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Desk study on potential protein production based on grass from meadows

BIOVALUE SPIR

Title:
Desk study on potential protein production based on grass from meadows

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Prepared by:
Danish Technological Institute
Agro Food Park 15
DK - 8200 Aarhus N
Bioressources & Biorefining
www.teknologisk.dk

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Authors:
Jørgen Hinge
Søren Ugilt Larsen
Jørgen Pedersen

Cover picture.
View from Nørreådalen in Denmark, an example of a valley with considerable meadow areas that are not being used and which is, therefore, a potential resource for harvest of biomass for e.g. protein production. The square plots are part of a field trial with application of potassium in order to increase the biomass production. (Photo: Søren Ugilt Larsen).

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1. Summary

In this desk study the potential protein production from §3 grasslands and adjacent intensive and extensive grasslands is estimated[1]. These areas may be divided into 'category b' which can be used for a combination of grazing and cutting and 'category c' which can be used for cutting. For the intensive and extensive grasslands adjacent to §3 grasslands, it may potentially be possible to harvest the 'entire area' or it may only be economically relevant to harvest the 'adjacent area' nearest to §3 grasslands.

The magnitude of the potential area varies considerably depending on the applied assumptions, including technical, economic and legislative issues. The total areal of grasslands available for harvesting grass for protein production is 149,039 – 454,300 ha, depending on which areas it eventually will be decided to include:

§3 grassland category	§3 area only (ha)	§3 area + adjacent intensive and extensive grasslands (ha)	§3 area + the entire area of intensive and extensive grasslands (ha)
Category c	149,039	274,436	410,282
Category b+c	193,057	356,797	454,300

The biomass yield per ha of meadow as well as the crude protein content of grass from meadows may also vary largely.

The potential annual production of crude protein from the different combinations of §3 grassland categories with and without adjacent intensive and extensive grasslands is 29,533 – 295,555 tons/year:

§3 grassland category	§3 area only (tons/year)	§3 area + adjacent intensive and extensive grasslands (tons/year)	§3 area + the entire area of intensive and extensive grasslands (tons/year)
Category c	29,533-45,665	144,369-160,501	268,774-284,906
Category b+c	36,196-56,314	186,337-206,455	275,437-295,555

Large-scale utilization of grass from meadows for protein production will depend largely on further development and optimization of technology for biorefining of protein from grass which may reduce the production costs. Moreover, utilization of meadow grass for protein production will depend on other economic parameters including future prices of protein and the alternative value of the grass from these areas.

[1] For definition of §3 grassland categories, intensive and extensive grasslands, see section 3.1.

2. Introduction

In Denmark, there is a very large consumption of protein feed in the animal production, and there is an annual import of approx. 1.5 million tonnes soybean meal (Termansen *et.al.*, 2015). There is great interest in replacing some of this imported protein feed by protein produced in Denmark, e.g. by refining protein from green biomass. One of the approaches to this is to replace the production of annual cereal crops such as wheat and barley by highly productive perennial grasses which may increase the overall production of biomass and protein and, at the same time, have positive environmental effects (Jørgensen & Lærke, 2016).

A possible source of grass biomass for protein production could be meadows and natural grasslands of which there are considerable areas in Denmark. Many of these areas were previously very valuable for grazing and hay production, but due to the structural changes in the agricultural production, a proportion of these areas are no longer being utilized. To maintain these areas as grassland, however, it is necessary to carry out management or 'maintenance', either by grazing or by cutting at least once per year. The lack of utilization of the biomass and the need to maintain these permanents grasslands suggest that harvest of the biomass for biorefinery could have plural purposes. Accordingly, Kristensen & Jørgensen (2012) have estimated that permanent grass on lowlands could contribute with 210,000-390,000 tonnes DM (Dry Matter) per year for biorefinery. The aim of this report is to analyse the potential for harvesting grass from meadows and other categories of permanent grassland for biorefinery of protein. The analysis is based on existing knowledge and the aim is to give estimates of the potential areas, potential DM yield and protein yield per hectare and, hence, the potential total protein production based on these areas. Moreover, the aim is to evaluate and discuss possible barriers and the possible feasibility of exploiting this source for protein production. The potential will depend on numerous factors such as geographic/topographic restrictions, potential alternative use of the areas, legislation and not least the efficacy and future developments in the technology for extracting protein from green biomass. Therefore, the estimates will also be subject to considerable uncertainty but will give an indication of the magnitude.

3. Estimation of potential available protein resources from meadows and natural grasslands

3.1. Total areas of meadows and natural grasslands

In this section, the total area of meadows and natural grasslands is assessed. The definition of meadows and natural grasslands may vary in different studies. In this context, it is especially interesting to consider grass from areas which are not being utilized today. However, it may also be relevant to include biomass from certain other areas, as discussed below.

In Kristensen & Jørgensen (2012), it is estimated that "extensive grasslands" cover 217.000 ha, of which 100.000 ha are permanent grass on lowlands. However, in this study the starting point for assessing the total area of available grasslands will be the so-called §3 grasslands.

According to §3 in the Danish Environmental Protection Legislation (‘Naturbeskyttelse-loven’) (Ministry of Environment and Food of Denmark, 2016), there are a number of different habitats, which need ‘special care’ in order to prevent the habitats from turning into forest – i.e. they should be maintained either by grazing by cattle or by regular cutting by use of machinery. Therefore, these areas are relevant in relation to potential protein resources, because the biomass could be collected and utilized, and because they need some kind of maintenance anyway. This also means that part of the harvest costs is already covered because of this obligation. The habitat types include heathers (‘heder’), mires/bogs (‘moser’), coastal meadows and coastal swamps (‘strandenge’ and ‘strandsumpe’), meadows (‘ferske enge’) and dry meadows (‘overdrev’)[2]. These types of areas are subsequently referred to as §3 grasslands. Different studies estimate the total Danish area of §3 grasslands at 341,807-343,252 hectares, see table 3.1.

Table 3.1. Total area of §3 grasslands in Denmark, estimated in two different studies.

Year	Source	Total area of §3 grasslands (hectares)
2012	Pedersen (2012)	343,252
2013	Levin (2013)	341,807

Adjacent to these §3 grasslands, there will often be two other types of land with grass. *Extensive grasslands* are primarily permanent pastures and other types of natural grasslands not covered by the §3 grasslands, whereas *intensive grasslands* are considered to be pastures in more intensive agricultural production. In his assessment of the total area of natural grassland, Levin (2013) includes the extensive and intensive grasslands, assuming that it would make sense to maintain (cut) these grasslands together with harvesting the §3 grasslands. In table 3.2, the total area of grasslands including §3 grasslands and adjacent extensive and intensive grasslands is summarized.

Table 3.2. Total area of §3 grasslands and adjacent grasslands, after Levin (2013).

Area category	Total area (hectares)
§3 Grasslands	341,807
Extensive grasslands, adjacent to §3 grasslands, with potential for combining the maintenance (cutting) of the §3 grasslands and the extensive grasslands	84,277
Intensive grasslands, adjacent to §3 grasslands, with potential for combining the maintenance (cutting) of the §3 grasslands and the intensive grasslands	176,966
Total	603,050

[2] The English terms for the habitat types are adapted from Levin et. al. (2014), table 3-4

It should be mentioned that the biomass eventually collected at the §3 grasslands are not necessarily grass biomass; the botanical composition may vary greatly and, therefore, biomass yield and protein content may vary significantly, see section 3.3.2. Levin (2013) has assessed the areas of different §3 grassland habitats, see table 3.3.

Table 3.3. Area of different §3 grassland habitat types, after Levin (2013).

Habitat type	Area (hectares)
Meadows ('Eng')	94,185
Heather ('Hede')	82,817
Mire/bog ('Mose')	89,908
Dry meadow ('Overdrev')	29,697
Coastal meadow ('Strandeng')	45,200
Total	341,807

3.2. Potential meadow areas for harvest of biomass

For the intensive and extensive grasslands (Table 3.2), we assume that the total areas may potentially be available for utilization. For the §3 areas, however, it will not be practically possible to cut and collect the biomass only from a certain proportion of the areas listed in table 3.3. Nygaard *et al*. (2012) splits the §3 areas in 3 categories in terms of their applicability:

a) Areas which can be used only for grazing
b) Areas which can be used for a combination grazing and cutting
c) Areas which can be used for cutting

When calculating the biomass production from §3 grasslands, Nygaard *et al*. found that only biomass from category c) areas will potentially be available for e.g. protein production, while for category b) areas, it is assumed that the harvested biomass will be used as winter feed for the cattle grazing the area in the summer, hence there will be no biomass available for other use. In this report, however, we will consider both category b) and c) areas potentially available for protein production, while the remaining part of the area (category a) can be used only for grazing. In table 3.4 we have, therefore, calculated the available areas for category c) and for category b) + c). The calculation takes into account that only a certain fraction of the total areas is likely to be available for utilization, as estimated by Levin (2013).

Table 3.4. Area of different §3 grassland habitat types available for cutting in Denmark (after Levin, 2013, and Nygaard *et al*., 2012). The fraction of the area available for cutting is as defined by Nygaard et al., 2012. See text above for definition of categories.

Habitat type	Area (hectares)	Category c)		Category b)+c)	
		Fraction available for cutting and utilization (%)	Area available for cutting and utilization (hectares)	Fraction available for cutting and utilization (%)	Area available for cutting and utilization (hectares)
Meadows ('Eng')	94,185	56	52,744	71	66,871
Heather ('Hede')	82,817	32	26,501	48	39,752
Mire/bog ('Mose')	89,908	35	31,468	52	46,752
Dry meadow ('Overdrev')	29,697	56	16,630	56	16,630
Coastal meadows ('Strandeng')	45,200	48	21,696	51	23,052
Total	341,807	44	149,039	56	193,057

With regards to the extensive and intensive grasslands, the total areas estimated in table 3.2 (84,277 and 176,966 hectares, respectively) can in principle be utilized for protein production. However, because it was argued in section 3.1 that the reason for taking these grasslands into account together with the §3 grasslands in the first place was to explore the advantages of maintaining and cutting the different grasslands together, it could also be argued that the area of extensive and intensive grasslands available for biomass production in this study should be reduced accordingly, i.e. assuming the same percentages of available areas as for §3 grasslands in table 3.4. In this case, the available extensive and intensive grasslands areas adjacent to the category c) grasslands would be 40,453 and 84,944, respectively. And similarly, the available areas adjacent to the category b) + c) areas would be 52,252 and 111,488 hectares, respectively.

Based on the above assumptions and calculations, the total areal of grasslands available for harvesting grass for protein production is 149,039 – 454,300 ha, depending on which areas it will be decided to include, as shown in table 3.5.

Table 3.5. Summary table of potentially available areas of §3 grassland categories with and without adjacent intensive and extensive grasslands.

§3 grassland category	§3 area only (ha)	§3 area + adjacent intensive and extensive grasslands (ha)	§3 area + the entire area of intensive and extensive grasslands (ha)
Category c	149,039	274,436	410,282

Category b+c	193,057	356,797	454,300

Certain meadow areas are periodically flooded which may prevent traffic and harvest of biomass. Here examples from Nørreådalen in Denmark, photos taken 21th September 2010. (Photo: Søren Ugilt Larsen).

3.3. Biomass production

In addition to estimating the potential harvestable meadow areas it is important to estimate the potential biomass yield and protein yield on these areas. In this section, we estimate the potential biomass production in terms of dry matter (DM) yield from different habitat types. In section 3.4, we estimate the potential protein yield.

The potential for biomass production from the various §3 habitat types described in section 3.1 is listed in table 3.6, as assessed by Nygaard *et al.* (2012). Also, the potential biomass production from extensive and intensive grasslands – as described in table 3.2 – is listed. The biomass production varies greatly between the types of areas, as shown in the table.

Table 3.6. Annual biomass production at various §3 grassland habitat types and extensive/intensive grasslands, after Nygaard *et. al.* (2012)

Habitat type / area type	Biomass production (kg DM/hectare)
Meadows ('Eng')	2,600-4,800
Heather ('Hede')	500
Mire/bog ('Mose')	500
Dry meadow ('Overdrev')	2,000
Coastal meadows ('Strandeng')	1,700-2,300
Extensive grasslands	5,250
Intensive grasslands	7,800

The potential biomass production is estimated by multiplying the harvestable meadow areas (table 3.4) with the biomass production (table 3.6). Depending on the assumptions, the potential biomass production from §3 grasslands is estimated to be in the range from approx. 236,000 to 450,000 tons DM/year, see table 3.7

Table 3.7. Potential annual biomass production from §3 grassland habitat types available for cutting (after Levin, 2013, and Nygaard *et al.,* 2012).

Habitat type /area type	Category c) Biomass production (ton DM/year)	Category b)+c) Biomass production (ton DM/year)
Meadows ('Eng')	137,134-253,171	173,865-320,981
Heather ('Hede')	13,250	19,876
Mire/bog ('Mose')	15,734	23,376
Dry meadow ('Overdrev')	33,260	33,260
Coastal meadows ('Strandeng')	36,883-49,901	39,188-53,020
Total	236,261-365,316	289,565-450,513

In table 3.8, the potential biomass production from the extensive and intensive grasslands are listed, with and without the reduction of the area as discussed in section 3.2.

Table 3.8. Potential annual biomass production from extensive and intensive grasslands adjacent to §3 grasslands.

Area category	Biomass production, entire area (ton DM/year)	Biomass production, reduced area, Category c) (ton DM/year)	Biomass production, reduced area Category b)+c) (ton DM/year)
Extensive grasslands	442,454	212,378	274,323
Intensive grasslands	1,380,335	662,563	869,606
Total	1,822,789	874,941	1,143,929

For comparison, Kristensen & Jørgensen (2012) estimated that permanent grass on lowlands could contribute with 210,000-390,000 tonnes DM per year. Hence, the chosen assumptions regarding potentially available areas are of major importance for the magnitude of the biomass potential.

Meadows and other types of grassland may differ considerably in botanical composition and often with large variation even within an area. The botanical composition depends on the naturally given growth conditions and the applied management practice and the composition may, on the other hand, have consequences for the productivity and potential protein yield from the area. Here examples from Nørreådalen in Denmark, photos taken 9th July 2010. (Photo: Søren Ugilt Larsen).

3.4. Protein content and protein yield

The quantity of harvestable protein per hectare of meadow depends on the protein content in the biomass and the quantity of the biomass. Harvest of the largest amount of protein per hectare will in principle be a matter of optimizing the product of these two parameters. In this section, the factors affecting the protein content and the protein yield per hectare will be discussed.

Protein content in biomass from grass and other plant species relevant in this study is depending on several factors. In general, it is assumed that there is a direct correlation between the nitrogen content and the crude protein content of the biomass. The crude protein content is, therefore, often calculated from an analysis of the total N-content (generally by the Kjeldahl method) (FAO, 2003). The protein content is calculated by multiplying the total N-content by 6.25, assuming 16 % N in the protein. Since the amino acid composition differs between proteins, the N content in the protein may vary between 13 and 19 % and the 'true' conversion factor is, therefore, also variable. Also, there may be variable contents of non-protein compounds. However, for the present study a conversion factor of 6.25 is assumed between N-content and protein content. Thus, if the N-content of the biomass is 2 % of the DM, the protein content of the biomass is assumed to be 12.5% of the DM.

In order to estimate the potential protein yield from the amounts of biomass calculated in section 3.3, it is necessary to make assumptions regarding N-content of the biomass harvested from meadows. In section 3.4.1-3.4.2, two of the main factors affecting the N-content and protein content of relevant crops are discussed, namely harvest time / cutting strategy and nutrient availability.

3.4.1. Harvest time and cutting strategy

In order to optimize yields from grasslands, the biomass is typically cut two or more times during the growing season, and the N-content may depend on the number of cuts per year and the timing of the individual cuts. The effect of timing of first cut in a three-cut strategy in grass-clover mixture was studied in three field trials in 2009, see figure 3.1 (Oversigt over Landsforsøgene, 2009a). When the timing for first cut was delayed from 8th of May until 29th of May, the DM yield increased gradually from 3.7 to 7.5 ton/ha, but since the protein content decreased concurrently from 16.7 to 10.6 %, the overall protein yield from first cut only changed moderately with an increase from 0.62 to 0.79 ton/ha. Hence, the time for cutting affects both the DM yield and the protein content, and although the increase in DM yield is counteracted by a decrease in protein content, the resulting protein yield may also be affected by the time of cutting.

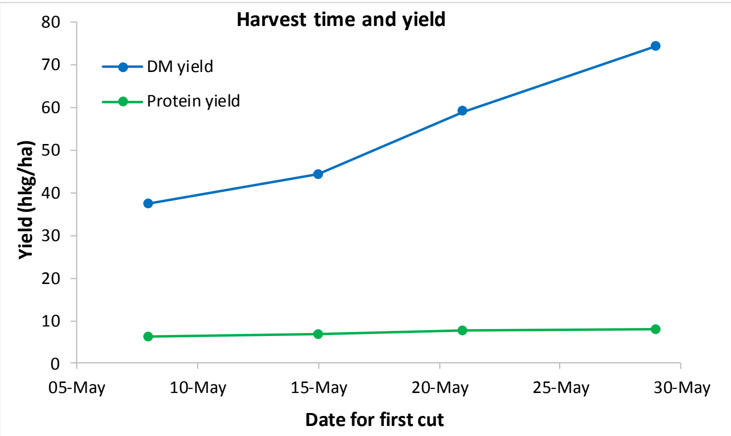

Figure 3.1. Dry matter yield and protein yield in first cut of a grass-clover mixture, depending on the time for first cut. Mean of three field trials in 2009. (Oversigt over Landsforsøgene, 2009, 'Tabelbilag L5).

The effect of the number of cuts per year has been illustrated in field trials with mixtures of *Festulolium* and red clover in 2008-2009 where a two-cut strategy (late June and mid-October) was compared with a three-cut strategy (early June, early August, mid-October)

(Oversigt over Landsforsøgene, 2009b). The annual DM yield was increased by on average 11 % by taking three cuts rather than two cuts per year, but since the average protein content was increased from 10.0 to 12.2 %, the annual protein yield was increased by 35 %.

The protein content may also vary between individual cuts within a year. In field trials with tall fescue and reed canary grass with three cuts per year, Larsen *et al.* (2016b) found considerable variation in the N content and DM yield between cuts as well as between harvest years, see table 3.9. Hence, the first cut contributed with on average 46 % of the annual protein yield whereas second and third cut contributed with 31 and 22 % of the protein yield, respectively.

Table 3.9. Variation in N content of tall fescue and reed canary grass, depending on harvest year and which cut. Average of the two species, four levels of N fertilization and two levels of PK fertilization (Larsen *et al.,* 2016b).

Year	**1st cut, early June**	**2nd cut, early August**	**3rd cut, early October**
		% N in DM	
2012	2.1	1.5	2.0
2013	2.3	1.9	2.3
2014	2.1	1.8	2.8
2015	2.4	2.3	2.4
Mean	**2.2**	**1.9**	**2.4**
		Biomass yield Ton DM/hectare	
2012	5.0	4.7	2.0
2013	4.0	2.2	2.0
2014	3.8	2.9	1.1
2015	2.2	2.5	1.8
Mean	**3.7**	**3.1**	**1.7**
		N-yield Kg N/hectare	
2012	108	74	40
2013	97	44	47
2014	80	52	32
2015	51	57	40
Mean	**84**	**57**	**40**

These examples clearly illustrate that cutting time and cutting strategy may have considerable impact on both the protein content and the quantity of harvestable protein per hectare. Although most of these examples are based on trials with intensive production of forage grass with fertilization, it is very likely that cutting time and cutting strategy will also be very important factors for the potential protein yield from harvesting meadow grass and, hence, may be possible tools for optimizing a protein production based on meadow grass, as far as allowed by practical conditions, economy and legislation, see section 4.1-4.2.

3.4.2. Nutrient availability

Larsen *et al*. (2016a) have described the effects of N and PK fertilizer application to perennial grass species (tall fescue and reed canary grass) in terms of biomass yield and N content of the biomass through a 3-year period, 2012-2014. With increasing N fertilization from 0-450 kg/ha/year, the N content increased with some 35-40%, typically from about 1.6% in DM to about 2.6% in DM with some variation. The quantity of N harvested with the crop in the trials also increased drastically with increased N fertilization – typically from about 53 kg N/ha/year to about 300 kg N/ha/year. There were of course variations depending on the species and whether PK-fertilizer was also added; for further details, please refer to Larsen *et al*. (2016a), table 20.1. However, the important conclusion here is that N availability has a massive influence on the protein content of the crop and the amount of protein that can be harvested per hectare.

In situations, where N availability is not a limiting factor for grass growth, the availability of other nutrients such as potassium (K) may be of importance to the biomass yield, and hence to the protein yield. Nielsen *et al*. (2016) have described the effect of adding vinasse, a K-rich by-product from e.g. sugar industry to meadows which were dominated by rough meadow grass and couch-grass, see table 3.10. The study showed that application of K to the meadows up to a certain level increased both the biomass and the N yield, whereas further K application did not increase the yields further.

Table 3.10. Biomass and N yield from meadows from 2012 through 2016, depending on the addition of K fertilizer. Nielsen *et al*. (2016)

Vinasse kg K/ha/year	Biomass yield ton DM/ha/year	N yield Kg N/ha
0	5,43	119
25-58	8,07	156
50-115	8,42	158

The optimization of nutrient availability with the intention of increasing protein yield from meadows and grasslands may indeed be relevant for the extensive and intensive grasslands referred to in section 3.2. On those areas, it is allowed to apply nutrients up to standard norms (reference) and the protein content in the harvested crop can, therefore, be optimized with the best combination of nutrients.

However, because application of nutrients to §3 grasslands is to some degree restricted by specific regulations (Miljø- og Fødevareministeriet, 2016, see also section 4.1), complete optimization of nutrient availability on those areas may not be possible.

The biomass production on meadows may in some cases be limited by the availability by other nutrients than N, e.g. K. This picture shows a field trial in Nørreådalen with application of K in order to increase the biomass production. This may also be relevant if the biomass is to be used for protein production. To the right, a plot with application of Ka and to the left, a plot without K and with considerable attack of crown rust. Photo taken 24th August 2011. (Photo: Søren Ugilt Larsen).

3.4.3. Other factors affecting biomass yield and potential protein production.

As described in section 3.4.2, optimizing nutrient availability by adding fertilizer is an important factor in increasing biomass and protein yield from meadows and adjacent grasslands. However, a number of other factors will also influence the nutrient availability. In figure 3.2, Van Duren *et al*. (2000) have illustrated the complex relations between different factors and their influence on nutrient availability.

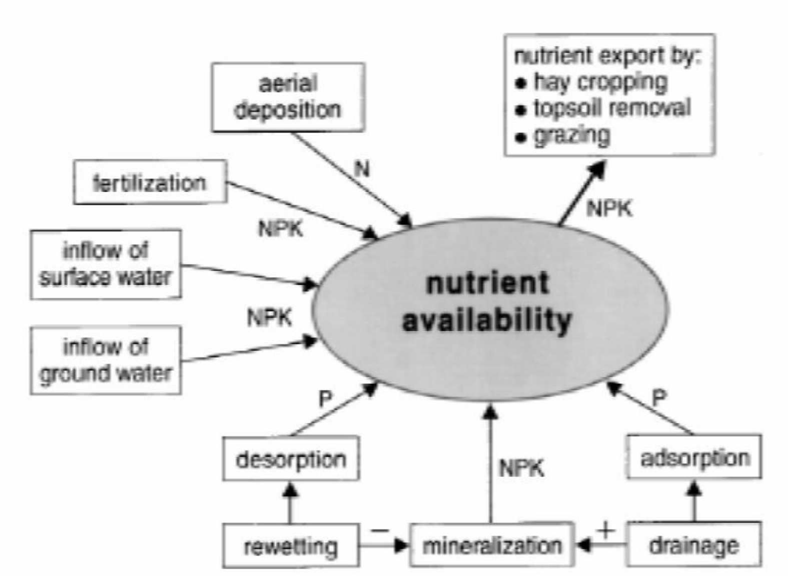

Figure 3.2. Factors affecting nutrient availability in wet and drained peat soils. (Van Duren et al. 2000)

Hydrology may influence the species composition of a given area. A certain soil moisture is necessary to maintain a vegetation rich in species, while deep draining may result in a reduction in number of species, as may oversaturation (Nielsen *et al*., 2006). As discussed earlier, species composition can affect biomass yield and thus potential protein production.

3.4.4. Potential protein yield

From section 3.4.1-3.4.3 it is evident that a number of factors may influence the crude protein yield of a given meadow area. Therefore, the potential for protein production from meadows in Denmark will be depending on not only the total area available and naturally occurring factors, but also on man-made decisions at two levels.

At one level, there is a comprehensive legislation, which regulates the agricultural practices to be applied, see section 4.1. First and foremost, there are limitations to amount of fertilizer which can be applied. For certain protected areas, no application of N fertilizer is allowed at all; this means that there are limited or no possibilities for increasing the protein yield from those areas. These man-made decisions (the legislation) are primarily motivated by environmental concerns.

On other areas (e.g. extensive and intensive grasslands, as defined in section 3.1), the yield of protein can legally be optimized by adding fertilizer within certain limits, and a second level of man-made decisions is therefore – for example - the farmers' decision whether or not (or to which degree) to apply fertilizer to those areas. This decision will typically be based on feasibility considerations, see section 4.2.

Here we define the potential protein yield from meadows as the total protein that can be harvested with the biomass, i.e. there is no distinction between the protein that may be extracted with the juice fraction and the protein that may follow the fiber fraction. In order to estimate the total potential protein production based on grass from meadows in Denmark, the following assumptions are made:

- The protein content is calculated by multiplying the total N-content by 6.25, assuming 16 % N in the protein (see section 3.4)
- N-content in §3 grasslands vary between 1.8-2.2% in DM; in our calculations, we'll use 2.0%
- N-content in extensive and intensive grasslands vary between 1.8-2.4% in DM; in our calculations, we'll use 2.1%

With these assumptions, the potential protein production from §3 (category c and category b)+c)), extensive and intensive grasslands is calculated in table 3.11a.

Table 3.11a. Calculation of potential annual protein production from different §3 grassland categories and adjacent intensive and extensive grasslands.

Area category	Calculation	Potential protein production (Tons/year)
1) § 3 grasslands, category c)	236,261-365,316 ton DM * 2.0 % N * 6.25	29,533-45,665
2) § 3 grasslands, category b)+c)	289,565-450,513 ton DM * 2.0 % N * 6.25	36,196-56,314
3) intensive and extensive grasslands adjacent to 1)	874,941 ton DM * 2.1 % N * 6.25	114,836
4) intensive and extensive grasslands adjacent to 2)	1,143,929 ton DM * 2.1 % N * 6.25	150,141
5) intensive and extensive grasslands, entire area	1,822,789 ton DM * 2.1 % N * 6.25	239,241

The total potential protein production based on grass from meadows in Denmark depends on whether it is assumed that:

- only category c) + §3 grasslands **or** both category b)+c) + §3 grasslands are available
  and
- only adjacent intensive and extensive grasslands **or** the entire area of intensive and extensive grasslands are available,

Hence, the potential total annual protein production based on grass from meadows may vary between 144,369 and 295,555 Tons/year, as calculated in table 3.11b. See also summary table 3.12.

Table 3.11b. Calculation of potential annual protein production from different combinations of §3 grassland categories and adjacent intensive and extensive grasslands.

Area category	Calculation (Tons protein/year)	Potential protein production (Tons/year)
6) § 3 grasslands, category c) + adjacent intensive and extensive grasslands	(29,533-45,665) + 114,836	144,369-160,501
7) § 3 grasslands, category c) + the entire area of intensive and extensive grasslands	(29,533-45,665) + 239,241	268,774-284,906
8) § 3 grasslands, category b)+c) + adjacent intensive and extensive grasslands	(36,196-56,314) + 150,141	186,337-206,455
9) § 3 grasslands, category b)+c) + the entire area of intensive and extensive grasslands	(36,196-56,314) + 239,241	275,437-295,555

Table 3.12. Summary table of potential annual protein production from different combinations of §3 grassland categories with and without adjacent intensive and extensive grasslands.

§3 grassland category	§3 area only (Tons/year)	§3 area + adjacent intensive and extensive grasslands (Tons/year)	§3 area + the entire area of intensive and extensive grasslands (Tons/year)
Category c	29,533-45,665	144,369-160,501	268,774-284,906
Category b+c	36,196-56,314	186,337-206,455	275,437-295,555

4. Legislation and feasibility

In section 3, the potential protein production based on grass from meadows in Denmark is estimated. There's quite a span between the lowest and the highest estimate, depending on the assumptions, which again reflects the importance of the framework conditions, such as legislation and fiscal incentives. Moreover, the extent to which the potential can be utilized may be highly affected by legislation and economic feasibility. In this section, we briefly discuss legislative and economic barriers as well as other possible barriers affecting the potential protein production from meadow areas.

4.1. Legislation

The legislation regarding management of meadows and grasslands (and agricultural land in general) is comprehensive, and it is beyond the scope of this study to give a complete overview of the entire legislation. However, a few important principles with particular impact on the potential protein production will be presented here.

§3 grasslands, as used in section 3 for estimating potential protein production, are defined in the Danish Environmental Protection legislation ('Naturbeskyttelseloven') (Miljø- og Fødevareministeriet, 2016). The legislation prescribes that the quality and status of these habitats must be protected, and management of the areas may not result in changes affecting plant and animal life. In principle, any legal practice carried out before the legislation was implemented can continue, so it is not necessarily prohibited to cultivate crops or fertilize all of these areas. In reality, however, this means that tight restrictions apply to many meadow areas, compared to other agricultural land and for instance for mires/bogs ('moser') and heathers ('heder'), fertilization is prohibited and can only happen after an exemption from the regulation. Also, on many §3 grasslands (i.e. heathers, meadows, coastal meadows), cutting can only take place between July 1st and April 30th. (Miljø- og fødevareministeriet, 2008) As a consequence, very few measures can be applied to optimize DM production, N-content and, therefore, protein production from §3 grasslands, beyond what is achievable within the given status and cultivation practices.

For *extensive* and *intensive* grasslands (as applied in this context – see section 3.1), the main principles for cultivation and maintenance follow the guidelines for "normal" agricultural practice in Denmark. This means that optimization is possible, for instance through fertilizer application or optimized harvest and cutting strategy, as described in section 3.4.1-3.4.2. This may turn out to be important if we wish to increase protein production from e.g. grass, because at present, feasibility is dubious, as described in Fog & Thierry (2016) and in the next section.

4.2. Feasibility

In recent years, there has been some focus on harvesting of grass from meadows for biogas production, and this concept has, for instance, been studied in the BioM project (2010-2012), using Nørreådalen as a study area (Lundegrén, 2012; Brieseid, 2012). Although grass from meadows is now to some extent being used for biogas production, the overall experience is that it is rather difficult to achieve economic feasibility, unless the nutrients after digestion in the biogas process are being used for e.g. fertilizer in organic agriculture. Feed protein extracted from meadow grass is likely to have a higher value than biogas and fertilizer, however, the production of protein from green biomass is also accompanied by considerable costs. Hence, preliminary economic analyses indicate that it is difficult to make production of protein from agricultural crops (grass in agricultural crop rotation) feasible (Fog & Thierry, 2016).

Similarly, Hermansen *et al*. (2017) have conducted economic analysis for highly productive grass species and concluded that there is "only a small window" where the operational economy in protein production from grass in a biorefinery becomes positive. This situation is when the residual juice can be utilized for energy generation and thus alleviating energy costs at the bio-refinery.

Harvest of grass from meadows may often be less rational than harvest of grass in rotational fields, since the fields may be relatively small due to the occurrence of streams og ditches or due to relatively wet conditions which may only allow traffic by relatively small machinery. Such conditions may weaken the economy further. On the other hand, grass from meadows may not have any alternative value, and there may even be a cost for maintenance of unutilized meadows which could be saved, if the biomass is gathered. Therefore, it is still likely that grass from meadows may become a feasible resource for refining of protein. This may be particularly relevant for relatively productive areas that are not too wet to harvest. Also, further development of the technology for protein refinery may reduce the production costs, and this may increase the relevance of the use of grass from meadows for this purpose.

Hence, there appears to be an overall need for further development and optimization of the concept of biorefining of grass for protein and other products, no matter if the grass is from rotational crop production or meadows.

An important challenge for harvest of biomass from meadows is that many areas may be wet, and this may impede or even prevent harvest of biomass. Various types of technology for harvest/management have been tested on meadow areas as exemplified from this demonstration in Nørreådalen on 24th August 2011. Compared to when the grass is only cut for maintenance purposes, the challenge is even larger when the biomass is to be gathered from the area since the load from trailers etc. increases the specific pressure from wheels or belts. (Photo: Søren Ugilt Larsen).

5. Conclusions

In conclusion, there appears to be large areas of meadows that could potentially be used for protein production. However, the magnitude of the potential area varies considerably depending on the applied assumptions, including technical, economic and legislative issues. In the presented scenarios, the potential total area of meadows available for protein production ranges from 149,039 to 454,300 ha.

The biomass yield per ha of meadow as well as the protein content of grass from meadows may also vary largely, e.g. depending on the possibilities for fertilizing the area. Variation in yield and protein content may add to the uncertainty of the technical potential for protein from meadow areas. In the study, the potential annual protein production from grass on meadows ranges from 144,369 to 295,555 tons crude protein.

Large-scale utilization of grass from meadows for protein production will depend largely on further development and optimization of technology for biorefining of grass for protein which may reduce the production costs. Moreover, utilization of meadow grass for protein production will depend on other economic parameters including future prices of protein and the alternative value of the grass from these areas.

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