Journal of Cleaner Production 294 (2021) 126304

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Economic viability of protein concentrate production from green biomass of intermediate crops: A pre-feasibility study

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A R T I C L E I N F O

Article history:
Received 6 July 2020
Received in revised form
3 February 2021
Accepted 5 February 2021
Available online 9 February 2021

Handling editor: Dr Sandra Caeiro

Keywords:
Intermediate crops
Green biomass
Protein extraction
Green and white proteins
RuBisCO
Economic assessment

A B S T R A C T

Green biomass is a major potential source of proteins for food and feed. This pre-feasibility study evaluates the use of green biomass of buckwheat, phacelia, hemp and oilseed radish grown as intermediate crops (IC) as a feedstock for production of protein concentrates to produce protein-rich food and feed products. We investigated the biomass yield, protein concentration and protein recovery potential of non-fertilized IC, nitrogen-fertilized IC and IC intercropped with legumes, harvested in late summer to autumn during 2017 and 2018 in southern Sweden. In addition, economic assessment of potential protein and fibre feed and food products were evaluated. The results showed that IC fertilized with 40 kg ha^{-1} N and intercropping with legumes contributed to a higher biomass dry matter (DM) yield of 4.9–5.8 t ha^{-1} as compared to between 2.2 and 3.1 t ha^{-1} for non-fertilized IC. Intercropping with legumes also resulted in higher protein yield of 154 g kg^{-1} vs. 103 g kg^{-1} for non-fertilized IC. Among IC, hemp, phacelia and oilseed radish showed up to ca. 25% higher DM yield and up to ca. 70% higher protein concentration as compared to buckwheat. Higher DM yield was obtained when IC were harvested in October and November than in August and September. Economic assessment was made on two feasible protein production pathways; (A) Green and white proteins and (B) total recoverable combined protein fraction (CPF). For all IC, cost per t DM was higher in August due to lower biomass yield as compared to other harvesting months. Nitrogen concentration was the main factor determining the size of revenues. Nitrogen concentration was 34% higher in 2018 compared to 2017 and therefore resulted in higher revenues in that year. Intercropping resulted in higher protein content and therefore contributed to lower breakeven prices of recovered green proteins for all IC. Breakeven price analyses showed that green protein and CPF were economically feasible to market as both bulk and premium products depending on lower ($\leq$2 € kg^{-1}) or higher (2–10 € kg^{-1}) price ranges, respectively. The results demonstrate that use of IC biomass could be a feasible option to produce high value protein-rich products, which can contribute extra income from IC for farmers.

1. Introduction

Currently, the diet patterns of modern society are rapidly changing. Plant-sourced protein-rich diets are increasingly replacing meat, and modern food alternatives with a balanced content of nutrients are gaining increasing attention (Kumar et al., 2017; Rosenfeld and Burrow, 2017). The changes in diet patterns are largely related to i) a perception that food consumption choices influence public health, ii) a desire to manage body weight (Rosenfeld and Burrow, 2017) and iii) ethical and environmental concerns regarding meat production (Schösler et al., 2012). This change in food choices may require an altered or increased production of crops containing suitable protein for human consumption. In addition, the increased desire of locally produced protein sources for feed purposes, results in an increased requirement of crops for protein-rich products (de Visser et al., 2014). Increased plant-based protein production requires either an increased yield per hectare or an increased use of agricultural biomass that is currently not used to produce plant protein-rich products. In this context, opportunities to use already available biomass, currently

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https://doi.org/10.1016/j.jclepro.2021.126304

without any specific use, for novel plant protein-rich products would be extremely valuable (Santamaría-Fernández et al., 2018; Tenorio et al., 2017). Intermediate crops (IC) are one example of such biomass, mainly grown to reduce nitrogen leakage, increase soil organic matter, and to benefit crop production (Fageria et al., 2005; Marcinkeviciene et al., 2013). IC used as catch or cover crops are usually incorporated into the soil as green manure. Therefore, harvesting of green biomass of IC for plant protein extraction may contribute to sustainable use of agricultural resources for production of protein-rich food and feed products.

Currently, bio-refinery technologies are evolving quickly, with the aim of converting any type of agricultural biomass into biofuels, biochemicals and biomaterials (Cherubini and Ulgiati, 2010; Dale et al., 2009). Similar technologies are also of relevance while exploring the opportunities of agricultural biomass and residues from various crops to be used for production of e.g. leaf protein concentrates or isolates for food and feed purposes (Berndtsson et al., 2019; Xu et al., 2017). The aerial parts of plants, especially green leaves, contain between 1.6 and 15% (wet weight) of proteins (Mielmann, 2013; Van de Velde et al., 2011), whereas stems and stalks tend to have low protein content as shown in Jerusalem artichoke (Johansson et al., 2015), *Amaranths* sp. (Hill and Rawate, 1982) and sugar beet (Tenorio et al., 2016). Crops such as alfalfa (Colas et al., 2013), tobacco (Teng and Wang, 2012) and sugar beet leaves (Tenorio et al., 2017) have been evaluated as sources for extraction of leaf protein for food-protein recovery to produce novel food products.

Extraction of the proteins abundantly available in green biomass has long been considered as a suitable although complicated option to sustain protein-rich food and feed production (Duckworth et al., 1961; Edwards et al., 1975). Since proteins are concentrated in the cells of green leaves, their efficient extraction requires disruption of cell wall structures. During the last decade, efforts have been made to not only increase the extraction efficiency of protein production but to also streamline the process to obtain different protein fractions and valuable side-products (Colas et al., 2013; Tenorio et al., 2017). The major interest expressed is to either extract or fractionate i) the main part of the proteins of the green biomass for feed purposes (Dale et al., 2009) or ii) the chlorophyll-related proteins from green leaves to obtain a nutritious and highly functional white protein for food use (Tenorio et al., 2017).

The recent trend of plant-based food and protein-rich feed warrants investigations in aspects of producing leaf protein concentrates for novel protein-rich food and feed products. To our knowledge, economic feasibility studies on the use of IC as protein sources for human and animal consumption are still lacking, although studies exist for purpose-grown crops (Bals and Dale, 2011; O'Keeffe et al., 2012). In order to fill this knowledge gap, to present novel resource-efficient ways of using IC biomass for the growing bioeconomy and to better integrate research with potential business scenarios, the aim of this study was to carry out a pre-feasibility assessment evaluating the economic viability of protein extraction from IC biomass and its use as a source of various protein-rich food and feed products. To build a business framework, impacts of the variation of protein content at various harvesting times of IC and use of the IC for food and feed protein production are presented. Based on the field experimental data analyses (total DM yield per hectare and protein content), the economic assessment discusses potential product applications and a comparison with similar products in the market.

2. Materials and methods

This study comprises of two parts. In the first part, data from field experiments and lab analyses on the cultivation of intermediate crops are presented. Data were analysed statistically to identify significant differences. In the second part, a systems study was performed to investigate the economic feasibility of implementing a large-scale production of proteins from IC. For that purpose, data from the first part was adjusted to represent what is achievable in a theoretical up-scaled technical production process. Additional production steps such as harvesting, transport and processing have been considered theoretically for a large-scale production and based on literature data. Results from the second part are meant to show the variation in the data and to identify important factors to influence the outcome in the cultivation part.

2.1. Field experiments

2.1.1. Experimental design

Field experiments were carried out during 2017 and 2018, in Norra Åsum, Kristianstad, southern Sweden. IC were sown on two neighbouring fields (55° 58′ 9.33″N; 14° 9′ 25.97″E) with sandy soil in a randomized block design, with and without fertilization of the IC. Plot size was 2 × 12 m and 2 × 6 m in 2017 and 2018, respectively. The treatments were replicated in four and three blocks in 2017 and 2018, respectively. Precipitation and growing degree days are presented in Table 1. In 2018, due to extremely dry weather, the field experiment was irrigated at 10 occasions with each 20 mm, so that the field experiment received approximately the same amount of water as the first experiment, which represents a more normal year in terms of precipitation.

The Field Research Station of the Rural Economy and Agricultural Society in Skåne, Kristianstad, carried out all field operations. In 2017, the pre-crop, spring barley, was harvested on 30 June and IC were sown on 7 July, and in 2018, the pre-crop, oats, was harvested on 2 July and IC were sown on 9 July. Both pre-crops were harvested as whole-crop cereals.

The study evaluated the following IC: buckwheat (*Fagopyrum exculentum* (Moench)), phacelia (*Phacelia tanacetifolia* Benth.), hemp (*Cannabis sativa* L. (Futura 75)) and oilseed radish (*Raphanus sativus* L. var. ol), were sown either as sole crops or intercropped in combination with one of the legumes, Persian clover (*Trifolium resupinatum* L.) or hairy vetch (*Vicia villosa* Roth.) (Table 2). A row distance of 12.5 cm was used and for intercropped IC, rows were alternated.

2.1.2. Sampling

IC biomass yield was estimated at specified harvest dates (Table 1), by hand harvesting of a randomly selected area (0.25 m^2) in each plot. The biomass was cut approximately 10 cm above the soil surface to simulate a mechanical harvest. Dry matter (DM) content was determined on sub-samples of the hand-harvested biomass by weighing before and after drying at 60 °C for 48 h.

Table 1
Harvesting dates, accumulated precipitation and growing degree days (GDD) for the field experiments.

Year	Seeding	Harvest date	Precipitation [mm]	GDD[a]
2017	7 July 2017	24 August	46	180
		15 September	161	683
		17 October	187	886
		15 November	272	959
2018	9 July 2018	12 September	77	881
		10 October	106	1085
		12 November	148	1200

[a] Base: 5 °C.

Table 2
Variety, seedling rate, fertilization and cultivation year of the different intermediate crops (IC) used in the present study.

Intermediate crop	Variety	Seeding rate[a] [kg ha^{-1}]	Fertilization[b] [kg ha^{-1}]
2017			
Buckwheat	Hajnalka	60	0 and 40
Phacelia	Stala	14	0 and 40
Hemp	Futura 75	25	0 and 40
Oilseed radish	Defender	15	0 and 40
2018			
Buckwheat	Hajnalka	60	0 and 40
Phacelia	Stala	14	0 and 40
Hemp	Futura 75	25	0 and 40
Oilseed radish	Defender	15	0 and 40
Buckwheat + Persian clover	Villana[c]	30 + 6	0
Phacelia + Persian clover	Villana[c]	7 + 6	0
Hemp + hairy vetch	Maral[c]	12.5 + 25	0
Oilseed radish + hairy vetch	Maral[c]	7.5 + 25	0

[a] Amounts in mixtures represent 50% of normal seeding rate.
[b] Digestate from a crop-fed biogas plant was dosed with respect to the NH_4–N content equaling 40 kg ha^{-1} N and resulting in a total fertilization of 63 kg ha^{-1} N.
[c] Variety of the legume, variety of the main IC as above.

2.1.3. Analyses of protein content

Protein content was determined for aerial parts (leaves and stalk) of all the studied crops. Samples were dried, milled and 3–4 mg of each sample was weighed into tin capsules. Nitrogen content was determined using the Dumas method through volatilisation of N on a Flash 2000 NC Analyser (Thermo Scientific, USA). Protein content was then estimated by applying a nitrogen conversion factor of 5.6 (Mariotti et al., 2008). Triplicates were used for measurements.

2.2. Statistical analysis

Total DM yield per hectare and protein content data of IC were analysed using Minitab statistical software (v17.1.0). Means for DM yield and total protein content of IC in response to nitrogen application, harvesting times and impact of legume intercropping, were analysed in an ANOVA by fitting a general linear model. Significant differences ($p < 0.05$) of means were analysed with the Tukey test to evaluate differences in DM yield and protein content in response to nitrogen application, harvesting time and choice of IC in a three way comparison.

2.3. Economic assessment

A cost-benefit analysis was carried out to estimate the economic feasibility of the use of IC for the valorization of protein for food and feed applications. A step-by-step calculation was applied that included all necessary machinery operations in the field, transport, storage and processing in a simulated protein extraction plant. Results from the economic assessment were based on only two years of field experiments and considered a roughly sketched processing line. Technical aspects of the applied processes were specified only roughly using literature data. Consequently, this kind of pre-feasibility study has a relatively large error margin, which typically ranges around ±30% (Bals and Dale, 2011).

Data for the assessment, i.e. literature results of field and laboratory studies were modified to reflect the technical potential of the crops, i.e. biomass yields were assumed to be 90% of hand-harvested yields. The data used in the assessment of the IC production are presented in Table A1 and Table A2 (appendix). Mineral nitrogen fertilizer was assumed to cost 1.0 € kg^{-1} (SBA, 2017). Assumptions for the storage of fibre pulp are given in Table A3 (appendix). A conversion factor of 1 SEK = 0.09433 EUR = 0.10545 USD was applied.

2.3.1. Feedstock production and supply

All field operations assumed the use of a 200 kW tractor. Two false seedbeds were assumed to be prepared, one and two weeks after harvest of the preceding crop in one pass by using a multi seedbed cultivator with an effective field capacity of 2.9 ha h^{-1}. The IC was then assumed to be sown and fertilized with mineral nitrogen, 40 kg ha^{-1}, in one pass by using a combi seed drill (4 m working width) with an effective field capacity of 2.0 ha h^{-1}. For 2018, irrigation equipment was used 4 h ha^{-1} at 10 occasions delivering 20 mm of water each. Harvest of the IC was assumed to be carried out in one pass, by using a tractor-drawn mowing and self-loading forage container wagon with a maximum capacity of 13 tonne (t) biomass or 40 m^3. A maximum harvesting speed of 15 km h^{-1} was assumed for biomass yields per hectare lower than approx. 15 t wet weight, or approx. 2.25 t DM. The harvested biomass was assumed to be transported by the tractor on field roads (1.0 km away at an average speed of 25 km h^{-1}) to a place where the container was switched to an empty one. The harvest capacity was between 1.0 and 1.5 ha h^{-1}, depending on the level of biomass yield per hectare. Three full containers, at a time, approx. 40 t of biomass were transported by a container trailer truck to the protein extraction plant (30 km away at an average speed of 60 km h^{-1}).

At the protein plant, the IC biomass was unloaded to a reception hopper connected to a conveyer belt directly feeding the biomass to a washing system. Transport density was estimated based on a DM density of 85 kg DM m^{-3} (Pettersson et al., 2009) and the corresponding DM content. The costs of machinery operation were estimated according to cost per hour and the amount of time used for the different field operations using standard cost recommendations (Maskinkalkylgruppen, 2019).

2.3.2. Protein production pathways

With well-developed extraction methods, proteins from leaves can be sequentially separated into two protein fractions; the green and white protein fraction with valuable side-products recovered during the production process (Tenorio et al., 2016, 2017). For the economic assessment, two protein production pathways were evaluated in this study: firstly, the production of two protein fractions i.e. green and white protein (Fig. 1, pathway A) and, secondly, an alternative, where the recoverable protein (both white and green fraction) is precipitated in a single step resulting in a production of total recoverable combined protein fraction (CPF) (Fig. 1, pathway B). The protein production pathway to obtain the protein fractions, include the following procedures (Fig. 1); a) cut biomass

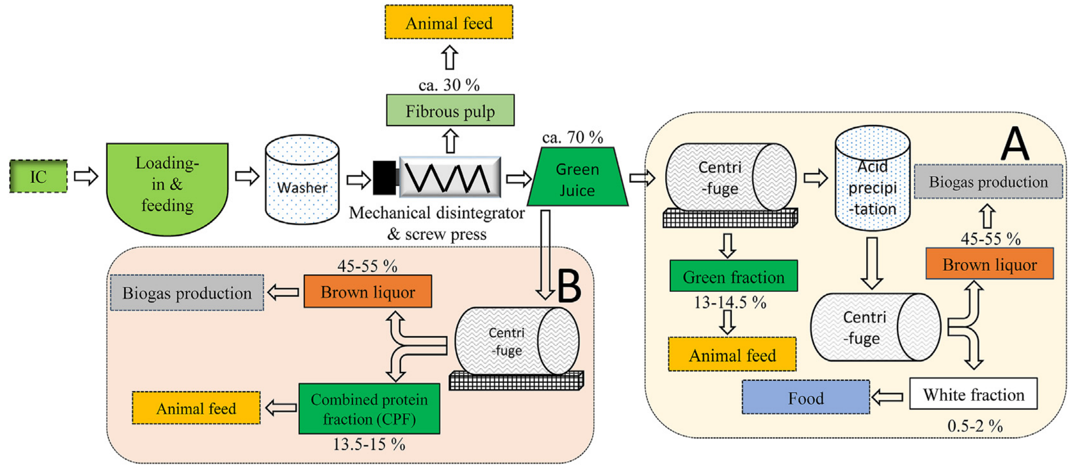

Fig. 1. Mass balance (wet weight %) flow diagram of intermediate crop biomass for production of (pathway A) green and white protein and (pathway B) combined protein fraction (green and white proteins extracted as one protein fraction). In both pathway A and B, brown liquor was obtained as a side-product.

is immediately transferred to the processing plant to avoid degradation and microbial growth, the time limitations for transfer depend on the ambient conditions, b) the cut biomass is unloaded to a feeding conveyor system that transports the biomass into washing and dewatering at constant feeding rate, c) the biomass is first washed to reduce microbial contamination and remove adhering soil, then fed into a screw-dewatering machine designed to disrupt the plant structure and separate the material into fibre pulp and green juice fractions.

For the protein production pathway B, the total protein content extract of the biomass after juicing has been used as the recoverable CPF, which is precipitated from green juice fraction by heat treatment at 80 °C followed by decanter centrifugation. For pathway A, the following additional steps are necessary; d) coarse particles and the green "chloroplast proteins" are separated from the green juice into a green solid fraction by a heat treatment (50–60 °C) followed by a decanter centrifugation, e) the supernatant from the decanter centrifuge is adjusted to pH 4–4.5 to precipitate the white protein fraction followed by disc centrifugation separating it from the clarified brown liquor. A wet weight mass balance of resulting fractions from evaluated protein production pathways is presented in Fig. 1. Besides the protein fractions, both protein production pathways result in the production of a fibre pulp and a brown liquor fraction.

For simulating the white and green protein pathway costs, data on an extraction process with mechanical pressing for fraction separation were used as presented by Bals and Dale (2011) (Appendix Table A4). Compared to the process suggested here, the Bals and Dale process includes both primary and secondary milling and pressing steps, which are energy and capital intensive (Bals and Dale, 2011). Using the economic data for the extended process, our calculations therefore rather overestimate costs. For simulating the recovery of CPF, a 20% cost reduction was assumed to account for a simpler process with a single protein precipitation step. This reduces the need for another centrifugation step, which is usually high in cost of energy and investment (Bals and Dale, 2011). Protein fractions were dried, before sale as products, to an average moisture content of 6% (for details see Table A5).

2.3.3. *Final products*

White protein concentrate is intended as a product for human consumption, primarily as an ingredient in the food industry. The DM protein yield depends strongly on precipitation conditions for protein recovery from green biomass and can range between 0 and 30%. A harsh protein precipitating condition (lower pH) leads to higher protein recovery although it may negatively affect protein functionality. Here, a maximised precipitation was assumed, resulting in a protein content of 29%, that was increased to 85% in the final product assuming additional purification steps (Edwards et al., 1975; Tenorio et al., 2016). Consequently, monetary valuation considered only the nutritional value, no functional value. After drying the precipitated protein from a moisture content of 30%, the product is considered an off-white powder with a moisture content of 4–8%, resulting in a long shelf life. A protein profile suitable for human consumption was assumed.

The green protein fraction (obtained in pathway A) and CPF (pathway B) are formulated similarly as powder for intended use as feed or feed ingredient. The protein content was assumed to be 29% in the final product (Tenorio et al., 2016). After drying the precipitated protein from a moisture content of 30%, the product has a moisture content of 4–8%, resulting in a long shelf life. A protein profile suitable for use as animal feed for both mono-gastric animals and ruminants was assumed. Fibre pulp is ensiled at a moisture content of 30% and intended for use as cattle feed with a protein content of approx. 3.3% wet basis.

Brown liquor is a residue product that is a mixture of soluble components with potential use as biogas substrate. However, due to low DM content (<10%) transport costs are high. Treatment to increase DM content needs to be balanced against product value. Depending on the transport distance, this by-product can become a cost or revenue. Therefore, revenues from this by-product have not been included in the economic assessment.

For assessing the economic feasibility, market-based costs were used to estimate potential sales prices for white protein and the fibre pulp (Table 3). White protein has been evaluated using sales

Table 3
Assumed protein revenues.

Product	Application	Chosen value (market range)[a] [€ kg^{-1}]
White protein	Food	11.2 (8.6–13.8)[a]
Fibre pulp	Feed	0.21 (0.14–0.28)[b]

[a]Price was assumed per kg of DM with a moisture content of 4–8%.
[a] Range as analysed on Alibaba.com (8 June 2019); when a default price of 1 US$ kg^{-1} product was given as the lower price range, this was corrected by assuming the lower price limit being at 50% or the upper price limit of the same product.
[b] Assumed to have the same value as that of untreated ley crop biomass used as ruminant feed.

prices corresponding to highly nutritional food additives sold as a premium product (protein isolate), while fibre pulp has been evaluated as a bulk feed product, i.e. used as cow feed additive, generating low revenues. For the green protein fraction and the recoverable CPF, the product value depends on the protein content, but also on other factors such as digestibility, amino acid composition and fibre content. Setting a price for these fractions would therefore require additional information on the feed value of the products, which was outside the scope of this pre-feasibility study. Therefore, for assessing the production pathway feasibility, the breakeven prices for the green protein fraction (pathway A) and the recoverable CPF (pathway B) were estimated for each crop, nitrogen fertilization level and harvest month. The breakeven price was here defined as the sales price per kilogram protein that would cover the difference between total production costs and revenues from white protein and fibre pulp.

2.3.4. Sensitivity analysis

A sensitivity analysis of the basic production factors including IC biomass yield, DM content at harvest and nitrogen content were carried out. The production factors were varied with +10% and −10% and the effect on the breakeven prices required for the green protein fraction and the recoverable CPF fraction was recorded.

3. Results and discussion

3.1. Biomass yield and protein concentration

Biomass DM and protein yield differed significantly among crops, harvest year and date, and nitrogen fertilization of IC (Table 4). Among the evaluated IC, buckwheat showed significantly the lowest DM and protein concentration per hectare (Table 4). Cultivation of IC in 2018 resulted in both significantly higher DM yield and protein concentration than when IC were grown in 2017 (Table 4), which might be the result of irrigation applied in regular intervals in 2018 due to unusual dry weather conditions as compared to those in 2017. Although irrigation only topped the water contribution from precipitation up to the more normal precipitation levels in 2017, there might have been an additional impact on biomass production originating from the regularity of the irrigation.

High DM yield per hectare was obtained when IC were harvested in October and November, with significantly higher harvest in October than in August and September, while no impact of harvest date was noted for protein concentration (Table 4). The lower DM yield obtained early during the season, is related to the very short period between sowing and harvesting that did not allow IC to produce much biomass. Previous studies have shown a high DM yield for hemp grown as main crop in the mid-autumn and thereafter a decrease in late fall or beginning of the winter due to frost at night, which negatively affect biomass yield (Kreuger et al., 2011; Prade et al., 2011).

Nitrogen application of 40 kg ha^{-1} and intercropping with legumes contributed to a significantly higher DM yield as compared to non-fertilized IC (Table 4). The intercropping with legumes also resulted in higher protein concentration in the biomass (Table 4). In previous studies, the positive effects of intercropping have been attributed to the fixation of nitrogen by the legumes, which leaves more soil nitrogen to the main crop (Hauggaard-Nielsen et al., 2016; Pelzer et al., 2012).

The present study aims at understanding the feasibility of protein fractionation from IC. The large variation in DM and protein concentration among type of IC, cultivation year, time of harvest and nitrogen fertilizer regime applied, is obviously both a limit and an opportunity considering protein recovery from IC. Besides the variation in DM and protein concentration, the water content of the aerial biomass at harvest also influences the protein recovery, i.e. higher water content in the plant and softer tissues contribute positively to efficient juicing and protein recovery from the fibrous structures of the plant material. As the plants grow, the water content in the plant normally decreases and plant tissues become increasingly lignified thereby complicating the juice extraction. Thus, selection of IC, nitrogen fertilizer regime, harvesting time and year of cultivation are clearly the factors that affect the feasibility of the use of IC for protein fractionation.

Based on the greening measure obligation implemented by the European Commission in 2015, about one third of the total agricultural land (amounting to 2.65 million hectares) has been cultivated with catch crops in the EU (EC, 2017). In Sweden, catch crops were cultivated on a total of about 70,000 ha in 2018 (Asplund and Svensson, 2018), although a potential area for IC of 194,000 ha have

Table 4
Mean values ± standard deviation for total dry matter (DM) biomass yield and mean protein concentration of IC during two years (2017 and 2018), harvested in Aug–Nov, for unfertilized IC (0N); unfertilized IC together with legumes (0N + L) and IC fertilized with 40 kg ha^{-1} N in the form of biogas digestate (40N).

Crop/Treatment	Biomass DM yield [kg ha^{-1}]	Protein concentration [g kg^{-1}]
Crop		
Phacelia	3028 ± 1349 a	110 ± 38.6 a
Hemp	4159 ± 2200 a	125 ± 42.0 a
Oilseed radish	5099 ± 3197 a	128 ± 35.6 a
Year		
2017[a]	3350 ± 1955 b	83.2 ± 23.8 b
2018	4668 ± 2272 a	126 ± 44.8 a
Harvest time		
Aug[b]	1413 ± 834 c	111 ± 17.9 a
Sep	3671 ± 1639 b	110 ± 29.5 a
Oct	4564 ± 2230 a	101 ± 45.1 a
Nov	4186 ± 2714 ab	116 ± 52.2 a
Fertilization[c]		
0N	2239 ± 1285 b (3196 ± 1235 b)	103 ± 36.8 b (119 ± 42.8 b)
0N + L	(4987 ± 1761 a)	(154 ± 44.9 a)
40N	4901 ± 2382 a (5820 ± 2808 a)	95.5 ± 29.0 b (104 ± 33.4 b)

[a] Values for harvests Sep–Nov.
[b] Values in Aug only for 2017.
[c] Values without brackets include both years, values in brackets presents data of year 2018. Means with same letters are not significantly different ($p < 0.05$) by Tukey post-hoc test.

been reported (Prade et al., 2017). By the assumption that IC should be used for production of white protein and that IC have an average protein potential of 500 kg ha^{-1} with a 10–21% fraction of recoverable protein, the IC food grade white protein potential yield is 9700–20,400 t per year in Sweden alone. Scaled to the EU, based on the current IC cultivation area, this potential grows to 133,000–278,000 t food grade white protein produced from IC per year. This corresponds to 1.5–3.1% of the EU28 annual protein consumption, with potential to increase if IC cultivation increases.

The EU is producing around 20% of the world's total pork, which creates a large demand for high quality protein suitable for mono-gastric animal production. This demand is currently fulfilled by imported protein-rich feed materials (Jørgensen and Lærke, 2016). Considering the EU's low self-sufficiency in protein-rich feed and consequent detrimental environmental impact of intensive animal husbandry for meat production, the EU has issued directives and resolutions to find sustainable protein-rich food and feed from different agricultural crops (Häusling, 2011; Weightman et al., 2011).

In this pre-feasibility study, in addition to the production of white proteins, the IC are also proposed as a suitable option to produce a green protein fraction and a combined protein fraction (CPF), which can be used as protein-rich feed or feed ingredient for mono-gastric animals and ruminants. This production of green proteins from IC may also contribute to reduce the EU's soya bean meal imports, which currently constitute ca. 69% of the total imported protein-rich feed materials (de Visser et al., 2014).

3.2. Possible uses of proteins fractionated from green biomass

The present study assumes the production of either both a green and a white protein fraction (Pathway A) or only a green protein fraction (Pathway B), produced from IC as green biomass, and feasibility calculations are made for both these pathways. The interest is currently increasing for the opportunities to use green biomass proteins as a sustainable, nutritional (with essential amino acids) and efficient source to meet the demands of functional proteins for food and feed production (Martin et al., 2019; Tenorio et al., 2016). Here, the white protein holds the highest value and is intended for human consumption. The white protein fraction is reported to contain essential amino acids beneficial for human health and also to provide structural features to the food, e.g. gelation, emulsification, foaming and water-binding properties (Martin et al., 2014).

The white protein fraction consists of approximately 50% of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), one of the most abundant proteins in the world (Bar-On and Milo, 2019; Barbeau and Kinsella, 1988). RuBisCO is an enzyme present in all green plants and has a key role in photosynthesis where it catalyses the primary binding of CO_2 (Martin et al., 2019). RuBisCO obtained from spinach leaves has demonstrated a low thermal gelation temperature, relatively low gelation concentration (compared to whey protein) and good foaming ability, suggesting it as an attractive alternative to dairy-based foams in different food applications (Martin et al., 2014; Williams and Phillips, 2014).

Differently from the white protein fraction, both the CPF (pathway B) and the green protein fraction (obtained in pathway A) contain green insoluble components and other impurities, negatively impacting taste and functionality when used for human consumption, and both these fractions are therefore considered suitable for mono-gastric and ruminant animal feed production. Leaf protein concentrate (LPC), from e.g. alfalfa, is already used as fodder in Europe and North America (Colas et al., 2013). Alfalfa LPC contains relatively high amounts (57.4 g.100 g^{-1}) of essential amino acids (e.g. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) compared to conventional corn (40.1 g.100 g^{-1}) and soya bean (45.3 g.100 g^{-1}) protein-based feed supplements (Dale et al., 2009).

The LPCs are reported as a good source of essential amino acids suitable for ruminant and non-ruminant animals (Akeson and Stahmann, 1965; Dale et al., 2009). Fractionation of white and red clover, alfalfa (Lucerne), and perennial ryegrass into green and white fractions indicated both green (60 °C) and white (80 °C) fractions have superior levels of sulphur containing amino acids compared to soya, making them attractive as mono-gastric feed (Damborg et al., 2020). Fibre fraction had improved levels of the limiting amino acids for ruminants compared to the starting materials making them attractive feed sources. Ruminants such as cattle and sheep have a digestive system where pre-gastric fermentation of feed can dramatically alter the amino acid profile for further digestion in the small intestine (Lu et al., 1983, 1988). Therefore, animals with high protein requirements, e.g. lactating cows, would benefit from being fed with CPF and green proteins due to the limited ruminal degradation of these proteins, which provide all the essential amino acids for digestion in the small intestine. According to a previous study, LPC from alfalfa tends to have a 20% slower ruminal degradation and greater digestibility in the small intestine compared to conventional soya bean protein (Lu et al., 1988). Similarly, inclusion of LPC has been reported to be a suitable alternative to fish meal proteins in diets of piglets to promote growth and weight gain (Duckworth et al., 1961).

According to Akeson and Stahmann (1965), the total recovery of protein varies with different plant species used, although, differences in digestibility and amino acid composition are minor. Based on the above, our assumption supported that the protein fractions resulting from IC biomass have suitable amino acid composition with a high digestibility. The attractiveness of LPC for use as animal feed depends mainly on factors such as consistency of quality (protein concentration and preserved nutritional profile), anti-nutritional compounds, physical form, price and feeding value (Dale et al., 2009). Thus, this pre-feasibility study focuses on the evaluation of both Pathway A with the production of both white and green protein concentrate and on Pathway B with a combined green protein concentrate as products in order to evaluate best possible uses of IC for protein recovery.

3.3. Nutritional and anti-nutritional components in protein fractions from IC

An understanding of how contents of both nutritional and anti-nutritional compounds are accumulated in the protein fractions of various IC is essential before the use of these in food and feed products. Also, in relation to a full calculation of economic values of the IC protein fraction, a full understanding of contents of various compounds in the protein fractions would be useful. The present study does not cover the full chemical analyses of the different fractions. Below, we are covering the knowledge from literature related to what chemical compounds can be expected to be found in the protein fractions from the IC evaluated in the present study.

Buckwheat seeds are normally consumed as food for their high protein content and phenolic compounds in the grains, although recent studies have shown that their aerial green parts also contain high amounts of phenolic compounds beneficial for human health (Kreft et al., 2013). For example, buckwheat leaves contain high amounts of rutin with high antioxidant activity (Holasova et al., 2002). Rutin is a phenolic compound in food that is reported to strengthen the immune system and decrease the risk of various cancers (Zhang et al., 2012). However, besides containing a variety of polyphenols with high antioxidant potential, fagopyrins could be present in green aerial parts of buckwheat. Fagopyrins are a group of compounds known to cause light sensitivity in humans when large amounts of green parts of buckwheat are consumed (Tavčar Benković et al., 2014). Since the solubility of fagopyrins is similar to that of chlorophyll and both compounds have a similar coagulation temperature of around 60 °C, there is a strong tendency that these compounds will end up in the green protein fraction aimed as animal feed rather than in the white protein fraction aimed for human consumption (Kreft et al., 2013; Tavčar Benković et al., 2014).

Radish is a cruciferous vegetable that contains high amounts of bioactive compounds in its napiform taproots and is consumed as food for its health benefits (Blažević and Mastelić, 2009; Goyeneche et al., 2015). However, there is limited information available on the chemical composition of oilseed radish leaves. A previous study reported that red radish leaves contain high amounts of minerals, polyphenols and ascorbic acid content as compared to its roots (Goyeneche et al., 2015). This suggests that during extraction of leaf proteins, minerals and bioactive compounds could end up in various protein fractions, which may increase the value of such products. Comparison of bioactive compounds present in buckwheat and oilseed radish with wheat grass (a commercial food product) suggests similarities in their composition (Kulkarni et al., 2006), indicating that protein extracted from aerial green biomass of oilseed radish may have potential in food and feed products.

Hemp leaves consist of ca. 24% of crude proteins, 19% of crude fibres, 11% of minerals and bioactive compounds (Audu et al., 2014). The amino acid profile of leaf protein extracts of hemp contains nine of the ten essential amino acids (except methionine) and is therefore of high nutritional value for food and feed purposes (Audu et al., 2014). Hemp leaves contain two groups of phytochemicals: cannabinoids such as cannabidiol (CBD), cannabinol (CBN) and tetrahydrocannabinol (THC) and non-cannabinoids such as flavones, alkaloids, terpenes and steroids (Audu et al., 2014; Khan et al., 2014; Pandohee et al., 2015). High amounts of cannabinoids such as THC are known to cause psychoactive effects, although industrial hemp varieties generally contain low amounts of this compound and the amounts being present are mainly found in the flowers. Therefore, the potential of these chemicals to cause negative health impacts are relatively low when hemp leaf protein extracts are considered for food and feed purposes.

There is lack of information in literature about the bioactive compounds present in the aerial green parts of phacelia. However, previous studies have reported the presence of polyphenols and minerals in sprouted phacelia seeds (Kruk et al., 2019; Pająk et al., 2019). Phacelia sprouts tend to have higher amounts of minerals and phenolic compounds with much higher antioxidant activity compared to the seed (Pająk et al., 2019).

In preparation of leaf proteins from green biomass, chlorophyll degradation products can result in the production of compounds undesirable for human and animal consumption. The first initial step of chlorophyll degradation occurs when cell structures are broken, e.g. by chopping or heating of the green biomass, leading to the release of enzymes and acids, which are exposed to chlorophyll protein complexes (Heaton and Marangoni, 1996). During leaf protein extraction, if high temperature-mediated coagulation is used, the chlorophyll is first converted into chlorophyllide. Production of chlorophyllide is directly related to activity of chlorophyllase enzymes in leaves. Crops with higher chlorophyllase activity tend to produce higher amounts of pheophorbides that are believed to cause photosensitization effects in albino rats (Lohrey et al., 1974). During the extraction of proteins from the green biomass, the acid treatment used to lower the pH to around 4 for precipitation of proteins can potentially convert the chlorophyllide into pheophorbide (Heaton and Marangoni, 1996; Holden, 1974).

To conclude, both nutritional and anti-nutritional compounds can be gathered in protein concentrates from IC, which might have both positive and negative impact on their usability as food and feed products and which might affect their economic value. The chemical compounds gathered most likely also differ for type of IC evaluated, which indicate the need of a full chemical characterization of the protein fractions from each IC before these can be marketed as products. Content of compounds in the IC protein fraction might influence its price both positively and negatively and might even warrant an additional purification step to avoid hazardous levels of anti-nutritional compounds. However, based on available knowledge the IC evaluated here seems to have potential opportunities for the production of nutritional protein fractions.

3.4. Economic evaluation of IC for protein-rich food and feed products

Economic assessment evaluating the extraction of white and green protein following pathway A, showed large differences in both costs and revenues between the different IC, harvest months, year of production, for both unfertilized and nitrogen fertilized crops (Fig. 2 a, b). The costs for the recoverable CPF production (pathway B) varied similarly, although with total costs being on average 1.7% lower (range −13.5% to +5.7%). As this is well within the error margin of the assessment, results for pathway B are not presented here. Revenues for the fibre pulp are identical between pathways A and B.

Generally, cost per t DM were high if IC were harvested in August, due to the low biomass yields per hectare that early after establishing the crop. For harvest during September, October and November, a decreasing cost was observed for Phacelia regardless of nitrogen fertilization or intercropping with a legume, but not for buckwheat, hemp and oilseed radish. For unfertilized buckwheat, harvest in September resulted in the lowest cost. However, for fertilized buckwheat, harvest in October and November resulted in lower production costs compared to September. The proportion of cost of cultivation in total costs also varied considerably, between 11 and 61% and between 10 and 60% for production of recoverable CPF and white plus green protein, respectively. Here, low biomass yields increased cost per t DM considerably, which also increased the proportion of cultivation costs. Processing costs for production of separate fractions of white and green protein were on average 15% (11−19%) higher compared to extraction of recoverable CPF. Irrigation increased cultivation cost on average 62% (45−91%) compared to if irrigation was excluded, total costs increased on average only 13% (4−25%).

Revenues per t of DM, excluding the green protein fraction, varied considerably between crops, nitrogen fertilization levels, harvest months and years for extraction of white protein and fibre pulp from pathway A (Fig. 2 a, b). Expectedly, the plant nitrogen concentration was the major factor determining the size of revenues, which is in line with earlier studies (Swanson, 1990). Irrigation together with the warmer and dryer weather in 2018 likely decreased nutrient leaching and reduced nitrogen dilution in the maturing crops as well as increased biomass yields. Nitrogen dilution in the plant biomass occurs naturally during plant growth, e.g. when the plant biomass formation continues but nitrogen uptake is declining, e.g. due to maturing processes (Plénet and Lemaire, 1999). Irrigation could therefore have contributed to prolonged nitrogen uptake. As a likely consequence, plant nitrogen content was on average 34% higher in 2018 compared to 2017. Revenues were consequently higher in 2018, with otherwise similar patterns for harvest months and fertilisation levels. Nitrogen fertilisation did not affect revenues per t positively, but produced more biomass per hectare, which lowered costs per t DM. In both years, phacelia, hemp and oilseed radish yielded higher

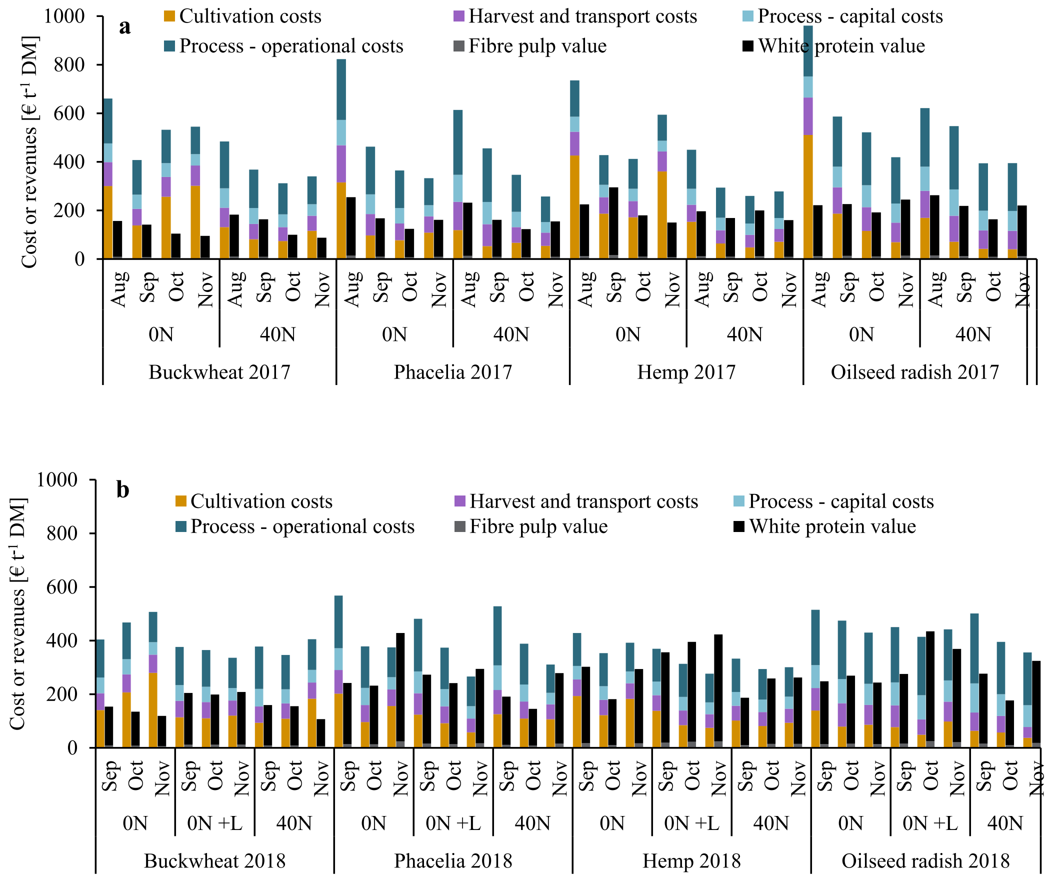

Fig. 2. Costs (left columns) and revenues (right columns) for production of white and green protein in € t^{-1} dry matter (DM) biomass from intermediate crops (IC) in 2017 (a) and 2018 (b) for human consumption and animal feed, respectively (pathway A). Months refer to the time of harvest of the IC. Revenues exclude those of the CPF and green protein fraction as explained in section 2.4.3. Data is shown for unfertilized IC (0N); unfertilized IC grown together with legumes (0N + L) and IC fertilized with 40 kg ha^{-1} N in the form of biogas digestate (40N).

revenues compared to buckwheat.

The breakeven prices per kilogram protein in the green protein fraction (pathway A; Fig. 3) and the recoverable CPF (pathway B; Fig. 4) showed large variations between crop, fertilization level and harvest month. A threshold price level was set at 2 € kg^{-1} protein; green protein fraction or recoverable CPF with breakeven prices below this level likely can be marketed as a bulk feed product. A second threshold price level was set at 10 € kg^{-1} protein;

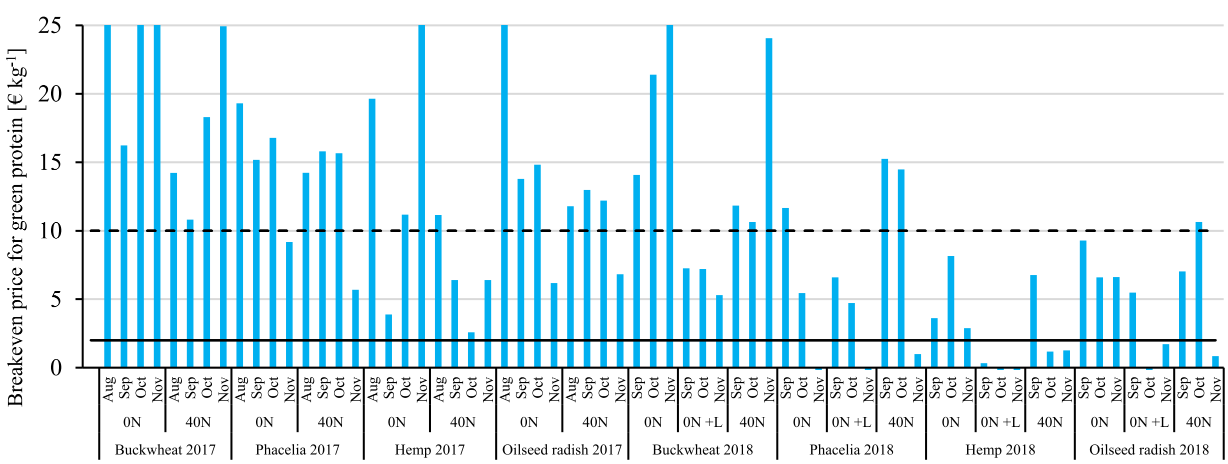

Fig. 3. Breakeven prices for the green protein fraction defined as the sales price per kilogram protein that would cover the difference between production costs and revenues from white protein and fibre pulp for pathway A as shown in Fig. 2. Treatments with values below the solid line could be marketed as bulk products with low sales prices, treatments with values below the dashed line need to be marketed as premium products with considerable higher prices. Treatments with values above the dashed line are considered too expensive in production.

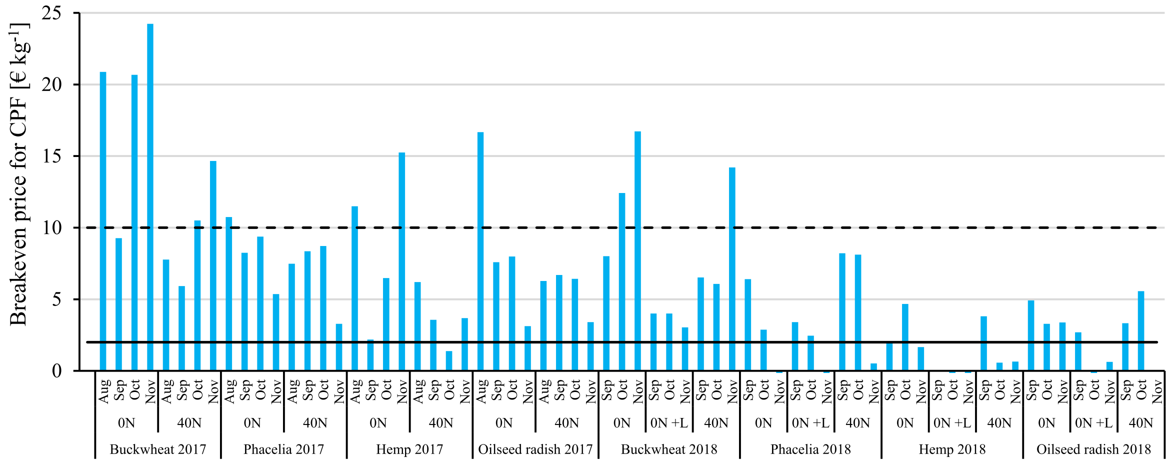

Fig. 4. Breakeven prices for the recoverable combined protein fraction (CPF) defined as the sales price per kilogram protein that would cover the difference between production costs and revenues from the fibre pulp for pathway B. Treatments with values below the solid line could be marketed as bulk products with low sales prices, treatments with values below the dashed line need to be marketed as premium products with considerable higher prices. Treatments with values above the dashed line are considered too expensive in production.

breakeven prices up to this level likely require marketing as a premium feed product, as is common in the horse feed market. Breakeven prices above 10 € kg^{-1} protein were considered too high for even marketing as premium products, given that packaging and distribution of the products was not included in the assessment.

All IC in both years were able to produce a CPF from pathway B (Fig. 4) that would be economically feasible to produce as a premium product. In 2018, the year with higher protein concentration in the plant biomass, protein concentrate production via pathways A and B was feasible even without revenues from the green protein fraction for phacelia unfertilized and intercropped with Persian clover in November; for hemp intercropped with hairy vetch and harvested during October to November; and for oilseed radish intercropped with hairy vetch (October).

Breakeven prices were 54 and 56% lower in 2018 compared to 2017, for pathways A and B, respectively. Breakeven prices for feasible IC options (below 10 € kg^{-1} protein) in pathway A were on average 2.0 € kg^{-1} protein higher compared with pathway B, with a variation between −1.1 and + 4.4 € kg^{-1} protein (Fig. 4). This was found despite the high revenue per kilogram of white protein, which can be explained by the relatively low extraction efficiency of white protein. Intercropping with a legume reduced breakeven prices considerably for all IC. In 2017, only hemp, fertilized and harvested in October, produced green protein feasible as a bulk product. Unfertilized IC harvested in August were not economically feasible for protein concentrate production, due to high feedstock costs per t DM.

For crops with a breakeven price below 10 € kg^{-1} protein, processing costs ranged approx. 1850–6890 and 1690–8620 € t^{-1} protein for pathway A and B, respectively. These costs can be compared to e.g. processing cost of 1367 and 2448 € t^{-1} protein extracted from microalgae, for enzymatic and alkaline extraction processes, respectively (Sari et al., 2016). Corresponding total costs for this selection ranged approx. 3500–10,970 and 3360–14,610 € t^{-1} protein, i.e. all protein fractions combined.

The sensitivity analysis showed that a −10% and +10% variation of the tested production factors (nitrogen content, IC biomass yield and DM matter content) affected the breakeven prices in production pathways A and B (Fig. 5). A variation of the nitrogen content effected the breakeven price the most, resulting in a variation of the breakeven price that was more than twice as large compared to the

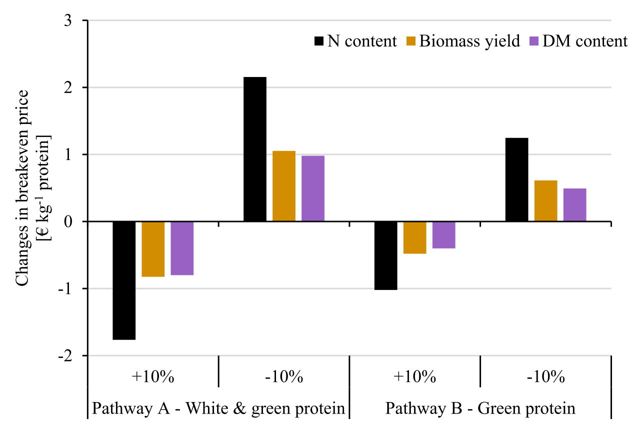

Fig. 5. Changes in breakeven price for the green protein fraction in pathway A and the recoverable combined protein fraction (CPF) in pathway B as caused by a +10 and −10% change of the production factors nitrogen content, biomass yield and dry matter (DM) content.

effects of the biomass yield and the DM content variability. In general, pathway A, production of white and green protein, was affected more strongly, prices varied approx. 70% more compared to the prices in pathway B, production of recoverable CPF. A 10% increase in nitrogen content strongly increased the number of IC treatments in pathway B that had breakeven prices below 10 € kg^{-1}, while variation of the other factors changed the number of IC treatments feasible as premium or bulk products in only 3 cases.

Transportation costs were negatively affected by lower DM content in the green biomass. Processing costs were mainly increased by lower protein content and lower DM content in the processing part. Here, hemp profited from high DM content compared with oilseed radish. This difference was however diminished by the approx. 20% higher biomass yields for oilseed radish that continued to increase into November, while hemp growth was interrupted by frost, which requires an earlier harvest in September or October. Optimization of IC biomass production towards an increased nitrogen (protein) content is desirable from a production point of view but needs also to take into account the current use of unfertilized IC as catch crops, reducing nitrogen leakage from the soil and the corresponding conflict of interest.

4. Conclusions

Green biomass from intermediate crops (IC) has a large potential as a raw material from which leaf proteins can be fractionated and valorized into protein-rich food and feed products. Factors such as harvesting time, nitrogen application and intercropping with legumes significantly impact the DM yield and protein content for the different ICs. High protein content in the green biomass of the IC and a high yield are the major determinant factors for efficient utilization of the biomass and to obtain sufficient economic revenues, when high value protein products for food and feed are produced. The results from this prefeasibility assessment lays a basis for exploitation of additional underutilized green biomasses/ residues (derived e.g. from broccoli, kale, sugarbeet etc.), thereby contributing to fulfill the increased societal demand for sustainably produced plant-based protein for utilization as food and feed. Furthermore, a biorefinery concept of the protein valorization also contributes to opportunities for the extraction of other valuable bioactive compounds.

Business development based on the present findings requires further investigations on how to achieve feedstock production with an increased protein content in a continuously changing climate. Intercropping with legumes is a promising track to increase the feedstock nitrogen content, while the role of fertilization is less clear. Through additional research efforts, requested by policymakers within environmental departments, clarity on nitrogen leakage as an effect from fertilization of IC and intercropping with legumes should be investigated. Furthermore, eventual integration of such inputs into the current agricultural support system for the cultivation of IC needs to be further elaborated on. In general, a better integration of greening measures supported under the common agricultural policy (CAP) with the ideas of a resource-efficient use of the potential feedstock for the growing bioeconomy originating from these measures would be desirable.

CRediT authorship contribution statement

Faraz Muneer: Writing – review & editing, wrote the manuscript with contribution from the co-authors. **Helena Persson Hovmalm:** Formal analysis, performed field experiments, sampling and analyses. **Sven-Erik Svensson:** Formal analysis, performed field experiments, sampling and analyses. **William R. Newson:** Writing – review & editing, contributed with critical commenting and writing the manuscript. **Eva Johansson:** Writing – review & editing, conceived the idea of the prefeasibility study and contributed with critical commenting and writing of this manuscript. **Thomas Prade:** Formal analysis, Writing – review & editing, performed field experiments, sampling and analyses. performed the economic assessment for this prefeasibility study. conceived the idea of the prefeasibility study and contributed with critical commenting and writing of this manuscript. wrote the manuscript with contribution from the co-authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by South Baltic Area Interreg projects, i) *Bioeconomy in the South Baltic area: Biomass-based Innovation and Green Growth* (BioBIGG), ii) *South Baltic Food Innovation: Developing food innovation capacity in Small and Medium Enterprises (SME's) in the South Baltic Region* and also funded by SLU Partnership Alnarp and project *Trees and Crops for the Future (TC4F).*

Appendices

Table A1
Data used for the economic assessment of the intermediate crop production and transport logistics.

Year	Crop	Fertilization	Harvest	Seeding material		DM content	Transport density	Storage density
		[kg ha^{-1} N]		[kg ha^{-1}]	[€ kg^{-1}]	[%]	[kg m^{-3}]	[kg m^{-3}]
2017	Buckwheat	40	Aug	60	23	13,8	620	989
			Sep	60	23	17,9	476	854
			Oct	60	23	24,0	356	727
			Nov	60	23	28,1	309	677
	Phacelia	40	Aug	12	12	9,3	927	1000
			Sep	12	12	11,7	740	1000
			Oct	12	12	18,7	458	835
			Nov	12	12	32,1	268	634
	Hemp	40	Aug	30	60	17,5	494	873
			Sep	30	60	25,0	341	711
			Oct	30	60	28,4	299	667
			Nov	30	60	30,1	283	650
	Oilseed radish	40	Aug	15	40	10,5	814	1000
			Sep	15	40	9,5	895	1000
			Oct	15	40	13,6	649	968
			Nov	15	40	13,4	638	985
2018	Buckwheat	40	Sep	60	23	17,9	476	854
			Oct	60	23	24,0	355	726
			Nov	60	23	28,1	303	670
		0	Sep	60	23	20,6	413	787
			Oct	60	23	21,6	393	766
			Nov	60	23	28,8	295	663
	Buckwheat + Persian clover	0	Sep	36	26	20,6	413	787

Table A1 (*continued*)

Year	Crop	Fertilization	Harvest	Seeding material		DM content	Transport density	Storage density
		[kg ha^{-1} N]		[kg ha^{-1}]	[€ kg^{-1}]	[%]	[kg m^{-3}]	[kg m^{-3}]
			Oct	36	26	21,6	393	766
			Nov	36	26	28,8	295	663
	Phacelia	40	Sep	12	12	11,7	729	1000
			Oct	12	12	18,7	454	830
			Nov	12	12	32,1	265	630
		0	Sep	12	12	13,5	630	1000
			Oct	12	12	18,3	463	841
			Nov	12	12	29,6	288	654
	Phacelia + Persian clover	0	Sep	12	26	13,5	630	1000
			Oct	12	26	18,3	463	841
			Nov	12	26	29,6	288	654
	Hemp	40	Sep	30	60	25,0	340	711
			Oct	30	60	28,4	299	667
			Nov	30	60	30,1	282	649
		0	Sep	30	60	25,4	334	704
			Oct	30	60	25,3	336	706
			Nov	30	60	31,3	271	637
	Hemp + hairy vetch	0	Sep	40	41	25,4	334	704
			Oct	40	41	25,3	336	706
			Nov	40	41	31,3	271	637
	Oilseed radish	40	Sep	15	40	9,5	890	1000
			Oct	15	40	13,6	626	1000
			Nov	15	40	13,4	633	1000
		0	Sep	15	40	12,7	671	1000
			Oct	15	40	11,9	717	1000
			Nov	15	40	14,0	609	994
	Oilseed radish + hairy vetch	0	Sep	33	32	12,7	671	1000
			Oct	33	32	11,9	717	1000
			Nov	33	32	14,0	609	994

[a] Storage density of the fibre pulp fraction.

Table A2
Machinery costs according to branch recommendations (Maskinkalkylgruppen, 2019).

Machinery	Specifications	Capacity	Costs[a] [€ h^{-1}]
Tractor	200 kW		81
Multicultivator	4 m (disc, tine, roller)	2.9 ha h^{-1}	138
Combi seed drill	4 m, 3300 L	2.0 ha h^{-1}	148
Irrigation	hose: 75 mm, 300 m	0.25 ha h^{-1}	8
Self-loading forage wagon	56 m^3	118 t h^{-1}	133
Front loader[b]	12 t, 110 kW	Compaction: 0.4 min t^{-1} Feed-in: 85 t h^{-1}	75

[a] Including costs for driver and fuel.
[b] Assuming an effective bucket volume of 4.5 m^3, 300 m transport distance, 20 km h^{-1} transport speed and a filling und unloading time each of 10 s per bucket load.

Fibre pulp storage as silage.

Costs for storage in bunker silos were estimated using an investment calculation on data given in Table A3.

Table A3
Economic calculations on bunker silo storage.

Variable	Unit	Value
Effective storage volume	[m^3]	17,505
Investment costs[a]	[€ m^{-3}]	17.0
Economic life span	[a]	20
Interest	[%]	6
Cost for plastic cover[a]	[€ m^{-2}.a^{-1}]	3.0
Bunker cost	[€ m^{-3}.a^{-1}]	2.2

[a] Source: Strid et al. (2012).

Storage density was estimated to be 646 kg m^{-3} according to Hjelm and Spörndly (2012):

$$Required\ storage\ \ density[\frac{kg}{m^3}] = DMcontent[\%] \cdot 3.5[\frac{kg}{\% \cdot m^3}] + 90[\frac{kg}{m^3}]$$

Table A4
Ranges of costs for protein extraction and drying of final products given as cost per t of initial feedstock. Actual values were dependent on DM and nitrogen content of the processed biomass as well as variations in energy consumption and prices.

Fraction	Operational cost [€ t^{-1}]	Investment cost[a][€ t^{-1}]	Technology used	References
Extraction				
White protein	18.7–23.5	8.0–9.6	mech. separation	Bals and Dale (2011)
Total green protein	15.0–18.8	6.4–7.7	mech. separation	Bals and Dale (2011)
Drying				
White protein	4.1–26.3	1.9–10.5	spray drying	own estimation[b]
Green protein	6.6–42.1	3.0–16.8	drum drying	own estimation[b]
Total green protein	11.5–73.6	5.2–29.5	drum drying	own estimation[b]

[a] for the drying processes estimated as 40 and 45% of high and low operational costs, respectively (Bals and Dale, 2011).
[b] Estimated based on the energy consumption of 3–7 MJ kg^{-1} evaporated water (Baker and McKenzie, 2005)and energy prices of 1.0–1.8 €-ct MJ^{-1} (SCB, 2019).

Table A5
Drying operations for different fractions.

Fraction	Drying method	Proportion of the initial biomass DM treated [%]	Moisture content[a][%]
Green protein	Drum drying	9.1	31
Total extractable protein	Drum drying	15.9	31
White protein	Spray drying	5.7	31

[a] Of the biomass entering and treated in the dryer, based on (Tenorio et al., 2016).

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