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Production of Green Biorefinery Protein Concentrate Derived from Perennial Ryegrass as an Alternative Feed for Pigs

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Abstract: Perennial rye grass is a widely used forage species in Ireland, on which the ruminant sector of agriculture is heavily dependent. While this species of grass is the primary source of fodder for cows, it is also abundant in plant protein, which could form a potential alternative ingredient in monogastric animal feed using a green biorefinery approach. In this study, perennial rye grass was processed using a novel biorefining process to extract value added products including protein as a potential replacement for soybean meal in monogastric feeds. Feed trials were conducted on a commercial farm with 55 weaner pigs for 31 days until slaughter. The diets comprised a control and a trial diet which integrated the green biorefinery protein concentrate. The effects of the new diet were determined by measuring the daily feed intake (DFI), average weight gain (AWG) and feed conversion ratio (FCR). Amino acid profiles of grass protein concentrate and soybean meal were comparable, with the latter having a slightly higher amount of total protein content, lysine and cysteine. The DFI and ADW indicated that the treatment diet was superior to the control. DFI for the treatment diet (1.512 kg/d) was 8% higher than the control diet (1.400 kg/d) by the end of the trial. Additionally, the ADW for the treatment diet was 6.44% higher than that achieved in the control sample. Meanwhile, FCR calculations indicated that the treatment diet is just as efficient as the conventional diet. Overall, the results of the study indicate positive potential for perennial ryegrass-derived green biorefinery protein concentrate as an alternative protein source for pig feed formulations in Ireland.

Keywords: green biorefinery; perennial rye grass; grass protein concentrate; monogastric feed; protein recovery

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1. Introduction

The continuing exponential increase in population coupled with a growing consumer demand for edible protein has resulted in a significant intensification of agriculture over recent decades, raising questions about the sustainability of the livestock sector. To meet this growing demand, the meat industry in Europe has become heavily dependent on the importation of protein-rich plant-based feed additives such as maize and soybean [1]. Ireland has a vibrant livestock industry comprising cattle, dairy, pig and poultry sectors [2]. Sustaining this sector is particularly reliant on imports of animal feed and related additives. In 2018, Ireland imported approximately 5.1 million tonnes of animal feed materials. Almost two-thirds of the animal compound feeds in Ireland are imported, compared to the UK (37%), France (27%) and Germany (26%). The pig, poultry and dairy sectors are particularly dependent on the import of genetically modified (GM) soy, maize and their by-products forming essential ingredients in animal feed formulations [3]. Almost 2.7 m tonnes of soya and maize GM products were imported into Ireland for animal feed applications in 2018, constituting approximately 50% of total feed imports. Up to 90% of the soybean and 80% of maize products are imported from Argentina, Brazil, Canada and the USA [4]. Significant quantities of non-GM maize and oilseed rape meal are also imported from continental Europe, including Ukraine [5].

According to new regulations by the EU, 30% of the protein for monogastric feed purposes must be sourced locally [6]. Although soy can be grown in Europe, it is usually only cultivated for commercial exploitation in the southern and eastern regions. Furthermore, soy cultivation in these regions cannot meet the animal feed demand of the whole of Europe. Therefore, northern Europe is heavily reliant on soybean meal imports [3]. Sources such as seaweed, mussels, insects and forage crops have been extensively investigated as potential sources of protein to decrease the dependence on soy imports for monogastric feed applications [7]. The European Commission published a report in 2018 detailing the development of plant proteins in the EU. Accordingly, this report investigated the supply and demand of protein within the EU. In 2016/17, the EU demand for plant protein amounted to 27 million tonnes of crude protein. Five major sources of plant protein were identified in this report, i.e., pulses, soybean complex, rapeseed complex, sunflower complex and dried fodder legume. Out of all these protein sources, soybean complex (soybeans, soybean oil and soybean meal) contributed to 14.3 million tonnes of the crude protein demand, with a huge share being imported. Meanwhile, a major share of the dried fodder legume, rapeseed complex and pulses were sourced from within the EU. The overdependence of the European Markets on imported soybean complex has called for member states to develop national protein strategies to guarantee long-term, sustainable food security.

EU member states such as Germany, France, Netherlands and Nordic countries such as Denmark and Finland have developed 'National Protein Strategies' with the aim to establish sustainable food security, efficient and resilient circular supply chains, support sustainable innovation and conserve natural resources and ecosystems. A common strategy among Germany, France and Finland is to encourage extensive cultivation and production of legumes as a potential source of protein [8,9]. This is in consensus with the EU report on sustainable sources of plant protein which explores the agronomic, environmental and climate benefits of legumes. Meanwhile, Denmark focuses on exploiting seaweed, non-native urchins and mussels as alternative sustainable sources of protein [10]. All the EU nations discussed in this section have emphasised the need for additional funding for protein crop strategies and R&D activities for developing alternative sustainable sources of protein for food and feed applications. Another common objective among these nations is the development of circular economies and sustainable supply chains to ensure that the products are sustainable and competitive in the global market.

Meanwhile, the EU strategy for the development of plant protein, known as the Supranational Protein Strategy, aims to support EU nations to grow protein crops and develop supply chains while creating a partnership between the government, industry and academia, encouraging customer behaviour towards sustainable food choices, developing practices and policies towards sustainable production and tapping into the benefits of sustainable production systems and agri-ecological practices. To achieve these aims, the EU has devised measures to support farmers in growing plant proteins by coordinating research into plant protein sources and providing funding to innovative projects that explore the sustainable production of plant protein, improving knowledge transfer, technical support and investments to provide rural development support on farms and promote the benefits of plant protein with respect to nutrition, health, climate and environment [11].

Circular bioeconomy relies on sustainably produced biomass as raw materials for the co-production of various products including food, feed, materials, chemicals and biofuels. Proteins for food and feed application are of great interest to establish a viable bioeconomy model which has the potential to be scaled-up to commercial operation. Agro residues are particularly interesting from a biorefining perspective since most oil seeds such as sunflower and rapeseed, which are widely cultivated in Europe for their oil content, leave behind protein-rich press cake on processing [12,13].

Wheat is the most cultivated crop in the EU and is rich in protein content [14]. This is followed by maize, barley, sunflower, rapeseed, soybean, pea, millet etc. [15]. Protein sources have been classified into five groups based on their protein content. Group 1 contains more than 50% of protein dry weight. A few examples of these protein sources are soybean protein and rapeseed protein concentrates. Protein sources which contain 25–45% of protein in their dry weight are classified as group 2 (e.g., rapeseed press cake, soybean meal, sunflower seed meal, microalgae etc.). Group 3 encompasses protein sources which have a protein content ranging between 10–20% dry weight. Examples include rapeseed hull, soybean pods, beet leaves and fresh grass. Meanwhile, protein sources with protein content between 5–10% dry weight are categorised as group 4 (e.g., rape straw, soy straw, corn stover, etc.). Lastly, protein sources where protein contributes to less than 5% of its dry weight is falls under group 5. An example of such a protein source is wheat straw [16].

Nutritional value and digestibility are two major factors that influence the utilisation of a protein source for food and feed applications. Plant-based proteins do not necessarily provide nutritional value in balanced proportions, which is a prerequisite for animal feed formulations. Essential amino acids such as lysine, cysteine and methionine have previously been found to be lacking in plant-based proteins [17]. The Protein Digestibility Corrected Amino Acid Profile Score (PDCAA) is a metric used to measure the nutritional value of protein derived from various sources. In the current scenario, soybean meal and canola have been identified as the best sources of plant protein based on their PDCAA scores. For this reason and for reasons of low-cost availability, soybean meal has been the major source of protein exploited for feed applications, followed by maize [18]. On the other hand, plant protein digestibility is low, which is a deterrent in their use in food and feed application. Furthermore, processing operations can further affect digestibility due to the loss of moisture content and the formation of complex disulphide bonds within the protein.

Soybean meal (SBM) is the primary plant-protein source for swine diets. The amino acid (AA) profile of SBM is well-balanced and complements the AA profile of grains such as corn and wheat, and these AAs are highly digestible for pigs [3]. The energy content of SBM has been reported as 3619 kcal/kg digestible energy (DE) and 3294 kcal/kg metabolizable energy (ME), which suggests that SBM has 105% and 97% of corn grain DE and ME values, respectively [19]. SBM plays an important role in animal feed production and has become one of the primary crops cultivated by farming communities across the world. However, the popularity of this oil seed for its protein content has resulted in daunting environmental problems. Since soy plants only produce one yield in their lifetime, their cultivation requires more land to meet demand. In 2017, 123.6 million hectares of land were used to grow 352.6 million tonnes of soybeans [20]. In the tropical countries where soy is largely produced, demand for soybean has resulted in vast areas of virgin land being cleared to grow this crop. This has led to widespread deforestation in South America, especially in Brazil, Argentina, Bolivia and Paraguay. In 2018, 57 million hectares of forest land were dedicated to soy production. Worldwide, soy cultivation takes up an area the size of France, Belgium, Germany and the Netherlands combined [21].

Along with deforestation, other concerns related to global soy production include carbon emissions, soil erosion and strained water resources. Conversion of forest land into agricultural land is one of the greatest contributors to carbon emissions. Forests absorb and store huge amounts of carbon dioxide which is released into the atmosphere when they are cleared to grow crops like soy. Additional emissions are associated with mechanised processing and export-related food miles [22]. Meanwhile, soya-related soil erosion is caused by the intensive agricultural practices such as ploughing and intensive irrigation. The lack of tree cover makes the land susceptible to wind which results in loss of topsoil. Over the course of time, agricultural land wanes in fertility, leading to a decrease in productivity threatening long-term global food security [23].

The environmental problems raised by global soy production call for drastic innovative measures to meet the demand for protein sources for animal feed applications. Alternative examples of other plant species that are viewed as potential plant protein sources include sunflower meal, beans, ground nuts, peas (pigeon peas, cow peas and chickpeas), sesame and green grains. However, from a national perspective, the climatic conditions in Ireland do not support the growth of soy and maize. To circumvent this problem, the EU and Ireland have partnered together through the Protein Aid Scheme launched in 2015 to subsidise farmers to grow other protein-rich crops such as beans, peas and lupins. However, only 8100 hectares in Ireland were dedicated to growing peas and beans in 2019. It is therefore safe to assume that this step, although promising, is unlikely to provide a viable solution to animal feed applications in the near future. On the other hand, grasslands constitute over 90% of the total agricultural lands in Ireland. Perennial rye grass, Italian rye grass and white clover are the three varieties of grass found in Ireland. Of these, perennial rye grass is the most commercially important form of grass as the other grass varieties are not commonly used.

Perennial rye grass is a temperate grass that is commonly found across Europe. This type of grass is rich in minerals and well-maintained crops have enough metabolizable energy. The climatic conditions in Ireland support the growth of various varieties of grass. The Republic of Ireland had a total of 4.9 Mha of grasslands in 2016 [24]. The agricultural economy of Ireland is heavily reliant on grasslands, in particular perennial rye grass varieties, and plays an important role in the ruminant industry [25]. Currently, the commercial application of this type of grass is confined as a cattle feed [26]. With a high metabolisable energy of 12.2 MJ/kg DM on average, this species of grass is the primary source of fodder for cows [27]. However, grass is also abundant in value-added products such as protein and can therefore form a potential raw material for small-scale green biorefinery processes [28].

Kromus et al. (2003) [29] describes green biorefinery as a concept to utilize green (grassland) biomass as raw material for the production of biobased products such as proteins, lactic acid, fibre and energy (via biogas). Green biorefineries process protein-rich green leafy biomass including grass (e.g., ryegrass, clover and lucerne) and fresh leaves (e.g., potato or beet leaves). The Green Biorefinery concept is currently at an advanced stage of development in several European countries, especially Germany, Denmark, Switzerland, the Netherlands and Austria. In 2001, the first green biorefinery began operation in Switzerland with a processing capacity of 5000 tonnes dry matter of grass per year [30] (Xiu and Shahbazi, 2015). BioWert, Gramitherm and Newfoss are among the commercial partners currently implementing green biorefineries in Europe, with a focus on non-protein products such as technical fibres for composites, insulation materials and paper [31]. Focusing specifically on efforts which have been undertaken to use green biorefineries to improve the protein availability of grasses, work in Denmark through Aarhus University and Netherlands through Grassa BV is the most advanced. The main focus of this approach is to separate soluble protein into a separate fraction which could be used by monogastric animals or in future plant food applications, while leaving sufficient protein within the fibres to serve as a high-quality ruminant feed. For example, through the Danish OrganoFinery project, a fermentation technology using the addition of a specific lactic acid bacterium for precipitation of proteins in the juice was developed with the resulting protein paste containing 5–7% of lactic acid, which was reported to be beneficial for the gut health of poultry and pigs [32]. A more recent study by Aarhus University presented a process using a screw press to separate white clover, red clover, lucerne and perennial ryegrass into a press cake and green juice fraction, with precipitation of protein from the juice performed by either a two-step heat precipitation (a) or acidic precipitation (b) [33]. A recent Danish study focused on biorefinery-extracted protein from organic grass-clover as an input to pig feed diets, found that the meat percentage measured at slaughter increased linearly with inclusion of grass-clover protein in the feed [34]. Meanwhile, two recent studies, one from Denmark and one from the Netherlands, have investigated green biorefinery press cake as an alternative feed in ruminant diets, with both studies demonstrating a comparable performance compared with un-refined silage, with reduced levels of n and p in cattle excrement [35,36]. These findings point to the potential of green biorefining as a route for increasing the protein potential of grassland.

The aim is of this study was to determine the potential perennial rye grass protein as a monogastric feed protein additive. The authors could not find any study that investigated the application of protein derived from perennial rye grass in feed applications. In addition, no such previous biorefining study has previously been undertaken in Ireland. Therefore, in this study, an innovative grass biorefining process was employed to extract green protein concentrate from perennial rye grass. The novel grass protein was analysed for its nutritional content and amino acid profile. Indicators such as daily feed intake, average daily weight gain and feed conversion efficiency were calculated. Grass protein was tested as a potential protein supplement in animal feed for pigs alongside conventional soybean meal to determine the performance of green protein feed in comparison with a conventional weaner diet.

2. Materials and Methods

2.1. Preparation of Grass Protein Concentrate

Fresh perennial rye grass was harvested from farms located in West Cork. The feedstock was processed using a novel green biorefinery process developed by Grassa BV. A schematic of the protein extraction process is provided in Figure 1. Specific details on the protein extraction process cannot be discussed in detail due to proprietary concerns. Briefly, fresh grass was loaded into the biorefinery via a loading dock washed with water upon entry to remove dirt, sand and impurities. The grass then underwent mechanical fractionation via an extruder which produced two primary products, a press cake and a green juice. The press cake, containing approximately 50–60% of the original, primarily insoluble protein, was ensiled and baled, serving as low emission feed for ruminants, with a high nitrogen use efficiency. The remaining 40–50% of protein was pressed into the green juice, along with nutrients, minerals and mainly fructan sugars. Heat coagulation via heat exchangers solidified the protein contained in the juice, which was then separated by vacuum filter. This green protein juice could be used directly as an input to wet feeding but in this trial was further dried to approx. 90%DM via belt dryer. This product, a green protein concentrate, can now be storable for more than one year and was integrated within pig feed rations for this study. The residual grass whey can be further processed to extract high value fructans and produce a mineral concentrate.

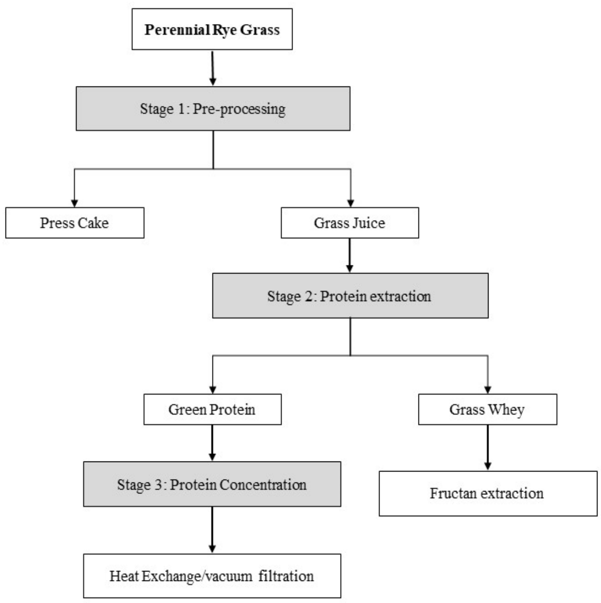

Figure 1. Schematic of protein extraction from perennial rye grass.

2.2. Characterisation of Perennial Rye Grass Protein Concentrate

Samples of green protein concentrate were subjected to proximate analysis. Proximate analysis was done by Dairy Gold analytical laboratory. Additionally, the amino acid profile of green protein concentrate was analysed at Sciantec, North Yorkshire, UK. The AA contents were measured by cation exchange chromatography after acid hydrolysis for 24 h [37].

2.3. Protein Concentrate Pig Feed Trails

To examine the feasibility of the grass protein as a potential replacement for soybean meal in pig feed diets, dry feed trials on a commercial pig farm were conducted to compare pig performance between control groups and treatment groups. Prior to the trial, the green protein concentrate was subjected to compositional analysis to assess the nutritional value. This information was used to replace conventional ingredients of the feed such as soybean meal and barley. The treatment feed was prepared to meet the nutritional requirements of the weaner pigs, details of which are provided in Table 1. The weaner diet was formulated on standardised ileal digestible amino acids. Protein is provided in the diet to supply amino acids (the building blocks of protein). There are 20 primary amino acids. Half of these are essential, in that they cannot be synthesised by the pig. The others are termed non-essential, as they can be synthesised in the body.

Table 1. Diet formulation for weaner pigs.

Nutrients	Concentration
Protein (%)	18.63
Oil (%)	5.77
Fibre (%)	3.11
Ash (%)	6.08
DE (MJ/kg)	14.42
NE (MJ/kg)	10.25
Lysine (%)	1.27
ILD Lysine (%)	1.15
Methionine (%)	0.42
Calcium (%)	0.75
Dig Phosphorus (%)	0.35
Sodium (%)	0.24
Vitamin A (IU/kg)	12,000
Vitamin D3 (IU/kg)	2000
Vitamin E (IU/kg)	125
Biotin (mcg/kg)	150
Phytase (FTU/kg)	1000

The dry feed trial was a comprehensive feed trial which focused on late stage finishing weaner pigs entering second stage weaner accommodation, aged nine weeks old and weighing 20 kg on average. The pigs were split into a treatment group and a control group with 54 pigs and 55 pigs, respectively, for approximately 30 days until slaughter. The control feed consisted of wheat, maize, barley, molasses, SBM, soy oil, soy hull and minerals in recommended amounts. The treatment feed, on the other hand, comprised green protein concentrate replacing a proportion of soybean meal, barley, and wheat by 27.3%, 25% and 8%, respectively, in comparison to the control. Additionally, the fraction of soy hull was increased by 33.3% in the treatment compared to the control. The compositions of the treatment feed and control feed are provided in Table 2. Weekly weigh-ins and feed intakes were recorded to allow the calculation of daily feed intake, average daily gain and feed conversion ratio for each treatment.

Table 2. Composition of control and treatment feed.

Raw Material	Control	Treatment
Barley (%)	30.00	22.50
Maize (%)	10.00	10.00
Wheat (%)	25.00	23.00
Molasses (%)	2.00	2.00
Hipro Soya (%)	22.00	16.00
Grass Protein Pellets (%)	-	15.00
Soya Hulls (%)	1.00	1.50
Lactoflo (%)	2.50	2.50
Soya Oil (%)	3.70	3.70
100 Weaner + Vita GP (3.8%)	3.80	3.80

2.4. Daily Feed Intake (DFI), Average Weight Gain and Feed Conversion Ratio

The daily feed intake for each treatment was calculated as follows:

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

Total feed intake is the feed delivered less the feed remaining at the end of the trial [38].

Meanwhile, weigh-ins of the pigs were conducted during the whole period of the trial, at the start and at the end of every week until the end of the trial, in order to calculate average daily weight gain.

Feed conversion ratio was calculated as follows [39]:

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

3. Results

3.1. Characterisation of Green Protein Concentrate

Proximate analysis was performed to determine the different components in the green protein concentrate such as crude fibre, ash, protein, starch and total solids. Accordingly, green protein concentrate comprised a crude fibre content of 6.1%. Furthermore, the protein content was recorded to be 33.9%, ash content at 11.8% and oil within the range of 10.5 to 13.2%. Interestingly, no starch content was found in the protein concentrate. The finished feed appeared dark green in colour.

3.2. Amino Acid Profiling of Green Protein

Table 3 provides a comparison of soybean meal nutritional qualities with other oil seed meals and green protein concentrate. The complete amino acid profile of the green protein concentrate is provided in Table 4. Accordingly, the novel protein concentrate was rich in glutamate+ glutamin (3.6%) aspartate (3.1%), leucine (2.8%) and alanine (2.1%). Interestingly, the gross energy was found to be 4347 kcal kg^{-1}.

Table 3. Nutrient requirements for pigs at different stages of life [12].

Essential Nutrients	Creep 6–10 kg	Weaner 10–20 kg	Grower 20–50 kg	Finisher 50–100 kg	Dry Sow	Lactating Sow
Crude protein	23–25	19–22	18–20	16–17	13.5–13.8	17–18
Crude fibre	1–3	2–4	2–5	3–4	4–5	2–5
Lysine	1.3–1.5	1.25–1.35	1.0–1.1	0.85–0.95	0.6–0.7	1.0–1.15
Arginine	0.52–0.6	0.5	0.4–0.44	0.34–0.38	0.24–0.28	0.4–0.46
Histidine	0.46–0.53	0.44–0.47	0.35–0.39	0.30–0.33	0.21–0.25	0.35–0.40
Isoleucine	0.78–0.9	0.75–0.81	0.6–0.66	0.51–0.57	0.36–0.42	0.6–0.69
Leucine	1.43–1.65	1.38–1.5	1.1–1.2	0.94–1.01	0.66–0.77	1.1–1.27
Methionine + cystine	0.78–0.9	0.75–0.81	0.6–0.66	0.51–0.57	0.36–0.42	0.6–0.69

Table 3. *Cont.*

Essential Nutrients	Creep 6–10 kg	Weaner 10–20 kg	Grower 20–50 kg	Finisher 50–100 kg	Dry Sow	Lactating Sow
Phenylalanine + tyrosine	1.24–1.43	1.2–1.3	0.95–1.04	0.81–0.90	0.57–0.67	0.95–1.09
Threonine	0.78–0.9	0.75–0.81	0.6–0.66	0.51–0.57	0.36–0.42	0.6–0.69
Tryptophan	0.23–0.27	0.23–0.33	0.18–0.20	0.15–0.17	0.11–0.13	0.18–0.21
Valine	0.98–1.1	0.94	0.75–0.83	0.64–0.71	0.45–0.53	0.75–0.86

Figures are represented as g per 100 g of feed.

Table 4. Amino acid profile of green protein in comparison with perennial rye grass protein [40]. Reproduced from [40], Journal of Dairy Sciences: 2013.

Amino Acid	Green Protein Concentrate	Perennial Rye Grass
Alanine	2.12	7.8
Arginine	1.84	6.0
Aspartic	3.09	10.4
Cystine	0.18	1.4
Glutamic	3.58	12.3
Glycine	1.79	6.2
Histidine	0.65	2.2
Iso-leucine	1.48	4.7
Leucine	2.75	9.4
Lysine	1.81	4.6
Methionine	0.65	2.2
Phenylalanine	1.84	5.7
Proline	1.52	6.0
Serine	1.38	5.0
Threonine	1.50	5.2
Tryptophan	0.61	-
Tyrosine	0.99	3.4
Valine	1.87	6.5

3.3. Daily Feed Intake

During the trial, the daily feed intake was recorded at the end of every week. The treatment feed was very well received by the pigs, and they ate well. The average weight of the pigs at the start of the trials was recorded to be 1.079 kg for the control diet and 1.132 kg for the treatment diet. There was a steady increase in the feed intake for both control and treatment diets as expected. During the first week, the feed intake for the control feed was recorded to be 0.991 kg/d. In comparison, this figure was 1.022 kg/d for the treatment diet. As the trial progressed, the difference between the daily feed intake for the control diet and the treatment diet increased considerably. By the end of the trial, the daily feed intake for the treatment diet (1.512 kg/d) was 8% higher than the control diet (1.400 kg/d). Dung consistency was normal, as can be observed in Figure 2, where the feaces of the treatment pigs appeared green. Pigs were clean and no major differences were observed when compared to the pigs on control diets.

(**A**) (**B**)

Figure 2. Dung consistency of treatment (**A**) and control weaners (**B**).

3.4. Average Daily Weight Gain

During the trial, the weaner pigs were weighed individually at the start of the trial and at the end of the week thereafter. The superiority of the treatment diet over the control can be observed from ADG comparisons. On the control diet, the pigs gained 0.592 kg/day during the first week. This rate increased as the trial progressed with an average daily gain of 0.646 kg/day after the second week, 0.699 kg/day after the third week and 0.682 kg/day at the end of the trial. On the other hand, the average weight gain of pigs on the treatment diet started slowly at 0.577 kg/day by the end of the first week but increased substantially as the trials progressed. The average weight gain by the end of the second week was 0.683 kg/day, which increased to 0.729 kg/day. By the end of the trial, a high average weight gain of 0.742 kg/day was achieved.

3.5. Feed Conversion Ratio

The feed conversion ratio for the trial is provided in Table 5. The initial value for FCR in the first week was reported to be 1.67 for the control diet. However, this value was higher for the treatment diet (1.77). Nonetheless, the FCR values for the succeeding weeks for both treatment and control were found to be similar. In general, the feed conversion ratio for both control and treatment diets increased each week as the trial progressed. There was no comparable difference between FCR values of the control diet and treatment diet by the end of days 15, 21 and 31.

Table 5. Daily feed intake, feed conversion ratio and average daily gain of weaners on treatment and control diets.

Date of Weighing	Daily Feed Intake (kg/d)		Feed Conversion Ratio		Average Daily Gain (kg/day)	
	Treatment	Control	Treatment	Control	Treatment	Control
Period 1	1.022	0.991	1.77	1.67	0.577	0.592
Period 2	1.247	1.182	1.83	1.83	0.683	0.646
Period 3	1.386	1.301	1.90	1.86	0.729	0.699
Period 4	1.512	1.400	2.04	2.05	0.742	0.682

4. Discussion

4.1. Green Protein Concentrate as an Additive for Pig Feed Preparation

Perennial rye grass was subjected to biorefining to extract green protein for monogastric feed applications. The biorefining activities involved extrusion followed by protein extraction and coagulation using heat. All the processes involved in the preparation of the protein concentrate used environmentally friendly, sustainable strategies. Further details about the biorefining process cannot be discussed due to proprietary issues. The pig's gut is an important factor for health and consistent performance. 'Gut health' is an underestimated factor that not only acts as a digestive organ but also has an immunological function. Modern livestock animals are typically fed high concentrate nutrient-dense diets to meet nutrient requirements [41]. However, high-concentrated diets cannot meet physiological requirements, as animals need dietary fibre for optimal health and digestion. A minimal level of fibre is a prerequisite for optimal nutrition and ingredients metabolism. Crude fibre is a measure of the fermentable components of the feed. Although low in energy this indigestible carbohydrate is important for the gut health of pigs and poultry [42]. It was therefore important to assess the amount of crude fibre in the green protein. Determination of these components allows us to make legitimate comparisons of feeds based on nutritional composition. The crude fibre content in the green protein concentrate was similar to that found in soybean meal (Table 5).

The ash content in the green protein concentrate was found to be 11.8%. In comparison, reported values of ash content in soybean meal fall within the range of 4.5–6.4% [42]. A high ash content in green protein concentrates may be attributed to the presence of sand due to improper washing of the grass feedstock. Ash content does not affect the digestibility of protein. On the other hand, high ash content can contribute to higher utilisation of digested protein [43].

Table 4 provides a comparison between the crude protein content of soybean meal and green protein concentrate. Soybean meal has a higher crude protein content which falls in the range of 44–48 g per 100 g. However, the crude protein in green protein concentrate was found to be 33.9%. This is an indication that soybean meal cannot be completely replaced by perennial rye grass protein concentrate when preparing pig feed rations. Another important aspect that has a significant impact on monogastric feed formulations is the synthetic amino acid content. There has been a steady increase in the use of synthetic amino acid in animal nutrition. More than half of the total global amino acid production is represented by animal feeds. Amino acids that are critical in the normal growth of the animal include lysine, threonine, tryptophan, methionine (and cystine), isoleucine, leucine, histidine, valine, arginine and phenylalanine (and tyrosine). However, the amino acids of greatest practical importance in diet formulations (i.e., those most likely to be at highest deficient levels) are lysine, tryptophan, threonine and methionine [44]. Table 1 represents the amino acid requirements and their respective levels in pig feed with respect to their age. Currently, soybean meal is considered as the best protein source for feed requirements for monogastric animals. Additionally, soybean meal provides a good amino acid balance due to the presence of high amounts of lysine, tryptophan, threonine and isoleucine. Furthermore, the digestibility of amino acids in soybean meal is high. While the total protein, lysine and cysteine contents are slightly higher in soybean meal in comparison with green protein concentrate, the methionine content and threonine content are at par. Besides, the latter has a higher percentage of crude fibre which, as mentioned earlier, is crucial for the pigs' gut health. From Table 5, the content of essential amino acids in the green protein concentrate is comparable with that of soybean meal.

From Table 4, it is evident that green protein concentrate contains significantly lower amounts of lysine and cysteine in comparison with soybean meal. It was therefore important to include soybean meal and soya hulls as part of the treatment diet to mitigate the deficient lysine and cysteine content in green protein. However, the methionine and threonine content in the green protein concentrate are comparable with that of soybean meal. Furthermore, the essential amino acid concentration (lysine, methionine, threonine and cysteine) in green protein concentrate was higher than that of sunflower meal, rapeseed meal and cottonseed meal, which are all sources of protein widely exploited as animal feed additives [45,46].

4.2. Daily Feed Intake

According to a study conducted by Pierozan, Agostini [47], the daily dry matter intake is dependent on the number of pigs per pen, type of feeder, origin and sex of the pigs. Pigs are generally fed in two modes: dry feed and wet feed. Both feeding modes have been connected with respective advantages. Approximately 70% of pigs in Ireland are on wet feed. Many producers use wet feeding as their units were built at a time when significant volumes of liquid by-products (e.g., liquid whey and skim milk) were readily available. Wet feeding offered the potential to feed such by-products with a balancer, thereby providing a cheap balanced diet. A study performed by Zoric, Johansson [48] reported the behavioural differences between pigs that were on wet feed and dry feed. Accordingly, although pigs from both systems performed well, the researchers preferred the dry feed system considering a welfare-based standpoint. Dry feeding systems are gaining popularity and have been adopted by several farms as they require less labour [49]. Hence, feeding experiments were conducted employing green protein concentrate as a dry feed additive.

In this study, daily feed intake for the treatment diet was found to be better compared to the control. From the results presented in Table 6, it was evident that the weaner pigs on the treatment diet consumed more food as compared to the control, indicating that the pigs preferred the treatment diet. This is indicative of the green protein imparting a better taste when incorporated in the feed. A recent study by Stødkilde, Ambye-Jensen [46] reported that adding green protein to the feed did not alter the taste to discourage the pigs from consuming it. Furthermore, addition of green protein from sources like clover grass improved the meat percentage and omega 3 fatty acids in meat.

Table 6. Crude fibre, crude protein and amino acid profile of oil seed meals [13].

Animal Feed Protein Sources	Crude Protein	Lysine	Methionine	Cysteine	Threonine	Crude Fibre
Soybean Meal	44–48	2.81–3.20	0.60–0.75	0.69–0.74	0.71–2.00	3.0–7.0
Sunflower Meal	24–44	1.18–1.49	0.74–0.79	0.55–0.59	1.21–1.48	12.0–32.0
Rapeseed Meal	36	2.00–2.12	0.67–0.75	0.54–0.91	1.53–2.21	10.0–15.0
Cottonseed Meal	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	33.9	1.81	0.65	0.18	1.5	6.1

Figures are represented as g per 100 g of feed.

4.3. Average Weight Gain

Variation in live weight and weight gain is undesirable in pig farming. It is essential to identify approaches that reduce the variation in live weight within a population compared to management approaches that attempt to minimize the impact of whatever level of variation exists [50]. In theory, it is only natural to observe variation in weights within a population at the end of finishing. Therefore, multiple approaches will need to be introduced to manage that variation. Reducing variation in growth rate is key in eliminating variation in live weight within the population. Some of the factors identified as influencers in weight variations among pigs include genetic factors, sex effects, birth weight, weaning age, feeding level and individual amino acid content (arginine levels) in the feed. Increasing the growth rate of all of the pigs in a population will not reduce variation but it will result in more of the lighter weight pigs reaching the minimum weight required by the market before the building needs to be emptied [51].

The average weight gain of pigs on the treatment diet was slightly lower than with that of weaners on control diet during the first week of the trial. This dip in AVG may be attributed to the need for the weaners to acclimatise themselves to the new diet. Interestingly, the AVG of the weaners on the treatment diet was found to be higher than that of control from week 2 of the trail. This trend continued until the end of the trial. The increase in AVG corresponds to the higher dry matter intake of weaner pigs on the treatment diet. A higher daily feed intake may have resulted in higher AVG. Consequently, the final weight gain achieved by the weaners on treatment diet was 6.44% higher than that of the control sample. The variation in average weight gain may be attributed to the inconsistency in the dry matter [49].

4.4. Feed Conversion Ratio

Feed conversion ratio is the measurement of the amount of feed required for the animal to gain one pound of body weight. A lower feed conversion ratio is an indication of the pigs efficiently turning feed into body weight. Several factors influence the feed efficiency in pigs. The amount of phosphorus fed to pigs should be maintained at an optimal level. Phosphorus in pig diets forms part of the structural compounds in bone and cell membrane and plays a vital role in energy metabolism and other metabolic pathways. Excess of phosphorus in the diet will result in the excretion of the mineral as faeces [50]. Pelleting of feed can improve the feed conversion ratio; quality pellets prevent the pigs from sorting and wasting the feed. Reduced pellet size can also assist digestion of the feed [51]. Finally, as was mentioned in the earlier section, lysine is the limiting amino acid in grain-based diets for pigs. Pigs require lysine in required amounts in order to effectively utilise other amino acids for growth [52].

From Table 6, the FCR values for the treatment and control diet were similar for the duration of the trial period except for the first week. This is an indication that the treatment diet is as efficient as the conventional diet fed to weaner pigs. The initial value of FCR for the treatment diet was higher than that of the control, perhaps due to the acclimatisation process required for the weaner pigs to digest the green protein. Nonetheless, from FCR values it can be observed that the conversion efficiency of the feed is not affected by the addition of the green protein concentrate. Additionally, the overall health of the pigs on the trial diet was similar to other pig groups reared on conventional diets [49].

5. Conclusions

A pig feed formulation which included green protein concentrate was found to be superior to soy-based pig diet. Characterisation of the green protein concentrate revealed that the crude protein content was comparable to that of soybean meal. All the indicators, i.e., the daily feed intake, feed conversion ratio and average daily weight gain, were consistently higher for the treatment diet compared to the control over the course of the feeding trials. This is an indication that the weaner pigs preferred the treatment diet over control. On the other hand, the lysine content in the green protein was not adequate enough to replace soybean meal in weaner diets. Overall, the results of the study, although preliminary, are quite promising and indicative of the potential of perennial ryegrass biorefined protein as a sustainable protein source for pig feed formulations. Further studies on the addition of this protein in pig feeds may include productive performances, ileal digestibility, etc., to further substantiate the efficacy of the green grass protein as a prospective feed protein source.

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