

The span in biomass yield from the grassland represents what can be expected from agricultural land in rotation under different conditions – see also table 2. The highly fertilized high yielding grass represents the expected result with improved, more productive species (e.g. tall fescue or festulolium) or varieties than is normally used today (Jørgensen & Lærke, 2016; Larsen et al., 2016). In this case the 200.000 ha of grass will produce 0.6 mill ton of protein-rich feed or almost 0.3 mill ton of crude protein. This corresponds to 27% of all imported crude protein per year.

At the same time 1.7 mill ton of fiber-rich feed for ruminants are produced with a protein content of approximately 17%. According to the preliminary results this can be expected to replace a medium quality of grass-clover silage. Using the dry matter yields from table 2 of approx. 10 ton dry matter per ha in current grass production this fiber food can replace 170.000 ha grassland or 130.000 ha of maize thus allowing, theoretically, a higher proportion of cereals to be produced at dairy farms.

The results in table 17 by no means represent the limits for including grassland in the rotation since the 200.000 ha is only 15% on the total cereal area of today. One can easily argue that another 200.000 ha could be grown instead of cereals outside Jutland. A main issue here will be to make proper use of the fiber fraction, possibly to biogas or bioethanol production. Likewise, a proportion of the grassland used to day for dairy production can be utilized for protein extraction.

A number of issues remain to be qualified to assess this scenario. Main issues are a better assessment of the technical efficiency of the bio-refinery plant including energy use, feeding value in practice of the feed stuffs produced, and costs related to logistics in practice. Once implemented it will also be very important for the environmental assessment, how the fiber-rich feed actually substitutes present crops on dairy farms.

9. Perspectives in organic farming

9.1 The challenge

In organic farming it is difficult to meet the need of proteins with the correct amino acid profile for pigs and poultry since in organic farming no use of synthetic amino acids are allowed to balance the diet. This means in practice that the livestock often are oversupplied with protein to meet the need of the individual essential amino acids. Consequently, the ammonia emissions from the manure are significantly higher than in conventional production (Hermansen et al 2015). At the same time the oversupply of protein is likely a contributing factor to the lower feed conversion of organic poultry and pig production compared to conventional production.

Organic livestock are fed primarily with organically grown feed. The above problem, along with the fact that the right amino acid supply can influence the behaviour and welfare of poultry, as well as the health of pigs, has meant that there is an exemption for pigs and poultry. These animals may be supported by up to 5% non-organic feed in order to better meet their nutrient needs. Typically, conventionally produced protein concentrate extracted from potatoes and / or corn gluten is used to balance the nutrient needs of the most vulnerable groups of animals, typically the young animals. The exemption, however, expires on schedule by the end of 2017, after which there is a further need to find relevant organic protein substances.

In the EU project 'Improved contribution of local feed to support 100% organic feed supply two pigs and poultry' (Smith et al 2014), a number of alternative feed sources were tested and evaluated. Overall, it was found that the self-sufficiency (considered at the EU level) with organic proteins to monogastric livestock was low - 50% for lysine and 40% for methionine. A number of feed materials can be used to fully or partially meet livestock amino acid needs, eg seeds of esparcette or 'grass seed pea', processed sunflower cakes where the protein is concentrated, mussel meal or meal form insects. A common feature of these solutions is that the feed material is expensive due to low yields (esparcette and grass seed pea) or the technology is not fully developed (insect meal) (Smith et al. 2014). It was concluded that green legumes like alfalfa were the most promising in terms of providing the necessary organically produced protein to meet the needs of pigs and poultry, because they are crops which can produce high yields even under organic production. In addition they are crops that fit well into organic crop rotation, and do not require synthetic nitrogen fertilizer. Use of whole green mass as feed (with the objective of supplying the animals with protein) results, however, in a lower feed conversion since the monogastric livestock cannot utilize the fiber part very well (Smith et al., 2014).

Both in Denmark and internationally, there is an increase in demand for organic food. In Denmark, the organic market share of eggs is 29% and of pork 3% (LF, 2016). The sale of organic pork rose, for example, by 36% from 2013 to 2014 (Statistics Denmark, 2016). Particularly for pig production a further significant increase to cover partly an increased domestic consumption, but mainly increased exports is expected (Friland, 2016). Similarly, a marked increase in demand for organic food in Sweden and the USA in the next few years is expected (Haman 2016). Just as there are 'deficit' of organic feed for pigs and poultry in Europe, the same is the case in the US (Haman 2016). This generally means higher prices for organic protein feeds - a development that is likely to be exacerbated, when the exemption to use non-organic feed supplement lapse in 2017.

Thus, the bio-refinery technology seems to represent a promising pathway to produce protein for organic monogastrics production.

9.2 Example of industry perspectives in organic livestock production

The organically managed land in Denmark amounts to approximately 180.000 ha – in latest year showing an increasing trend. Of these approx. 66.000 ha are located on dairy farms and 15.000 ha on farms for horticulture. The remaining area (100.000 ha) is used for arable and mixed farming as well as pig and poultry farming (Jensen & Pedersen 2015).

The largest proportion of land is used for grass/clover grass/other green fodder (100.000 ha), while approx. 50.000 ha is used for cereal production. Thus, contrary to the situation in conventional farms, organic farms have much more grass-clover in the crop rotation (to support the supply of nitrogen through biological N-fixation) and less cereal.

A typical dairy farm has around 60% grass-clover, 20% cereals and 20% whole crop silage or maize silage in the rotation (Kristensen 2015), and grass-clover constitutes the main silage type used during winter. The high proportion of grass-clover in the rotation facilitates a high intake of fresh grass through grazing during summer, but at the same time makes much -grass-clover available for conservation due to the high growth in early summer.

Based on these numbers it could be considered to use half of the grass-clover produced on organic dairy farms for bio-refinery (corresponding to a theoretical area of 20.000 ha).

It can be deducted from the above that the non-dairy and non-horticulture farms also have a relatively low proportion of cereals in the crop rotation – around 40%. Therefore it is probably not feasible in general to reduce this proportion too much further. However, like for dairy farms it could be an option to use part of the (surplus) grassland on these farms for bio-refining purposes.

Assuming that in total 40.000 ha of organically managed grassland could be used for bio-refinery and using the data from table 15, 56.000 ton of protein concentrate could be achieved. Assuming Danish organic pig and poultry production includes 8000 sows with finishers and 550.000 hens producing 12 mill kg eggs per year, the need for domestic use can be estimated to 18.000 ton. Thus there would be room for an export of 38.000 ton. As previously mentioned, there is a shortage of protein feed for monogastrics in the EU generally, so one can assume good market opportunities.

Since the technology mentioned is not fully developed, there are no consolidated financial calculations on costs. Still, Tvedegaard (2016) estimated that after deducting all costs for processing etc there would be approximately 3000 DKK per ha to pay the farmer to grow one ha with grass-clover. This example was based on a decentralized solution of 3000 ha organic grass-clover, where there is a pressing and juice production at three stations, delivering the juice into a bio-refinery. This solution is chosen to minimize transportation costs relative to one central solution because preliminary calculations have shown that transport costs are very crucial for the overall economy. An amount of 3000 DKK per ha clover is not an attractive application seen by farmers, so despite the technical biological perspective there is a great need to optimize the overall solution.

10. Ongoing commercial, research and development activities

Tabel 18 gives an overview of recent and current activities within biorefining of grass and legumes and table 19 lists Danish research competences within the field of green biomass refining.

Tabel 18. Overview of recent and current activities within biorefining of grass and legumes.

Company/ Organisation	**Country**	**Focus area**	**Status**
GRASSA (company)	NL	Development of mobile biorefinery units producing protein paste. Including recovery of phosphorous from the residual juice	Ongoing development, partner in BioValue innovation project
BioPos (research institute)	GE	Fermenting juice from silage grass for lactic acid production.	Ongoing development
IBERS (University)	UK	Using high sugar grasses for fermentation into chemical building blocks	Ongoing development
INNOFEED (Private-public)	Fi	Investigates options to use ensiled grass for biorefining into a variety of inventive feed products	Research project started 2015 running to 2018 http://www.ibcfinland.fi/projects/biorefining-ensiled-grass-into-i/
BioValue SPIR (Private-public)	DK	Optimizing sustainable production, separation and conversion of biomass, hereunder green biomass. Specific project on Products from green biomass (protein, storable fibers, inorganic elements)	http://biovalue.dk/projects/project-2/ Ongoing with 16 partners
DLF (Company)	DK	Plant breeding for specific qualities of grass and clover	Commercial equipment (current activity: BioValue partner)
Hamlet Protein (Company)	DK	Fermentation, conversion and separation of protein-rich feedstock into Safe Proteins or Animal feed (reduced anti nutritional factors)	Commercial equipment (current activity: BioValue partner)
KMC (Company)	DK	Physical separation and precipitation of products from potato processing. Testing if green biomass processing could compliment the potato campaign.	Commercial equipment (current activity: BioValue partner)
DLG/ Sejet Plantbreeding/ DANGRØNT Products A/S	DK	Primary production of feedstock and agricultural supplies/plant breeding/ processing grass and alfalfa etc. into green hay and pellets	Commercial grass drying equipment (current activity: BioValue partner)

Arla Foods (Company)	DK	Primary production of milk, focusing on finding alternative protein sources for a more sustainable dairy production	Commercial scale biorefinery production upgrading all side streams from dairy products (current activity: BioValue partner)
SEGES (Advisory service)	DK	Participating in research activities, developing web based model for handling cost, logistics and storage of biomass	Ongoing modeling work. Building new advisory competences on green biorefinery
GreenField International ApS (Company)	DK	Developing protein drink from soluble grass protein	Ongoing BioValue Innovation project
NybroTørrer (Company)	DK	Growing, harvesting and processing grass and alfalfa into green hay and pellets. Participated in large scale production of grass-clover pulp for dairy cow feeding trial	Ongoing BioValue Innovation project
Dacofi Holding ApS (Company)	DK	Testing new press techniques for separation of green biomass into liquids and solids	Ongoing BioValue Innnovation project
Lihme Protein Solutions ApS		Protein extraction, separation and refinement technologies in the green biorefinery	Ongoing BioValue Innovation project
OrganoFinery	DK	From green crops to proteins, biogas and fertilizer, organic protein extraction using lactic acid fermentation, test of different grasses and legumes, feed trials in poultry	http://icrofs.dk/en/research/danish-research/organic-rdd-2/oreganofinery/ (ongoing with 8 partners)

Table 19. List of Universities projects with competences or facilities in green bio-refinery.

Organization	Type	Focus areas (beyond green bio-ref)	Equipment
Aalborg University	Academic Institution	Development of processes for Biomass conversion for production of animal feed, bioenergy, biochemicals, biomaterials, healthy feed and food ingredients	Pig gut simulation for testing prebiotic effects of oligosaccharides; fermentation bioreactors
Aarhus University	Academic Institution	Value added products, bio-energy, lipids, biogas, biogas upgradation, protein refining, Synergy in biorefining, biomass production.	Green biorefinery for production of protein enriched animal feed from green clover/grass, Facilities for tests of green biomass production and environmental impact related hereto and facilities to animal nutrition experiment
Danish Technical University	Academic Institution	Development of processes for Biomass conversion, product separation and product development; for production of bioen-	Pretreatment pilot plant; Upscaled fermentation bioreactors

		ergy, biochemicals, biomaterials, healthy feed and food ingredients	
Roskilde University	Academic Institution	Characterization of enzyme performance; design of cellulytic enzymes	www.ruc.dk
University of Copenhagen	Academic Institution	Optimisation of biomass, plant breeding and growth (soil, nutrients), biomass supply, biomass characterisation, pretreatment, hydrolysis and fermentation. Biofuels, Proteins and biochemicals Protein from green biomass – variation in yield and quality among grass genotypes	Facilities for high throughput amino acid analysis based on microwave assisted protein acid hydrolysis for screening of protein quality
University of Southern Denmark	Academic Institution	Development of industrial extraction and separation processes for high value secondary metabolites, fertilizers, proteins and biofuels, pilot scale biogas reactors	www.sdu.dk
Danish Technological Institute (DTI)	Independent R&D institute	Biomass and Biorefinery, pretreatment, enzymatic hydrolysis, fermentation, biomass analyses and chemical characterization, logistics, pilot scale equipment for biorefinery, biomolecule extraction, animal feed production, and solid biofuel.	Up-scaling facilities for pretreatment, storage stability and biomass conversion product development Pilot plant being constructed for e.g. protein extraction from sugar beet leafs

11. Conclusion

Producing proteins from green biomass at a commercial level for feed involves many factors from the production of the most relevant green biomass in relation to yield, quality, costs and environmental impact, the logistics, the refining process, the incorporation in feed mixtures and the implementation in the feeding at farm level. While a vast amount of knowledge exists or is in progress on these issues, also at all steps there is lack of knowledge to evaluate with a high certainty the overall economic and environmental consequences in case this technology is to be implemented.

First and foremost there is need for the bio-refining process to run at a commercial scale to obtain better knowledge on resource use and efficiency in the process which to a high degree will determine the profitability. There is a need to figure out the practical possibilities of using the protein paste as input in wet-feeding systems for pigs, since this will influence energy demands in the process to a high degree. Likewise there is a need to investigate how best to utilize the high moisture residual juice for energy recovery in biogas production to counteract the energy use at the factory level. Another major uncertainty lies in the way the green biomass is treated from being cut and to arrival at the bio-refinery plant. At the fresh stage the proteins in the green biomass are intact but degradation starts right after cutting. From a logistics point of view, costs will be significantly reduced if the biomass can be predried at the field, but the consequences of this in relation to obtaining intact proteins in the bio-refining process needs to be illuminated in much more detail.

As regards biomass supply in relation to environmental impact there is a need better to understand how long lasting grass or grass-clover fields can be established and maintained without losing nitrate and carbon. Likewise, there is a need to explore the potential of using cover crops as feedstock supply and - related to that - the environmental impact of new types of cover crops and management practises.

Finally, there is a need to explore the possibilities of extracting other valuable components from the green biomass, upgrade proteins for human consumption and/ or upgrade fibre for higher value products in order to improve the overall profitability of the bio-refining process.

12. References

Akiyama H, Yan X & Yagi K, 2010. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N2O and NO emissions from agricultural soils: meta-analysis. Global Change Biology 16, 1837–1846.

Amby-Jensen, M. & Adamsen, A.P. 2015. Green Biorefinery concept pro-ducing local feed protein feasibility study and comparison of central vs. de-central implementation. 11th International Conference on Renewable Re-sources and Biorefineries (RRB11), York, UK, June 3-5, 2015. Available at Re-searchgate.net.

Amore A, Ciesielski PN, Lin C-Y, Salvachúa D, Sànchez, NV, 2016. Development of Lignocellulosic Biorefinery Technologies: Recent Advances and Current Challenges. *Australian Journal of Chemistry* , -.http://dx.doi.org/10.1071/CH16022

ANON, 2013. Klimaplanens virkemiddelkatalog. Potentialer og omkostninger for klimatiltag. Udgivet af Klima-, Energi- og Bygningsministeriet. www.kebmin.dk.

Askegaard M, Eriksen J. 2007. Growth of legume and nonlegume catch crops and residual-N effects in spring barley on coarse sand. Journal of Plant Nutrition and Soil Science. 170:773-780

Azmir, J., Zaidul, ISM, Rahman, MM, Sharif, KM, Mohamed, A, Sahena, F, Jahurul, MHA, Ghafoor, K, Norulaini, NAN, Omar KMN, 2013. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, Volume 117, 426-436

Bernardo R, 2010. Genomewide Selection with Minimal Crossing in Self-Pollinated Crops. Crop Science 50: 624–627. doi: 10.2135/cropsci2009.05.0250

Bojesen, M., Jensen, M.V., Lillethorup, T.R., Gylling, M. & Jakobsen, A.B., 2016. *Helårsforsyning af bioraffinaderier: Udbudskurver for biomasse fra halm, majs, pil og slætgræs.* Frederiksberg: Institut for Fødevare- og Ressourceøkonomi (IFRO), Københavns Universitet. Rolighedsvej 25, 1958 Frederiksberg C. IFRO rapport nr. 252.

Bunzel, K., Kattwinkel, M., Schauf, M., Thrän, D., 2014. Energy crops and pesticide contamination: Lessons learnt from the development of energy crop cultivation in Germany. Biomass Bioenergy 70(0), 416-428.

Chiesa, S., & Gnansounou, E., 2011. Protein extraction from biomass in a bioethanol refinery - Possible dietary applications: Use as animal feed and potential extension to human consumption. *Bioresource Technology, 102*, 427-436.

Clarke,B.B.,White,J.F., Hurley,R.H., Torres,M.S., Sun,S. and Huff,D.R. (2006). Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease* **90**, 994-998.

COWI, 2015. *Analyse af det regulerings- og støttemæssige landskab for biomasseanvendelse.*

Damborg, V.K., Stødkilde, L., Jensen, S.K. and Weisbjerg, M.R., 2016. Protein and fibre characterisation of the fibre-rich fraction from biorefining of legumes and grass. EGF seminar 2016, September, Trondheim Norway.

Dotsenko, G, and Lange, L., 2015. Protein recovery from white clover and ryegrass screw press pulps by aqueous and enzymatic extractions. (Submission September 2015)

DS, 2015. Danmarks Statistik. Høsten af korn, raps og bælgsæd.

Dubgaard A, Jespersen HML, Laugesen FM, Hasler B, Christensen LP, Martinsen L, Källstrøm M & Levin G, 2012. Økonomiske analyser af natur-plejemetoder i beskyttede om-råder. Fødevareøkonomisk Institut, Rapport nr. 211, 120 p.

Eriksen J., Askegaard M., Rasmussen, J., Søegaard K., 2015. Nitrate leaching and residual effect in dairy crop rotations with grass-clover leys as influenced by sward age, grazing, cutting and fertilizer regimes. Agriculture, Ecosystems and Environment 212: 75-84.

Eriksen, J., Vinther, F.P., Søegaard, K., 2004. Nitrate leaching and N2-fixation in grasslands of different composition, age and management. The Journal of Agricultural Science 142, 141-151.

European Environment Agency, 2007. Estimating the environmentally compatible bioenergy potential from agriculture. Copenhagen, EEA Technical report 12/ 2007.

Geurts, J.J.M., Smolders, A.J.P., Verhoeven, J.T.A., Roelofs, J.G.M., Lamers, L.P.M., 2008. Sediment Fe:PO4 ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Freshwater Biology, 53, 2101-2116.

Greve, M.H., Christensen, O.F., Greve, M.B., Kheir, R.B., 2014. Change in peat coverage in Danish cultivated soils during the past 35 Years. Soil Science 179, 250-257.

Gylling, M., Jørgensen, U., Bentsen, N.S., Kristensen, I.T., Dalgaard, T., Felby, C. & Johansen, V.K., 2016. *The + 10 million tonnes study: increasing the sustainable production of biomass for biorefineries.* Frederiksberg. Department of Food and Resource Economics, University of Copenhagen.

Hansen EM, Eriksen J, Vinther FP. 2007. Catch crop strategy and nitrate leaching following grazed grass-clover. Soil Use and Management. 23:348-358.

Heffner EL, Sorrells ME, Jannink JL, 2009. Genomic Selection for Crop Improvement. Crop Science 49: 1–12. doi: 10.2135/cropsci2008.08.0512

Hermansen, JE., Munkholm, LJ, Bruus, M, Eriksen, J, Poulsen, HD, Kronvang, B, Bak, JL, Dalgaard, T, Kristensen, HL, Rasmussen, A, Adamsen AP, Hansen, B, Brüsch W, Thorling, L. Magid, J, Rasmussen SK, Jensen, LS, 2015. Miljø. I: Økologiens bidrag til samfundsgoder. ICROFS, pp. 107-168.

Hoffmann, C.C., Kjaergaard, C., Uusi-Kamppa, J., Hansen, H.C.B., Kronvang, B., 2009. Phosphorus retention in riparian buffers: Review of their efficiency. Journal of Environmental Quality, 38, 1942-1955.

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

Hvid, SK. 2012 Efterafgrøder til biogas er ikke rentabelmed aktuelle priser på biogas. Planteavlsorientering 092. www.landbrugsinfo.dk

Hyttel, O., 2015. Biomassehøsten omkring biogasanlægget Krogenskær ved Brønderslev. Notat fra Naturstyrelsen J.nr. NST-4104-00144.

IPCC, 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Institute for Global Environmental Strategies, Hayama, Japan.

Jensen, B. Z., 2016. Kan efterafgrøder gøres mere attraktive via managementændringer? Kandidatspeciale i Agroøkologi – Agro-Environmental Management, 68 p.

Jensen, J.D., 2016. *Bio-refining agricultural crop products into high-value materials – Economic impacts on the agricultural sector.* (Not yet published). Department of Food and Resource Economics, University of Copenhagen. Rolighedsvej 25, DK-1958 Frederiksberg C, Denmark.

Jensen, P.N., Blicher-Mathiesen, G., Rolighed, J., Børgesen, C.D., Olesen, J.E., Thomsen, I.K., Kristensen, T., Sørensen, P., Vinther, F.P., 2016. Revurdering af baseline. Teknisk rapport fra DCE – Nationalt Center for Miljø og Energi nr. 67.

Joosten, H., Tapio-Biström, M.L., and Tol, S., 2012. Peatlands-guidance for climate change mitigation through conservation, rehabilitation and sustainable use. FAO and Wetlands International, Rome, Italy.

Jørgensen U, 2012. Baggrundsnotat: Beregning af effekter på nitratudvaskning. http://ifro.ku.dk/publikationer/saerlige_ifro-udgivelser/10miotons/

Jørgensen U, Elsgaard L, Sørensen P, Olsen P, Vinther FP, Kristensen EF, Ejrnæs R, Nygaard B, Krogh PH, Bruhn A, Rasmussen MB, Johansen A, Jensen SK, Gylling M, Bojesen M, 2013. Biomasseudnyttelse i Danmark - potentielle ressourcer og bæredygtighed. DCA - Nationalt center for fødevarer og jordbrug, DCA Rapport nr. 033, 127 p.

Jørgensen U, Olesen JE. 2013. Behov for grøn revolution i landbruget. Ingeniøren 49,13.

Jørgensen U, Schelde K 2011. Næringsstoftab efter omlægning fra omdrift til dyrkning af flerårige energiafgrøder: Status på viden om produktion på høj- og lavbundsjord. Intern rapport - markbrug nr. 31; 24 pp.

Jørgensen U, Solati Z. & Lærke PE, 2016. Fordobling af produktionen pr. ha. I: Plantekongres 2016. s. 354-355.

Jørgensen, L., Kjærsgaard, H. D., Wachmann, H., Jensen, B. B., & Back Knudsen, K. E., 2001. Effect of pelleting and use of lactic acid in feed on Salmonella prevalence and productivity in weaners.

Jørgensen, U., & Lærke, P., 2016. Perennial grasses for sustainable European protein production. In: *Perennial Biomass Crops for a Resource-Constrained World*, Springer Switzerland, 33-41.

Kahn, SH, Iqbal J, 2016. Recent advances in the role of organic acids in poultry nutrition, Journal of Applied Animal Research, 44:1, 359-369

Kandel T, Elsgaard L, Andersen MN, Lærke PE., 2016. Influence of harvest time and frequency on light interception and biomass yield of festulolium and tall fescue cultivated on a peatland. European Journal of Agronomy 81:150-160.

Kandel TP, Elsgaard L, Karki S, Lærke PE., 2013. Biomass yield and greenhouse gas emissions from a drained fen peatland cultivated with reed canary grass under different harvest and fertilizer regimes. Bioenergy Research 6 (3):883-895.

Karki, S., Elsgaard, L., Audet, J., Laerke, P.E., 2014. Mitigation of greenhouse gas emissions from reed canary grass in paludiculture: effect of groundwater level. Plant and Soil 383, 217-230.

Kaye, J.P., Hart, S.C., 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology and Evolution, 12, 139-143.

Kiel, P., Andersen, M., Lübeck, M., 2015. A method of providing functional proteins from a plant material. PCT DK2015 050185. WO2015197078.

Kjaergaard, C., Heiberg, L., Jensen, H.S., Hansen, H.C.B., 2012. Phosphorus mobilization in rewetted peat and sand at variable flow rate and redox regimes. Geoderma, 173, 311-321.

Knudsen, L.,2015. Kvælstofnormer indstillet til Normudvalget. Internt notat

Kristensen IT, Jørgensen U, 2012. Baggrundsnotat: Forudsætninger for og beregning af biomassescenarier for landbruget: + 10 mio. tons planen - muligheder for en øget dansk produktion af bæredygtig biomasse til bioraffinaderier. 26 p. http://ifro.ku.dk/publikationer/saerlige_ifro-udgivelser/10miotons/

Kristensen, T., 2015. Beregning af grovfoderudbytte på kvægbrug ud fra regnskabsdata. DCA rapport 57, 27 pp

Larsen SU, Jørgensen U, Lærke PE (2016). Biomass yield and N uptake in tall fescue and reed canary grass depending on N and PK fertilization on two marginal sites in Denmark. In: *Perennial Biomass Crops for a Resource-Constrained World*, Springer Switzerland, 233-242.

Li X, Petersen SO, Sørensen P, Olesen JE. 2015. Effects of contrasting catch crops on nitrogen availability and nitrous oxide emissions in an organic cropping system. Agriculture, Ecosystems & Environment. 199: 382-393.

Losche, M., Salama, H., 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. 1. Carbohydrate and protein fractionation. *Grassland in a* *changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation*, (pp. 521-523). Kiel.

Lærke, P.E., Kandel, T., Tybirk, K., 2012. Tørvejorde kan bruges til energiafgrøder. Teknik & Miljø: Stads og Havneingeniøren. 9, 34.

Maamouri, A., Louarn, G., Gastal, F., Beguier, V., & Julier, B., 2015. Effects of lucerne genotype on morphology, biomass production and nitrogen content of lucerne and tall fescue in mixed pastures. *Crop & Pasture Science, 66*, 192-204.

Madsen, M.B., Søegaard, K., 1991. Kvælstof til græs i 1. og 2. brugsår. Tidsskrift for Planteavl 95, 239-253.

Manevski, K., Lærke, P.E., Jiao, X., Baby, S., Jørgensen, U., 2016. Biomass productivity and radiation utilisation of innovative cropping systems for biorefinery. Agricultural and Forest Meteorology (accepted).

Meuwissen THE, Hayes BJ, Goddard ME, 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819–1829.

Mikkelsen, M.H., Albrektsen, R., Gyldenkærne, S., 2014. Danish emission inventories for agriculture. DCE rapport nr. 108.

Näsi, M., & Kiiskinen, T., 1985. Leaf Protein from Green Pulse Crops and Nutritive-Value of Legume Protein-Concentrates for Poultry. *Journal of Agricultural Science in Finland, 57*, 117-123.

Nielsen, L., 2012. Baggrundnotat: Udbytte niveauer ved forskellig drift af lavbundsjord, 7 pp. http://ifro.ku.dk/fotos/media_library/foi/docs/publikationer/rapporter/eksterne_rapporter/ti_mio_tons_plan/bpl4

Nielsen A.L., Hald A.B., Larsen S.U., Lærke, P.E. & Møller H.B. 2013. Potassium as a mean to increase production and NP-capture from permanent grassland on organic soils. Grassland Science in Europe, Volume 18, pp. 569-571. Proceedings of the 17th Symposium of the European Grassland Federation, Akureyri, Iceland, 23-26 June 2013. http://cabdirect.org/abstracts/20133335432.html;jsessionid=F45C8F34A461C5765819BBFD6A8B604A

Nielsen, O.-K., Plejdrup, M.S., Winther, M., Nielsen, M., Gyldenkærne, S., Mikkelsen, M.H., Albrektsen, R., Thomsen, M., Hjelgaard, K., Hoffmann, L., Fauser, P., Bruun, H.G., Johannsen, V.K., Nord-Larsen, T., Vesterdal, L., Møller, I.S., Caspersen, O.H., Rasmussen, E., Petersen, S.B., Baunbæk, L., Hansen, M.G., 2014. Denmark's National Inventory Report 2014. Emission Inventories 1990-2012 - Submitted under the United Nations Framework Convention on Climate Change and the Kyoto Protocol. Aarhus University, DCE – Danish Centre for Environment and Energy, 1214pp. Scientific Report from DCE – Danish Centre for Environment and Energy No 101.

O'Hanlon, K, Knorr, K, Jørgensen, LN, Nicolaisen, M & Boelt, B, 2012. Exploring the potential of symbiotic fungal endophytes in cereal disease suppression. *Biological Control*, vol 63, no. 2, pp. 69-78., 10.1016/j.biocontrol.2012.08.007

Olesen JE, Jørgensen U, Hermansen JE, Petersen SO, Eriksen J, Søegaard K, Vinther FP, Elsgaard L, Lund P, Nørgaard JV, Møller HB, 2013. Effekter af tiltag til reduktion af landbrugets udledninger af drivhusgasser. DCA - Nationalt center for fødevarer og jordbrug, DCA Rapport nr. 027, 52 p.

Olesen, J.E., Jørgensen, U., Hermansen, J.E., Petersen S.P., Søegaard, K., Eriksen, J., Schønning, P., Greve, M.H., Greve, M.B., Thomsen, I.K., Børgesen, C.D. & Vinther, F.P., 2016. *Græsdyrkningen klima- og miljøeffekter*. Nationalt Center for Fødevarer og Jordbrug, Aarhus Universitet. DCA notat til FMVM.

Olesen, JE, Jørgensen, U., Hermansen, JE, Petersen SP., Søegaard, K., Eriksen, J., Schønning, P., Greve, MH., Greve, MB., Thomsen, IK., Børgesen, CD., Vinther, FP., 2016. Græsdyrkningen klima- og miljøeffekter. DCA notat til FMVM, 18 pp

Pedersen, EJN., and Møller, E., 1976 Almindelig rajgræs og klæøver i renbestand og i blanding. 6. Beretning fra Fællesudvalget for Statens Plateavls- og Husdyrbrugsforsøg, 27 pp

Pirie NW, 1987. Leaf Protein and its By-products in Human and Animal Nutrition, Cambridge University Press.

Pisulewska, E., Hanczakowski, P., Pisulewski, P., & Szymczyk, B., 1991. The changes of the yield, composition and nutritive value of leaf protein extracted from vetch and cereal mixtures during three years cultivation. *J. Sci. Food Agric., 55*, 197-205.

Powlson, DS., Stirling,CM., Jat, ML., Gerard, BG., Palm, CA., Sanchez, PA., Cassman, KG., 2014. Limited potential of no-till agriculture for climate cahnge mitigation. Nature Climate Change 4, 678 - 684

Pugesgaard, S., Schelde, K., Larsen, S.U., Lærke, P.E., Jørgensen, U., 2015. Comparing annual and perennial crops for bioenergy production influence on nitrate leaching and energy balance. Glob. Change Biol. Bioenergy 7, 1136-1149.

Qiao C, Liu L, Hu S, Compton JE, Greaver TL & Li Q, 2015. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. Global Change Biology 21, 1249–1257.Santamaría-Fernández, M., Molinuevo-Salces, B., Kiel, P., Steenfeldt, S., Uellendahl, H., Lübeck, M. Lactic acid fermentation for refining proteins from green crops and obtaining a high quality feed for monogastric animals. Submitted to Bioresource Technology (October 2016)

Schardl, C. L., Grossman, R. B., Nagabhyru, P., Faulkner, J. R., and Mallik, U. P., 2007. Phytochemistry 68 (7):980-996.

Schardl,CL, Leuchtmann, A and Spiering, MJ, 2004. Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology* **55**, 315-340.

Scott DB and Schardl CL, 1993. Fungal symbionts of grasses: evolutionary insights and agricultural potential. *Trends in Microbiol.* **1**, 196–200.

Smith, J., Gerrard, C. and John Hermansen (Eds), 2015. Improved contribution of local feed to support 100% organic feed supply to pigs and poultry (ICOPP): Synthesis report.

Søegaard, K. , Kristensen T., 2015. Udbytter på græsarealer – omdriftsgræs og vedvarende græsarealer. Bestilling fra NaturErhvervsstyrelsen 27. juli. Journal 103385.

Søegaard, K., 1994. Kombinationer af slæt og afgræsning i græs og kløvergræs. SP rapport nr 4, Statens Planteavlsforsøg, 71 pp.

Søegaard, K., 2004. Kvælstof til kløvergræs ved forskellige benyttelsesformer. DJF rapport Markbrug nr. 106, 47 pp.

Sørensen, C G; Bochtis, D D; Green, O; Oudshorn, F.W., 2010. A scheduling model for forage harvesting. Grassland Science in Europe, Vol 15, Pages 443-445 og Sørensen, C.G.; Nielsen, V. 2005. Operational Analyses and Model Comparison of Machinery Systems for Reduced Tillage. Biosystems Engineering, 92: 143-155

Solati Z, Jørgensen U, Søegaard K. 2016a. Protein productivity and extractability of legume and grass species during spring growth. In: Höglind M, Bakken AK, Hovstad KA, Kallioniemi E, Riley H, Steinshamn H, Østrem L, eds. The multiple roles of grassland in the European bioenconomy: Proceedings of the 26th General Meeting of the European Grassland Federation. Wageningen. s. 116-118.

Solati, Z., Jørgensen, U., Eriksen, J., Søegaard, K., 2016b. Estimation of protein extractability in legume and grass species. Submitted for Journal of the Science of Food and Agriculture. .

Sørensen, JN., Grevsen, K., 2016 Strategies for cut-and-carry green manure production. Acta Horticulturea In press

Soussana, J.F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays, D., 2004. Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management 20, 219-230.

Stødkilde-Jørgensen, L., Damborg, V.K., Jørgensen, H., Lærke, H.N. and Jensen, S.K., 2016. Nutritional evaluation of white clover as protein feed for monogastric animals. (Manuscript).

Taghizadeh-Toosi, A., Olesen, J.E., 2015. Modelling soil organic carbon in Danish agricultural soils suggests low potential for future carbon sequestration. Agricultural Systems (submitted).

Tanneberger, F., Wichtmann, W. (eds), 2011. Carbon credits from peatland rewetting: climate, biodiversity, land use. Schweizerbart Science Publishers, Stuttgart.

Termansen, M., Gylling, M., Jørgensen, U., Hermansen, J., Hansen, L.B., Knudsen, M.T., Adamsen, A.P.S., Ambye-Jensen, M., Jensen, M.V., Jensen, S.K., Andersen, H.E. & Gyldenkærne, S., 2015. *Green Biomass.* National Centre for Food and Agriculture (DCA), Aarhus University.

Thomsen IK, Elsgaard L, Olesen JE, Christensen BT., 2016. Nitrogen release from differently aged Raphanus sativus L. nitrate catch crops during mineralization at autumn temperatures. Soil Use and Management. 32(2):183-191

Thomsen, MH, Bech, D., Kiel, P, 2004. Manufacturing of stabilised brown Juice for L-lysine production - from university lab scale over pilot scale to industrial production. Chem. Biochem. Eng. Q. 18, 37–46

van de Velde F; Alting, A.; Pouvreau, L., 2011. From wasteproduct to food ingredient: the extraction of the abundant plant protein Rubisco. *New Food,* **2011,** pp 10-14.

VFL, 2015. *Håndbog til driftsplanlægning 2015.* SEGES Forlag, Agro Food Park 15, 8200 Aarhus N.

Wachendorf, M., Büchter, M., Trott, H., Taube, F., 2004. Performance and environmental effects of forage production on sandy soils. II. Impact of defoliation system and nitrogen input on nitrate leaching losses. Grass Forage Science 59, 56-68.

Whitehead D.C., 1995. Grassland Nitrogen. CAB International.

Young, C. A., Felitti, S., Shields, K., Spangenberg, G., Johnson, R. D., Bryan, G. T., Saikia, S., and Scott, B., 2006. Fungal Genetics and Biology 43 (10):679-693.

Young, C. A., Tapper, B. A., May, K., Moon, C. D., Schardl, C. L., and Scott, B., 2009. Applied and Environmental Microbiology 75 (7):2200-2211.

Zak, D., Gelbrecht, J., Zerbe, S., Shatwell, T., Barth, M., Cabezas, A., and Steffenhagen, P., 2014. How helophytes influence the phosphorus cycle in degraded inundated peat soils – Implications for fen restoration. Ecological Engineering 66, 82-90.

DCA - National Centre for Food and Agriculture is the entrance to research in food and agriculture at Aarhus University (AU). The main tasks of the centre are knowledge exchange, advisory service and interaction with authorities, organisations and businesses.

The centre coordinates knowledge exchange and advice with regard to the departments that are heavily involved in food and agricultural science. They are:

Department of Animal Science
Department of Food Science
Department of Agroecology
Department of Engineering
Department of Molecular Biology and Genetics

DCA can also involve other units at AU that carry out research in the relevant areas.

SUMMARY

The report summarizes our present knowledge on the bio-technical as well as economic issues in relation to value creation of green biomass in Denmark through high value protein production. The report describes the availability and quality of relevant green biomass, the environmental impact related to the crop production, the mass balances in the bio-refining processes, the feeding value of the protein recovered as well as the remaining fibre-fraction, prospects of the recovered proteins for human food, and operational costs . While a vast amount of knowledge exists or is in progress on these issues, also at all steps there is lack of knowledge to evaluate with a high certainty the overall economic and environmental consequences in case this technology is to be implemented.

First and foremost there is need for the bio-refining process to run at a commercial scale to obtain better knowledge on resource use and efficiency. There is a need to figure out the practical possibilities of using the protein paste as input in wet-feeding systems for pigs, since this will influence energy demands in the process to a high degree. Likewise there is a need to investigate how best to utilize the high moisture residual juice for energy recovery in biogas production to counteract the energy use at the factory level. As regards biomass supply in relation to environmental impact there is a need better to understand how long lasting grass or grass-clover fields can be established and maintained without losing nitrate and carbon. Likewise, there is a need to explore the potential of using cover crops as feedstock supply and - related to that - the environmental impact of new types of cover crops and management practises. Finally, there is a need to explore the possibilities of extracting other valuable components from the green biomass, upgrade proteins for human consumption and/or upgrade fibre for higher value products in order to improve the overall profitability of the bio-refining process.