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Synergetic Benefits for a Pig Farm and Local Bioeconomy Development from Extended Green Biorefinery Value Chains

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Abstract: As the global population rises, agriculture and industry are under increasing pressure to become more sustainable in meeting this growing demand, while minimizing impacts on global emissions, land use change, and biodiversity. The development of efficient and symbiotic local bioeconomies can help to respond to this challenge by using land, resources, and side streams in efficient ways tailored to the needs of different regions. Green biorefineries offer a unique opportunity for regions with abundant grasslands to use this primary resource more sustainably, providing feed for cows, while also generating feed for monogastric animals, along with the co-production of biomaterials and energy. The current study investigates the impact of a green biorefinery co-product, leaf protein concentrate (LPC), for input to a pig farm, assessing its impact on pig diets, and the extended impact on the bioenergy performance of the pig farm. The study found that LPC replaced soya bean meal at a 50% displacement rate, with pigs showing positive performance in intake and weight gain. Based on laboratory analysis, the resulting pig slurry demonstrated a higher biogas content and 26% higher biomethane potential compared with the control slurry. The findings demonstrate some of the local synergies between agricultural sectors that can be achieved through extended green biorefinery development, and the benefits for local bioeconomy actors.

Keywords: bioeconomy; biorefinery; pigs; soya bean; grass; protein; biogas; biomethane

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1. Introduction

The world currently faces a climate and biodiversity emergency brought about, in part, by an unsustainable food system, which has an immense environmental footprint [1,2]. Globally, livestock farming is the single greatest source of environmental impacts in agriculture, accounting for 77% of all land used for food production, with 14.5% of global greenhouse gases (GHGs) linked to livestock production (rising to 25% if land use change is included) [3]. In Europe, agriculture is a significant contributor to GHGs with about 10% of all Europe's GHGs generated by agriculture [4]. This varies by country, with countries at the lower end, such as Netherlands, U.K., and Greece, below 10%, and other countries such as Lithuania and Latvia recording over 20% of their emissions from agriculture [5]. Ireland has the highest proportion of national agricultural emissions of all EU member states, with over 30% of total national emissions coming from agriculture [5,6], the vast majority of which arises from livestock sectors [6].

The European Green Deal sets out Europe's pathway towards a climate-neutral future, which involves reducing net emissions by at least 55% by 2030 and ultimately achieving climate neutrality by 2050 in line with its commitments under the Paris Climate Agreement [7]. These ambitious European targets place pressure on the agriculture sector to reduce its emissions at the member-state level, with several member states already taking sector-specific action in response.

The development of a circular bioeconomy underpinned by sustainable renewable biological resources from primary production and its associated technological, product, and systemic solutions has been proposed as offering a solution to support a reduction in emissions within the broader agricultural and livestock sectors, while at the same time offering new business and innovation opportunities in traditional primary sectors [8]. Solutions vary from synthetic and seaweed-based additives, which can potentially inhibit rumen methane emissions [9,10], to the displacement of fossil-based materials and fuels with new bio-based materials and biofuels produced from agricultural by-products [11–13], to nature-based solutions [14,15]. Many synergies for bioeconomy development exist within primary sectors, and some of these are explored in Figure 1. Using this approach, many local agricultural and societal needs may be met using available local primary resources, thereby increasing the self-sufficiency of the region, while reducing emissions by using local and shorter supply chains. This approach may be seen as an expansion of the concept of bio-districts or eco-regions which, as described by Dias et al. [16], aims to stimulate a collective approach to sustainable resource management by adopting organic farming practices at a territorial level to ensure benefits are distributed across the region, stimulating rural stakeholder networks and strengthening existing links between rural stakeholders. This can contribute to sustainable local development that prioritizes resource conservation and ecological integrity as inherent characteristics of economic logic, and can contribute to greater quality of life for direct stakeholders and local communities [16]. The potential of adapting this bio-district approach to support local bioeconomy development is exemplified in Figure 1 below, which shows potential synergies between (1.) grassland, (2.) monogastrics, and (3.) marine sectors, which can all provide goods and services for the overall bio-district.

One opportunity for potential local symbiosis between primary sectors exists in the development of green biorefinery models. Many protein sources are not being used optimally and may be more efficiently used by deploying biorefinery approaches [17]. In green biorefinery systems, green biomasses, such as grasses and legumes, can be processed into multiple co-products, including leaf protein concentrate (LPC), which is suitable for both ruminant and monogastric animals and for use in aquaculture [18–20]. This approach can help to improve the overall resource efficiency of the green biomass by providing an ensiled fiber press cake for cows, which can offer a reduction in nitrogen and phosphorous emissions while maintaining milk productivity [18,20,21], and by providing an additional LPC co-product that can potentially replace soya bean meal in pigs, poultry, and other monogastrics [22,23]. The opportunity to use available grasslands in Europe more efficiently to produce a local, homegrown protein could resolve a key challenge for European agriculture, which is heavily reliant on imported sources of unsustainable feed. The European Union (EU) is reliant on animal feed imports from North and South America for livestock production, due to domestic deficits [24]. In addition, such a model can provide further bio-materials including fiber for insulation materials, bio-composites and packaging, high-value prebiotic materials, and brown juice or grass whey [25,26]. This whey can subsequently be used in different applications, for example, in biogas production or as fertilizer [25,26]. The interest in biogas production and its upgrading to biomethane, which can substitute natural gas and vehicle fuel, has become a trend in Europe, but its potential is still underexploited [27–29]. This interest has been further heightened by the Russia–Ukraine war. The REPower EU Plan, introduced by the EU Commission in 2022, focuses on reducing energy dependence on Russia by setting a target of 35 billion cubic meters of biomethane production by 2030, thus replacing the need for the import of natural gas [30].

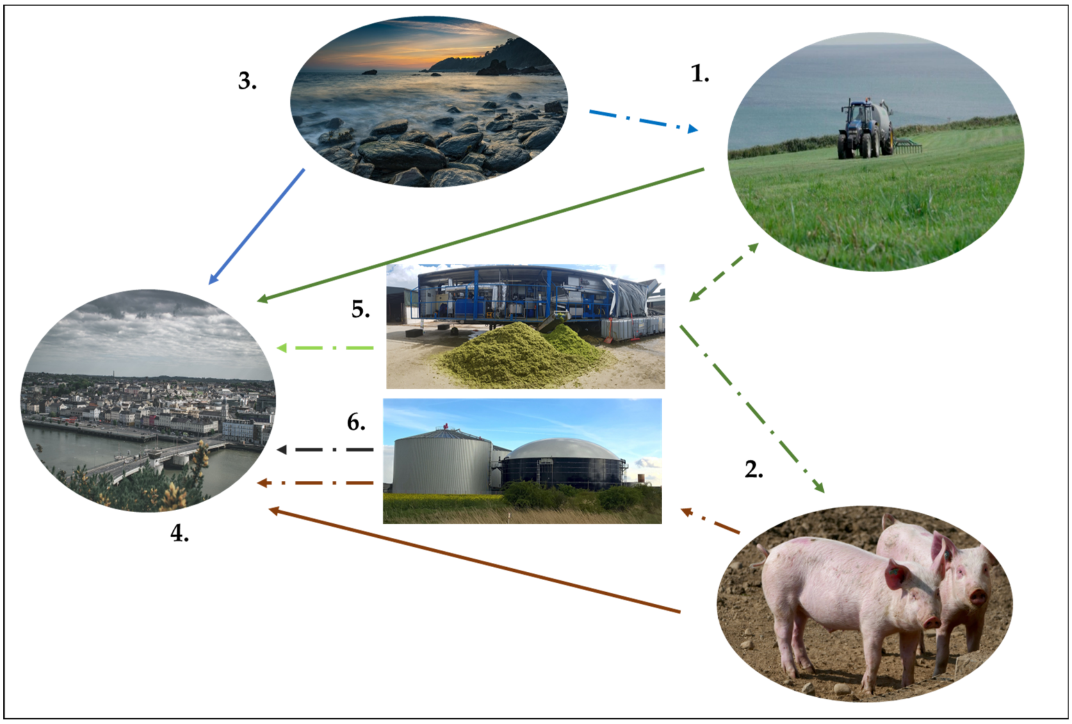

Figure 1. A bio-district approach for bioeconomy development. The figure shows potential synergies between (1.) grassland (ruminants), (2.) monogastrics, and (3.) marine sectors, which can collectively provide goods and services for the overall local bio-district (4.). Conventional products (traditional food products, such as milk, beef, fish, and pork) are highlighted by continuous lines while dotted lines indicate new value chains and products. A green biorefinery (5.) and anaerobic digestion unit (6.) are included as examples of enabling technologies that can support new local value chain development. In sector (1.), grass is supplied into a green biorefinery which can create low-emission feeds for ruminants and monogastrics (and potentially aquaculture feed), replacing imported feed sources. Other bio-material products may also be produced such as fiber insulation materials. Conventional dairy and meat products are also produced from the grassland chain. In sector (2.), pig slurry along with other animal slurries and food waste from the municipality can be supplied to an anaerobic digester to create heat, electricity, and/or biofuels for the bio-district. Pork is also produced. In sector (3.), coastal/marine resources can supply food products such as fish, while processed marine biomass, such as microalgae and Asparagopsis, can be used as fertilizer, feed, or anti-methanogenic additives for ruminants within the grassland chain.

This article explores the potential benefits of green biorefinery co-product LPC for a pig farm, partially displacing imported soya bean meal within a conventional pig diet. The impact of this diet change on pig performance is considered, as is the resulting pig slurry including its impact on the biomethane potential of slurry, a key factor for the pig farm which supplies its slurry to a local community anaerobic digestion plant. While a previous study has investigated the potential of green biorefinery LPC as an alternative pig feed [31], within this current study the inclusion rate of LPC was greatly increased to understand the impact of a higher inclusion rate. Furthermore, while other studies have investigated the biogas and biomethane potential of green biorefinery by-products such as grass whey, brown juice, de-FOS whey [25,26], and press cake [25], and various studies have looked at pig slurry [32–34], no similar studies investigating and analyzing the impact of the resulting biorefinery LPC pig slurry from this value chain were found within the literature This represents a novel aspect of the current research. In this way, this paper explores, in more detail, the potential benefits that green biorefineries implemented within the cattle sector can deliver for the pig sector.

2. Materials and Methods

Fresh grass was harvested from farms located within a 10 km radius of Afferden, Limburg, in the Netherlands during July and August of 2021. The feedstock, a 75–25% perennial ryegrass–clover mix, was processed within 12 h. The processing was carried out with the innovative "green biorefinery" process developed by Grassa BV. The biorefinery is a fixed unit demonstration facility with 4 tonnes per hour of fresh input processing capacity. A schematic of the process is included as part of Figure 2 below. Briefly, fresh grass was washed with water upon entry to the biorefinery to remove sand, with the water being recycled within the process. The grass was then subject to wet fractionation using an extrusion process. This created two primary products, a protein-rich "press cake" containing 50–60% of the initial grass protein, and a green, grass-derived juice. The press cake was further preserved through ensiling and baling, to be later used as ruminant feed, which exhibits a high nitrogen use efficiency and reduced nitrogen and phosphorous emissions [20]. The remaining protein is contained within the green juice. The protein fraction of the green juice was solidified using a heat coagulation process. This solid protein portion was extracted using a vacuum filter and then dried to in excess of 90% dry matter (DM) creating a storable LPC. The LPC was incorporated into appropriate feed for the pigs involved in this study. The remaining grass whey retains valuable sugars, e.g., fructans, and minerals after the protein fraction has been removed, and these can be extracted through further processing. The Grassa green biorefinery process is presented in Figure 3a below. The LPC was later tested as a feed ingredient at Carhue Piggeries in Timoleague, Co., Cork, Ireland. The piggery is also linked with Timoleague AgriGen Biodigester, where it currently supplies its generated pig slurry for the purposes of biogas production (Figure 3b below). The green biorefinery process is highlighted in Figure 2 below, with the main focus points of the current study, testing of LPC input to the pig farm and impacts on pig and biogas production, highlighted by a dotted line.

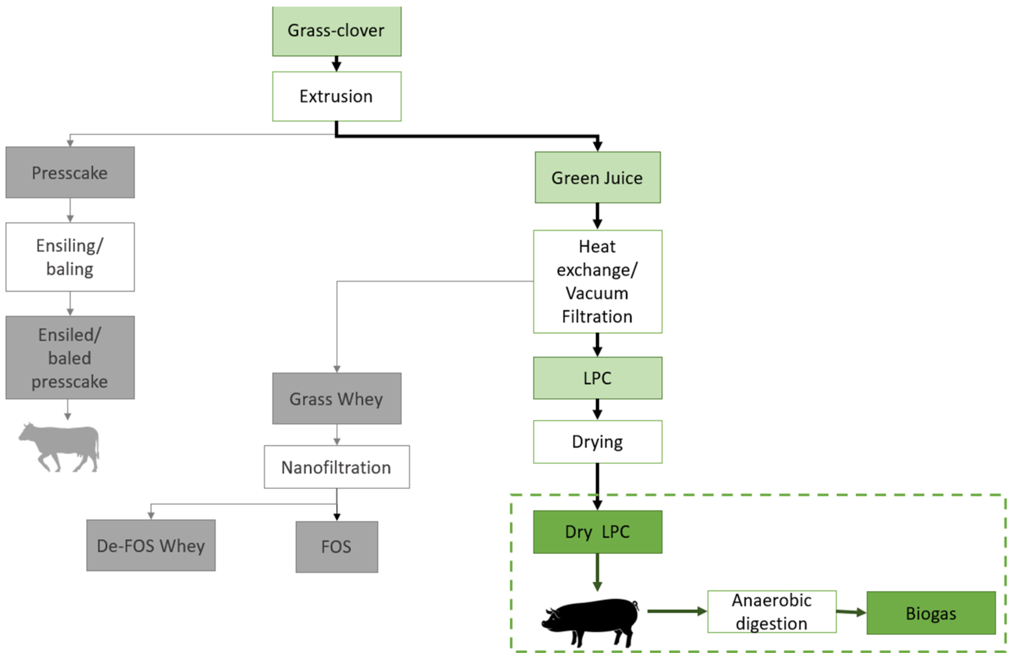

Figure 2. Schematic of green biorefinery value chain with main focus areas of the current study highlighted with a dotted line.

(**a**) (**b**)

Figure 3. (**a**) Grassa 4-tonne per hour pilot green biorefinery plant production unit for LPC; (**b**) Timoleauge AgriGen Biodigester for supply of slurry to produce biogas.

2.1. Analysis of Green Biorefinery LPC as an Alternative Feed for Pigs

2.1.1. Characterization of LPC

A proximate analysis of LPC was performed by Dairygold's analytical laboratory at Mallow, Co., Cork, Ireland. Crude fiber was analyzed using the Fibretec method based on ISO 6865:2000 [35]. Protein was determined using the Semi-Micro-Kjeldahl method based on ISO 5983:2000 [36]. Calcium, magnesium, sodium, and potassium were determined using atomic absorption based on ISO 6869:2000 [37]. Moisture content was determined using an oven at 102°C based on ISO 6496:1999 [38]. Phosphorous was determined using colorimetric UV spectrophotometry following ISO 6491:1998 [39]. Starch was determined using the polarimetry method based on ISO 15914:2004 [40]. Oil content was determined using the Soxhlet method following ISO 6492:1999 [41]. Crude ash was determined using a muffle furnace based on ISO 5984:2002 [42].

The LPC was analyzed by Sciantec, North Yorkshire, U.K., to assess the amino acid profile. Cation exchange chromatography was used to measure the AA content, following 24 h of exposure of the LPC to acid hydrolysis.

2.1.2. LPC and Control Diet Pig Feed Trial

To explore the potential of the LPC biorefinery co-product as a partial substitute for soya bean meal in pig diets, pig feeding trials were conducted at a privately owned piggery, Carhue Piggeries at Timoleague, Co., Cork, Ireland. Pigs were fed from two separate silo's via Schauer's Spotmix feeding system, in which feed is dispensed dry and is combined with water at the valve above the feeding trough and is presented to the pigs as wet feed. This system facilitates the feeding of multiple diets across pen groups without cross contamination or feed freshness issues. Pigs were fed ad libitum with feed troughs probed by the computer multiple times per hour with the same probe preventing over-feeding by regulation by the same feeding probe. Based on the characterization of the LPC, treatment and control feed rations were designed by Makeway Nutrition Ltd. with input from contributors with a view to reducing the soya bean usage of a conventional pig weaner ration by 50%. The nutritional requirements of the weaner pigs were considered in the preparation of the treatment diet in order to make it comparable to the control diet and sufficient for the pigs' needs. The treatment diet is provided in Table 1 alongside the control diet.

Table 1. Treatment and Control diet formulations for weaner pigs.

Raw Material	Control (%)	Treatment (%)
Barley	30.00	29.20
Maize	10.00	10.00
Wheat	25.00	25.00
Molasses	2.00	2.00
Hipro soya	22.00	11.00
Soya hulls	1.00	1.00
Whey permeate powder	2.50	2.50
Soya oil	3.70	3.10
Grass protein (42.8% CP)	0.00	12.40
Limestone flour	0.80	0.68
Salt	0.40	0.40
Lysine hydrochloride	0.00	0.08
Methionine	0.00	0.04
104 Weaner + Vita GP (2.6%)	2.60	2.60
Total	100.0	100.0

The focus of the trial was on weaner pigs aged approximately 9 weeks old and weighing 17 kg on average at the start of the testing phase. The pigs were randomly split into a treatment group of 110 pigs to be fed the treatment feed and a control group with 110 pigs to be fed the control diet over a 31-day period. The control diet was comprised of wheat, maize, barley, molasses, soya bean meal (in the form of hipro soya), soy oil, soy hull, and minerals in recommended amounts (Table 2). The treatment feed included LPC replacing 50% of the hipro soya level contained in the control diet, which represented the most significant change between the two diets (Table 2). Small amounts of synthetic lysine and methionine (0.08% and 0.04% of diet, respectively) were added to the LPC diet to prevent the crude protein of the diet from exceeding 18% crude protein and differing greatly from the control diet. All other amino acids are non-limiting. Additionally, a small amount of limestone flour was removed from the grass protein diet due to the high calcium value within the LPC. A comparison of constituents from the control and treatment diet is displayed in Table 2. The two diets were dispensed from separate feed silos using a computerized feed system which dispensed weighed amounts of feed to each group of pigs.

Table 2. Main constituents of treatment and control diets.

Constituent	Value	Control (%)	Treatment (%)
Protein	%	17.87	18.00
Oil	%	5.34	6.07
Fiber	%	3.34	3.35
Ash	%	5.51	5.86
DE	MJ/kg	14.32	14.24
NE	MJ/kg	10.25	10.24
Lysine	%	1.26	1.25
ILD lysine	%	1.15	1.15
Calcium	%	0.70	0.71
Dig phos	%	0.36	0.36
Sodium	%	0.23	0.23

The weight of pigs and feed intake were recorded on a weekly basis. These data were necessary in order to calculate the feed intake and weight gain on a daily basis (daily feed intake and average daily gain), and to evaluate the feed conversion ratio per treatment.

The daily feed intake (DFI) [43] for each treatment was evaluated by dividing the total feed intake by the size of the group, i.e., the number of pigs. The total feed intake was calculated at the end of the trial by deducting the feed remaining from the amount of feed delivered.

$$\text{Daily Feed Intake} = \frac{\text{Total Feed Intake}}{\text{Number of pigs on treatment}}$$

The pigs were weighed throughout the trial, at the start and end of every week, to calculate the average daily gain (ADG).

To calculate the feed conversion ratio (FCR) [44], the DFI was divided by the ADG for each treatment group.

$$\text{Feed Conversion Ratio} = \frac{\text{Daily Feed Intake}}{\text{Average Daily Weight Gain}}$$

2.2. LPC and Control Slurry Biogas and Biomethane Analysis

To assess the potential effect of the diet change on the slurry feedstock from the perspective of anaerobic digestion, a biogas and biomethane analysis of the feedstock was undertaken. Carhue Piggeries currently sends its produced pig slurry to Timoleague Agri Gen, a community-based anaerobic digestion facility located at Timoleague Co., Cork, Ireland approximately 2 km from the piggery site. At this facility, the slurry is co-digested along with food waste to produce biogas which is converted to heat and electricity. The site produces 500 KW of renewable electricity. The digestate produced from the digestion process is rich in nitrogen (N), phosphate (P), and potassium (K), and this biofertilizer can be land spread as a substitute for chemical fertilizer. To complete the analysis, approximately 2 kg of mixed slurry resulting from the treatment and control pig feeding trial batches from Carhue piggeries were collected and sent for analysis by Celignis Analytical, located at Castletroy, Co., Limerick, Ireland.

2.2.1. Determination of Total and Volatile Solids for Slurries

Slurries from weaner pigs feeding on LPC and the traditional soya bean meal diets were analyzed for biomethane potential. The samples were extracted simultaneously from the manure cellars when the feeding period ended. Prior to the BMP analysis, both slurries underwent a proximate analysis for determination of the total solids, volatile solids, and ash content. The proximate analysis followed European standard protocols as described in reference methods EN 14774-1:2009 [45] and EN 14775:2009 [46].

2.2.2. Biomethane Potential (BMP) of Slurries

An Anaero BMP unit consisting of 15 slots for 1 L digesters (700 mL working volume) was utilized for determination of methane potential from the slurries. The digesters were constantly stirred with stainless steel paddle systems and were placed in a 37 °C water bath. The BMP analysis followed the German standard method protocols (VDI 4630). Celignis propriety inoculum, which treats different sources of substrates including grass, whey, sewage sludge, manure, and other lignocellulosic biomass, was utilized for the BMP analysis. The inoculum was sieved to remove residual organic matter and degassed for at least seven days to remove any remaining organic matter. An inoculum to substrate ratio of 4:1 was prescribed in the VDI 4630 reference method. The BMP analysis of each slurry was performed in triplicate with no pH adjustments. pH adjustments with special reagents were not required because the pH of the substrate–inoculum mix was within the optimum range for anaerobic digestion. The BMP analysis of LPC and soya bean meal pig weaner slurries was monitored within 28 days of digestion.

2.2.3. Biogas Analysis and Biomethane Calculations

In the Anaero BMP unit, a tipping bucket flowmeter system coupled with a recording computer was used to measure biogas production from the various digesters. The flow meter had 15 chambers (buoyancy bucket design in every chamber), and each chamber contained a salt solution that prevents the dissolving of carbon dioxide, hydrogen sulfide, and ammonia from the biogas generated during this study. The biogas from the flowmeter was collected in 2 L Tedlar bags and methane, carbon dioxide, hydrogen sulfide, ammonia, and oxygen were analyzed using a Biogas 5000 gas analyzer. On the 3rd, 7th, 14th, 21st, and 28th days of digestion, the gas composition was analyzed and utilized to determine the biomethane potential of the slurries, applying methods used by Ravindran et al. [25] to evaluate the biomethane potential of green biorefinery products (Equations (1)–(5) [47–51]).

$$\text{Cumulative biogas produced} = \sum_{\text{Day } 0}^{\text{Day } 28} (\text{biogas produced per day}(\text{mL})) \tag{1}$$

$$\text{Biogas yield} = \frac{\text{Cumulative biogas produced (mL)}}{\text{FM or TS or VS fed (g)}} \tag{2}$$

$$\text{Average CH}_4 \text{ percentage} = \text{Determined from Biogas analyzer 5000} \tag{3}$$

$$\text{Cumulative CH}_4 \text{ produced} = \sum_{\text{Day } 0}^{\text{Day } 28} (\% \text{ Average CH}_4 \times \text{biogas produced per day}(\text{mL})) \tag{4}$$

$$\text{Methane yield} = \frac{\text{Cumulative methane produced (L)}}{\text{FM or TS or VS fed (kg)}} \tag{5}$$

3. Results

3.1. LPC and Control Diet Pig Feed Trial Results

3.1.1. Characterization of Grass-Derived LPC

A number of important LPC properties including crude fiber, ash, protein, starch, and total solids were determined through proximate analysis. Based on the analysis, the LPC had a high DM content, containing only 5.5% moisture. The sample contained 42.8% crude protein on a DM basis, 3.9% crude fiber, 9.4% ash, 2.8% potassium, 0.37% phosphorous, and oil content of 12.1%. Neutral detergent fiber was 43.5%, and acid detergent fiber was 7.63%.

3.1.2. Amino Acid Profile of LPC

The amino acid profile of the LPC is provided in Table 3. The analysis shows that the LPC was rich in glutamic acid (3.7%), aspartic acid (3.53%), leucine (3.10%), and alanine (2.24%). Table 4 provides a comparison between the constituents of LPC compared with soya bean meal and other common feedstuffs. Overall, the LPC compares quite well with soya bean meal, providing comparable levels of protein, lysine, methionine, and threonine, although with lower cystine. The LPC also compares favorably with rapeseed meal, with LPC containing higher crude protein and crude fiber contents than rapeseed meal (Table 4). Comparing the composition of the LPC with the results of LPC produced by Ravindran et al. [31] from perennial rye grass, the overall CP is significantly higher (43% versus 34%) and more consistent with the CP content of soya bean meal, as indicated in Table 4. The sample also has a higher content of production-limiting amino acids lysine, methionine, and threonine. The increase in protein content may be partly resulting from the removal of small fibers from the protein products and soluble salts such as potassium, which are washed using a vacuum filter in the current biorefinery process. The DM content of the LPC from this study is higher when compared with Ravindran et al. [31] (94.5% versus 87%) due to a new drying process that was implemented in the current biorefinery process. The protein was dried at 70 °C in a belt dryer with a residence time of 24 h; Maillard reactions occurred, as well as polymerization reactions in unsaturated fats. The crude fiber of the current LPC is lower than that found by Ravindran et al. [31], but it is still in the comparable range with soya bean meal (Table 4) [33]. The ash content was reduced from 11.9% to 9%. This reduction may be attributed to lower sand contained on the feedstock or improved washing of feedstock during pre-processing.

Table 3. Amino acid profile of LPC.

Constituent	%
Cystine	0.21
Aspartic	3.53
Methionine	0.72
Threonine	1.71
Serine	1.54
Glutamic	3.77
Glycine	1.91
Alanine	2.24
Valine	2.09
Iso-leucine	1.71
Leucine	3.10
Tyrosine	1.00
Phenylalanine	2.07
Histidine	0.78
Lysine	2.03
Arginine	2.07
Proline	1.65

Table 4. Crude fiber, crude protein, and selected amino acid profile of various feedstuffs in comparison with LPC from the current study [52] (g/100 g).

Animal Feed Protein Sources	Unit	Crude Protein	Lysine	Methionine	Cysteine	Threonine	Crude Fiber
Soya bean meal	g/100 g	44–48	2.81–3.20	0.60–0.75	0.69–0.74	0.71–2.00	3.0–7.0
Sunflower meal	g/100 g	24–44	1.18–1.49	0.74–0.79	0.55–0.59	1.21–1.48	12.0–32.0
Rapeseed meal	g/100 g	34–36	2.00–2.12	0.67–0.75	0.54–0.91	1.53–2.21	10.0–15.0
Cottonseed meal	g/100 g	24–41	1.05–1.71	0.41–0.72	0.64–0.70	1.32–1.36	25.0–30.0
Grass protein concentrate	g/100 g	42.8	2.03	0.72	0.21	1.71	3.9

Once mixed into rations at the Barryroe feed mill, finished feed appeared dark green in color compared with the control sample (Figure 4 below).

Figure 4. Control feed (**left**) compared with treatment feed (**right**)—note the green color of the treatment feed.

3.1.3. Feeding Trial

Pig weights were recorded at the beginning of the trial. The initial average weight of the control group pigs was 17.388 kg, while the initial average weight for the treatment group pigs was 17.246 kg. As expected, feed intake increased in both groups during the course of the trial, and by week 5 the average trial end weight was 35.092 for the control diet and 35.450 for the treatment diet. The total weight gain per pig since weaning is presented in kg in Table 5and shows a comparable weight gain for pigs on the control diet versus those on the treatment diet, with pigs gaining 19.494 kg and 19.554 kg, respectively, by week 5.

Table 5. Total weight gain per pig since weaning on treatment and control diet.

Date of Weighing	Total Weight Gain Per Pig Since Weaning (kg) Treatment	Control
Week 1	4.656	4.413
Week 2	8.557	9.178
Week 3	13.104	14.178
Week 4	18.204	17.704
Week 5	19.554	19.494

The dung consistency appeared similar in both batches,; however the treatment dung had a notable green color. Overall, the pigs on the treatment diet appeared healthy.

Daily Feed Intake

The DFI was measured weekly during the trial. The results indicate that the pigs readily accepted the treatment feed. During the first week, week 1, the control group DFI was 1.060 kg/d and the treatment group DFI was similar: 1.079 kg/d. During week 2, the DFI dipped lower (1.155 kg/d) for the treatment diet than the control diet (1.167 kg/d). Subsequently, the DFI of the treatment diet overtook that of the control diet from week 3 onwards and was 3% higher at the end of the trial (1.427 kg/d versus 1.391 kg/d, respectively, for treatment and control).

Overall, the difference in DFI between the treatment and control group was not significant. A slightly larger amount of the treatment feed was eaten by the pigs in the treatment group than the amount of control feed eaten by their counterparts in the control group, which suggests that the weaner pigs liked the treatment diet. These results are described in Table 6. A similar trend was also seen in the study by Ravindran et al. [31], with pigs also consuming more treatment feed [31]. These findings indicate that the incorporation of green protein in the diet enhances the attractiveness of the feed, e.g., due to changes in taste, smell, or other sensory characteristics. Stødkilde et al. [23] found that the addition of green protein to pig feed rations did not negatively change the taste, i.e., pigs were not discouraged from consuming it [23]. Moreover, inclusion of green protein from specific feedstocks, e.g., clover grass, has also been found to improve the meat yield and omega-3 fatty acid content of pig meat, which may have positive health benefits [24].

Table 6. Comparison of treatment and control groups describing daily feed intake, feed conversion efficiency, and average daily gain.

Date of Weighing	Daily Feed Intake (kg/day)		Feed Conversion Efficiency		Average Daily Gain (kg/day)	
	Treatment	Control	Treatment	Control	Treatment	Control
Week 1	1.079	1.060	1.62	1.68	0.665	0.630
Week 2	1.155	1.167	1.89	1.78	0.611	0.656
Week 3	1.285	1.265	2.06	1.87	0.624	0.675
Week 4	1.384	1.349	2.13	2.13	0.650	0.632
Week 5	1.427	1.391	2.19	2.14	0.652	0.650

Average Daily Weight Gain

Average daily gain (ADG) is an evaluation of the average daily increase in the live weight of an animal and is recorded across the growth period of the animal. This can provide insight into the animal's growth rate, and evaluate the time at which it should reach market weight [53]. Ravindran et al. (2021) noted a number of factors that can influence pig weight, including genetic differences, sex effects, weight at birth, age at weaning, feeding level, and specific amino acid content (e.g., arginine) in feed [31].

During the feed trials, pig weight was measured individually, first at the beginning of the trial and at the end of each following week. In week 1, pigs in the control group gained 0.630 kg/day, increasing to 0.656 kg/day after week 2, 0.675 kg/day after week 3, 0.632 kg/day after week 4, and 0.650 after the final week. For pigs on the treatment diet, the ADG started well at 0.665 kg/day at the end of week 1, but reduced to 0.611 kg/day by week 2. From that point, the ADG increased to 0.624 kg/day by end of week 3, 0.650 kg/day by end of week 4, and 0.652 kg/day by the end of the trial, slightly higher than the control diet. Overall, the variances in average weight gain between the weeks is considered to be negligible and are generally consistent across both diets. The results are presented in Table 6. Over the course of the trial, the analysis showed comparable performance in weight gain for pigs on the control and treatment diets, with pigs gaining 19.49 kg in the case of the control diet and 19.55 kg in the treatment diet by the end of the trial. The ADG of the treatment group pigs was marginally higher than the control group pigs during week 1, but dipped in the following week as the control ADG increased. This may have been a result of acclimatization of the pig's microbiome to the new protein diet. Over the course of the remaining weeks, the ADG increased in the treatment batch week on week, culminating at week 5, at which point the treatment pigs had a slightly higher (0.652 kg/day) ADG compared with the control batch (0.650 kg/day). Over the course of the trial, the ADG between both trials were quite evenly matched and without significant differences. While there were some variances in ADG week on week, these were relatively minor and did not indicate a major trend. Certain factors such as time of day with weighing or gut fill can have a big impact on the weekly weighings during trials; however, over an extended period these factors do not contribute the same variance.

Feed Conversion Ratio

The feed conversion ratio (FCR) describes the quantity of feed required for pig weight to increase by one pound. Lower FCR values indicate that pigs are efficiently converting feed into body weight, while a high FCR can be a sign of the pigs being unable to convert the feed into body weight effectively [31]. A number of factors influence the feed conversion ratio in pigs. Pierozan et al. (2020) found the number of pigs per pen, feeder type, origin, and sex of the animals to be determinant factors [54]. According to Hancock and Behnke (2000), feed conversion efficiency, and thus FCR, can be enhanced by pelleting feed as pigs will not sort and waste feed pellets [55]. Smaller pellets are also more digestible [55]. Lastly, dietary components such as lysine and phosphorous levels are important. Lysine is essential for pigs in order to effectively utilize other amino acids for growth [56]. Additionally, phosphorus at optimum levels is required for the proper development of muscle and optimal energy use, with excess phosphorus being excreted as feces [57].

The FCR increased weekly for both groups over the course of the trial. Initially, the FCR was recorded in week 1 as 1.68 for the control group, and 1.62 for the treatment group, which was slightly lower. In subsequent weeks, the FCR increase for both diets was quite comparable, with the FCR by the end of the trial being slightly lower for the control diet (2.14) compared with the treatment diet (2.19). The comparable FCR during the trial indicates that the treatment diet compares well to the conventional diet fed to the pigs. The comparative FCR by the end of the trial indicates a slight improvement in FCR compared with previous research on LPC [31]. The difference that exists may indicate that the regression equations used for energy estimation of the grass protein may need to be refined slightly. However, once again, the difference between the groups is minor and the performance is largely in line with the control diet. This is a field of primary concern to pig farmers as optimizing FCR can have a significant positive effect on overall profitability.

3.2. LPC and Control Slurry Biogas and Biomethane Analysis Results

The biomethane potential is a measure of the biomethane that can extracted from an organic substrate. Pig slurry is a well-recognized source of animal manure for biogas production when co-fed with carbohydrate-rich feedstock. Pig slurries have high biomethane potential (275–450 L/Kg VSfed) when compared with other animal manures except for poultry litter (460 L/Kg VSfed) [58].

The biogas and biomethane production profiles from both LPC and soya bean meal pig weaner slurries are shown in Figure 5. The maximum biogas and biomethane yields for both slurries was achieved after 25 days of digestion. The biogas production from both LPC and soya bean meal pig weaner slurries were determined to be 495 $\pm$ 12.52 L/Kg VSfed and 478 $\pm$ 7.93 L/Kg VSfed, respectively (Table 7). An ANOVA analysis conducted on the biogas yield showed no statistical significance (p-value (0.1206) > 0.05). This indicated no significant difference in biogas production from both LPC and soya bean meal pig weaner slurries. From Figure 5, it can be observed that 90% of biogas production was achieved after 10 to 11 days. The daily biogas production was similar for both LPC and soya bean slurries which recorded a high of 66 L/Kg VSfed. This aligns with the findings of Santos et al. [59] and Miroshnichenko et al. [60], which indicated high daily biogas production in anaerobic digestion of some pig slurries [59,60]. However, there was a significant difference in the daily methane production, which was between 10% to 50% higher in LPC slurry than soya bean meal slurry. This was attributed to the high methane content of produced biogas from the LPC, which was about 22% to 35% higher than soya bean meal slurry (statistical significance at p-value 0.0335 < 0.05) (Table 7). Biogas from anaerobic digestion of LPC slurry had methane contents ranging from 70% to 73% compared with the soya bean meal slurry which had biogas methane contents from 52% to 59%. The high methane content obtained for LPC could be attributed to the higher volatile solid content of the LPC pig slurry, especially with regard to the high-protein diet fed to the pigs. The significant difference in biogas methane content for both slurries led to a higher biomethane potential of 355 $\pm$ 9.45 L/Kg VSfed for LPC pig weaner slurry compared with 281 $\pm$ 7.11 L/Kg VSfed for soya bean meal pig weaner slurry (statistical significance at p-value 0.0004 < 0.05). The BMP results indicated that slurry from weaners feeding on the LPC treatment diet performed significantly better (26% increase in methane yield) than the conventional slurry from weaners on soya bean meal. This is potentially a major benefit in addition to the successful replacement of the soya bean mealdiet with the LPC treatment diet and suggests that co-digestion with carbohydrate-rich substrates at the Timoleague, Co., Cork, community anaerobic digester has the potential to yield improved performance with LPC pig slurry. Another key positive point was the high methane yield obtained from LPC slurry from pig weaners compared with the reported literature on methane production from pig slurry. Biomethane production from pig slurry/manure, especially from weaners, tends to have a lower methane yield compared with that obtained from this study. The studies by Browne et al. [61] and Miroshnichenko et al. [60] for pig slurries from weaners yielded considerably lower biomethane potentials of 38.0 L/Kg VSfed and 75.5 L/Kg VSfed, respectively [1,60]. On the other hand, studies from Santos et al. [59] and Rodríguez et al. [33] indicated high biomethane yield for pig slurries [33,59]. These studies mostly digested pig slurries from pig fatteners and pregnant sows which tend to yield high biomethane production. The differing biomethane yields for pig slurry/manure seem to be highly dependent on the type of pig (i.e., weaners, fatteners, pregnant sows, and suckling sows) excreting the slurry with another key factor being the type of meal fed to the varying range of pigs. Irrespective, the biomethane yield from the LPC pig weaner slurry performed considerably better than the reported literature on pig weaner slurries and was about 20% less than the highest reported study of various pig slurries/manures.

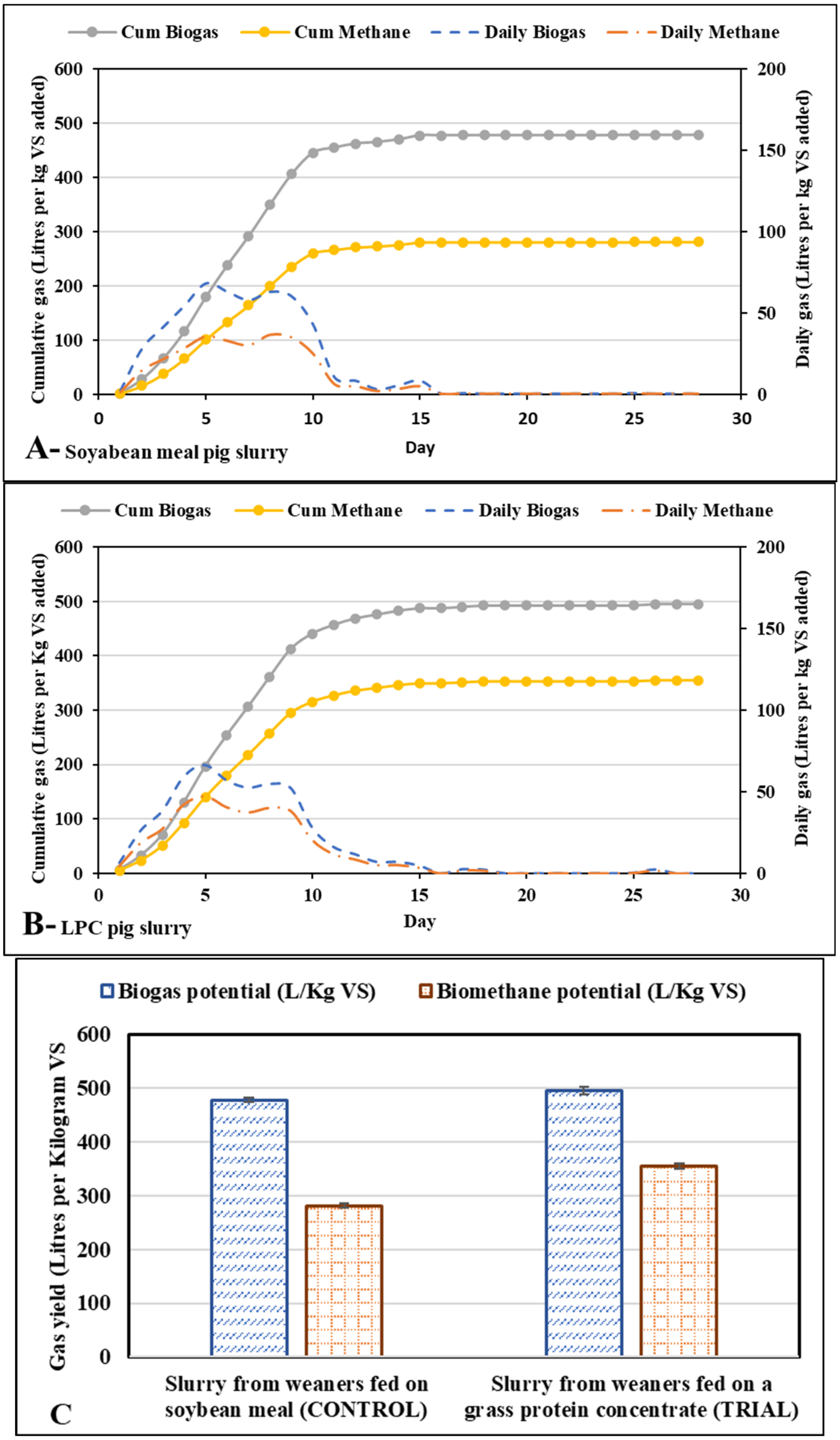

Figure 5. Daily and cumulative biogas and biomethane production profiles for (**A**) soya bean meal pig slurry; (**B**) LPC treatment meal pig slurry; and (**C**) biogas and biomethane potential of treatment and control pig slurry.

Table 7. Summary data for biogas and biomethane production from soya bean meal pig slurry and LPC treatment meal pig slurry.

Measurement	Control Diet Slurry	Treatment Diet Slurry
Biogas potential—replicate A (L/kg VS_{fed})	470.33	499.79
Biogas potential—replicate B (L/kg VS_{fed})	486.20	504.45
Biogas potential—replicate C (L/kg VS_{fed})	478.03	480.81
Average biogas potential (L/kg VS_{fed})	478.19 ± 7.93	495.02 ± 12.52
Average biomethane potential (L/kg VS_{fed})	280.85 ± 7.11	354.87 ± 9.45
Biogas potential (L/kg DM)	316.09 ± 5.25	349.65 ± 8.85
Biomethane potential (L/kg DM)	185.65 ± 4.70	250.66 ± 6.67
Biogas potential (L/kg FM)	8.52 ± 0.15	11.37 ± 0.29
Biomethane potential (L/kg FM)	5.01 ± 0.13	8.15 ± 0.21
Biogas composition (CH_4 %)	52.5–58.7	70.9–71.7
Biogas composition (CO_2 %)	41.3–47.5	28.3–29.1

3.3. Bioenergy Assessment of LPC Pig Slurry and Soya Bean Meal Pig Slurry Feed AD Systems: A Case Study

To demonstrate the potential impact of the above findings, in this case study, two AD scenarios are considered to ascertain bioenergy production from the usage of LPC pig slurry and soya bean meal pig slurry as feedstock to a community AD plant close to the pig farm where the LPC slurry was produced. This bioenergy assessment consists of mass and energy balance with the major assumptions listed in Table 8 [62]. The results of the bioenergy assessment are displayed in Figure 6 and discussed in Section 4.

Table 8. Assumptions made for bioenergy assessment of LPC pig slurry AD and soya bean meal pig slurry AD systems.

	Components	Conditions/Assumptions
Anaerobic digester	Operating temperature	36 °C
	Lower heating value (LHV) of methane	10 kWh/Nm^3 CH_4
Energy requirement of anaerobic digester	Electrical energy required for mixing slurry	10 $kWh_{electric}$/t slurry
	Thermal energy for heating digester	$E_{thermal} = Cp \times m \times \Delta T$
	Boiler efficiency	0.9
Energy requirement for digestate centrifugation	Electrical energy demand	3.5 $kWh_{electric}$/t digestate
	Moisture content in solid digestate	0.7
Energy requirement for amine scrubber biogas upgrading	Methane content in the upgraded biogas	96%
		Methane losses neglected
	Electrical energy demand	0.09 $kWh_{electric}/m^3$ biogas input
	Thermal energy demand	0.45 $kWh_{thermal}/m^3$ biogas input

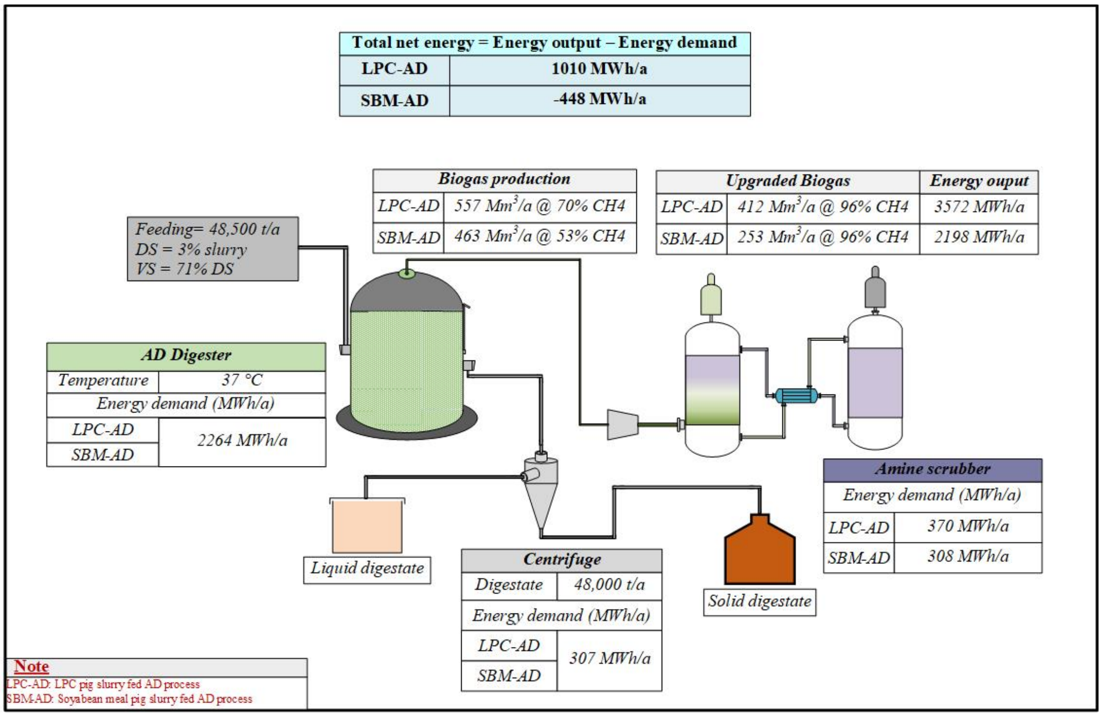

Figure 6. A bioenergy case study for an AD plant fed with LPC pig slurry and soya bean meal pig slurry.

4. Discussion

The results of these experiments demonstrate some potential for extended green biorefinery value chains to have positive benefits for the pig sector by supplying LPC as a sustainable alternative to soya bean meal and enhancing the potential for biogas production from pig slurry residues. Pig farmers, like most sectors of agriculture, are under pressure to become more sustainable. It is estimated that 68% of greenhouse gas emissions in the pork-production chain occur at the pre-farm gate phase [63]. A recent study from McAuliffe et al. [64] found that the primary aims of environmental performance improvements in the pig sector are reducing the crude protein content of pig feed and producing bioenergy through anaerobic digestion of pig slurry [64].

From a sustainability perspective, the potential to displace soya bean meal with an indigenous source of protein, such as LPC, could bring some key benefits. Soya bean meal is a major global commodity crop and is widely used in animal feed production, being one of the primary crops cultivated by farming communities across the world. Its production is mainly in the USA, Argentina, Brazil, China, and India, with only a small amount of cultivation taking place in Europe [17]. The soya bean market is closely associated with land use change for agricultural expansion and forest loss, particularly in South America [65]. It is estimated that approximately 2.31 million hectares of forest disappear annually to make way for soya bean production [66]. Despite a moratorium introduced in 2008 to avoid the purchase of soya beans from deforested land, by 2020 a further 133,000 ha of soy in the Amazon, planted on land deforested after this date, has been produced, which is linked to 69 million tonnes of CO_2 emissions [67]. A recent study from Franchi et al. [68], applying consequential life cycle assessment (LCA) to compare LPC and SBM for use in poultry diets, found a significantly lower environmental footprint in the case of LPC. A separate consequential LCA study from Parajuli et al. [69] found that a livestock production system that partially displaced Brazilian soya bean imports through integrated green biorefineries coupled with biogas facilities to produce feed and biomethane, and combined crop and livestock production, generated a lower environmental impact compared with the preexisting "conventional" system (livestock production without biogas production or green biorefinery) [69].

Despite the negative impacts, South America still accounts for more than 50% of global soya bean production, of which 70% is exported, with around 21% of these exports coming to the EU for use in animal feed [65]. A recent study from Escobar et al. (2020), mapping carbon emissions embodied in Brazil's soy exports, found that the EU has a significantly higher footprint associated with soya bean imports compared with China; this mainly due to the source of soya beans being linked to deforested areas [70]. To highlight the impact that source can have on potential sustainability impact, referencing the life cycle inventory database Agri-Footprint 6, an economic allocation, and a point of substitution on the system, the footprints for Argentinean-, Brazilian-, and U.S.-sourced soya bean meal are 4.13 kg CO_2/kg, 4.28 kg CO_2/kg, and 0.53 kg CO_2/kg, respectively.

Despite these sustainability issues, the EU is still very dependent on imported soya beans. Soya bean meal is one of the most widely used individual protein sources in Europe, accounting for 29% of crude protein for animal feed in the EU (including the United Kingdom) during the period 2019–2020 [71]. This results in the average European person consuming 61 kg of soy indirectly every year. However, Europe has very low domestic soya bean production, accounting for just 3% of the total demand in the period 2019–2020, demonstrating a low self-sufficiency rate [71]. Overall, the EU's lack of indigenous protein results in approximately 17 million tonnes of proteins being imported on an annual basis, with the majority (13 million tonnes) deriving from soya bean [72]. This has prompted the European Commission to support the development of native protein ingredients such as peas, lupins, and faba beans [72]. On the other hand, grassland is a very available resource across the continent of Europe, with permanent grasslands making up 35% of total arable land use in EU-28 [73]. A study from Mandl [74] investigated the potential of grasslands to deliver additional protein for Europe. Focusing only on 15% surplus grassland, and assuming a yield of 8 t DM/ha, it is estimated that there is a grass surplus in Europe of approximately 20 million t DM/ha, creating an additional crude protein equivalent of approximately 3 million t DM [74]. This potential protein availability can be further increased to unlock more protein from all EU grasslands providing for ruminant and monogastric needs. The integration of legumes alongside grass for biorefining can further increase the sustainability of green biorefinery systems. If we consider, based on the more recent work of this paper, that by biorefining we can give almost 45% of the protein originally present in grass to pigs without reducing the impact on milk production from feeding the press-cake co-product to cows; then, assuming a grass yield of 10 t DM/ha/yr with 20% protein content, we can estimate an LPC biorefinery co-product yield from dairy farming of about 0.5 tonne protein/ha. This would mean that the 3 M tonnes can be obtained from only 6 Mha, or that the 17 M tonnes of protein that we import in Europe can be obtained from 34 Mha, being about half of all grassland in the EU.

Further environmental and self-sufficiency gains may be achieved using this extended green biorefinery model by increasing the potential of renewable energy from pig slurry, with the findings from this study indicating a 26% increase in biomethane yield from slurry produced from pigs on the treatment diet.

In Figure 6, a case study assesses two AD plant scenarios which considers the conventional soya bean meal pig slurry and the current study LPC pig slurry as AD feedstock to a community digester. The bioenergy case study includes the anaerobic digester, a centrifuge for digestate separation and an amine scrubber for biogas upgrading. The community digester can process a high throughput of 48,500 tonnes per annum of slurry, hence this feedstock capacity was assumed for both the LPC and soya bean meal pig slurries. Employing the biomethane yield from (Table 7), 357 and 220 thousand cubic meters of methane is produced per annum from LPC and soya bean meal slurries, respectively. This translates to 3572 MWh and 2198 MWh per annum of renewable bioenergy for the LPC and soya bean meal slurries, respectively. The gross bioenergy from the LPC pig slurry feed AD scenario was about 63% higher than that of the conventional soya bean meal pig slurry feed AD scenario. Furthermore, considering the parasitic energy demand of both scenarios, only the LPC pig slurry feed AD plant generated a positive net bioenergy of 1010 MWh per annum. This indicated the superiority and efficacy of the LPC pig slurry in utilization to produce renewable energy as compared with the conventional soymeal pig slurry.

According to the European Biogas Association [75], up to 41 billion cubic meters (bcm) of biomethane could be produced from sustainable feedstocks in Europe by 2030, rising to 151 bcm in 2050 [75]. These targets would enable the fulfillment of the European Commission target of 35 bcm biomethane production per annum by 2030, as outlined in the REPowerEU plan. Out of this total, the majority, 38 bcm by 2030 and 91 bcm by 2050, are estimated for anaerobic digestion, with 33% of the contribution anticipated to come from animal manure feedstocks [75].

While the overall CH_4 potential of pig slurry as a biogas feedstock can be comparatively low by comparison with other substrates, the utilization of pig slurry for on-farm energy production can have positive impacts on pig farm operations while contributing to a circular economy, also enabling the recirculation of nutrients to local farms [76]. Using LCA to investigate strategies for addressing greenhouse gas (GHG) emissions and fossil energy consumption in European pig production, Nguyen et al. [63] found that using pig manure as a feedstock source for anaerobic digestion had the ability to displace 53% of fossil energy usage and reduce GHG emissions of the farm by 27% over the baseline pig farm scenario [63]. Various other studies have investigated the feasibility and benefits of pig slurry anaerobic digestion for on-farm usage. Freitas et al. [77] investigated the potential of co-digestion with elephant grass silage, or corn silage, as well as the use of a biochar additive in comparison with monodigestion of pig slurry [77]. Using co-substrates and additives enabled greater production of electricity compared with monodigestion, but also resulted in significant environmental impacts associated with co-substrate use, primarily as a result of fossil fuel use during the silage production chain. When comparing digestion with the direct spreading of slurry, Zhang et al. [32] found that digestion of pig slurry alone resulted in a 48% decrease in direct emissions of GHGs (190 tonne CO_2e) compared with direct land application, due to the recovery of methane [34]. Using LCA, Jiang et al. [78] compared the co-digestion of pig manure and food waste with alternative management strategies including pig manure land use, food waste mono-digestion, and composting, and found that the co-digestion approach performed better in most environmental impact categories [78]. Using an average size piggery for comparison, comprising 762 sows with 16,000 t/yr pig manure, the same study found that the global warming potential (GWP) only became negative when the inclusion of food waste in feedstock was greater than 2000 tonnes per annum [78]. A further recent benefit of interlinking the green biorefinery approach with pig systems was reported by Regueiro et al. [79] who demonstrated that both unfermented and fermented whey or brown juice from the grass biorefinery can be used to stabilize pig slurry in storage by contributing to reducing the pH. A reduction below pH6 reduces ammonia emissions as well as methane emissions from the stored slurry.

The development of such anaerobic digestion models may also help to meet the needs of the local communities or districts in which they are based. Community-based anaerobic digestion models, which can help to meet the heating and electricity requirements of the community, for example, heating community buildings, are well established. In addition, such models may help to meet the mobility needs of the community, through the development of biomethane-based transport systems in pressurized or liquefied forms. In Europe, biomethane-based transport is at the furthest stage of development in Sweden, where half of the biogas production is used for transport [80], including public transport buses where it supplies the fuel for over 20% of the distance travelled [81]. Biomethane may also help to reduce the emissions and dependency of local manufacturing industries which are heavily dependent on natural gas, including pulp and paper, and some food processing industries.

In addition to the environmental sustainability and self-sufficiency benefits, the development of such local bioeconomy value chains may also help to relieve cost pressures for local pig farmers. According to the European Commission [82], EU pigmeat production is due to decrease by 5% in 2022 in large part due to rising input costs [82]. This situation has been compounded by the Russia–Ukraine war, as both Russia and Ukraine were key exporters of fertilizers and grain and oil crops, including wheat, maize, and sunflower, and Europe has also been dependent on natural gas from Russia. Between Q2 of 2021 and Q2 of 2022, following the Russian invasion of Ukraine, the average price of goods and services used within agriculture in the EU jumped by 36% for the same agricultural inputs [83]. An economic analysis of small-scale green biorefineries from Cong and Termansen [84] found that using LPC can be economically feasible for both pig farmers and the green biorefinery [84]. In order for these models to be successful and implementable, economic feasibility is an important outcome. The study found that LPC will decrease the average feed cost by 5%. Coupled with this, rising natural gas prices, and a need to reduce dependency on imported Russian gas, has changed the landscape for biomethane in Europe, making biogas more cost-competitive and even cheaper than natural gas in the current environment [85]. This combined with a significant increase in biomethane potential, demonstrated by this study, may offer a greater opportunity for pig farmers to produce, use, and supply renewable energy. While this paper primarily underlines the synergies and benefits that may be found by connecting ruminant and swine sectors in extended local green biorefinery models, as noted earlier, several additional synergies may be found by connecting additional value chains in local bioeconomy models, further improving the resilience of local regions.

5. Conclusions

Overall, this work demonstrates significant potential for the development of local bioeconomy value chains based on a green biorefinery model, increasing the resource efficiency of grasses and legumes, building synergies, and meeting input requirements to increase the sustainability and resilience of the pig sector. The LPC co-product from the green biorefinery was produced with a high-crude-protein content of 43.9% and has been integrated within the pig treatment diet replacing 50% of the soya bean meal present in the control diet, achieving a slightly higher daily feed intake and average weight gain compared with the control batch on conventional weaner diets. In addition, the slurry produced by pigs on the treatment diet achieved higher biogas and biomethane production rates compared with the slurry from pigs on the control diet. Since sustainability improvements in the pig diet and anaerobic digestion of slurries are identified as key components to increasing the sustainability of pig production systems, a local bioeconomy model may help to meet this objective, while providing additional co-products for use in the ruminant and industrial sectors. Given the abundance of green biomass that exists in Europe, the potential to unlock additional protein from these through local green biorefineries may offer significant potential to increase feed resilience for the pig and broader livestock sector.

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